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Histological eosinophilic gastritis is a systemic disorder associated with blood and extra-gastric eosinophilia, Th2 immunity, and a unique gastric transcriptome

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Abstract

Background—The definition of eosinophilic gastritis (EG) is currently limited to histological EG based on the tissue eosinophil count.

Objective—We aimed to provide additional fundamental information about the molecular, histopathological, and clinical characteristics of EG.

Methods—Genome-wide transcript profiles and histological features of gastric biopsies as well as blood eosinophil numbers were analyzed in EG and control patients (n = 15 each).

Results—The peak gastric antrum eosinophil count was 282.7 ± 163.9 eosinophils/400X high-power field (HPF) in EG and 11.0 ± 8.5 eosinophils/HPF in control patients ($P = 6.1 \times 10^{-7}$). EG

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patients (87%) had co-existing eosinophilic inflammation in multiple gastrointestinal segments; the esophagus represented the most common secondary site. Elevated peripheral blood eosinophil numbers (EG $1.09 \pm 0.88 \times 10^3$ [K]/ μ l vs. control 0.09 ± 0.08 K/ μ l, $P = .0027$) positively correlated with peak gastric eosinophil counts (Pearson $r^2 = .8102$, $P < .0001$). MIB-1⁺ (proliferating), CD117⁺ (mast cells), and FOXP3⁺ cells (regulatory and/or activated T cells) were increased in EG. Transcript profiling revealed changes in 8% of the genome in EG gastric tissue. Only 7% of this EG transcriptome overlapped with the eosinophilic esophagitis (EoE) transcriptome. Significantly increased *IL4*, *IL5*, *IL13*, *IL17*, *CCL26* and mast cell-specific transcripts and decreased *IL33* were observed.

Conclusion—EG is a systemic disorder involving profound blood and gastrointestinal tract eosinophilia, Th2 immunity, and a conserved gastric transcriptome markedly distinct from the EoE transcriptome. The data herein define germane cellular and molecular pathways of EG and provide a basis for improving diagnosis and treatment.

Keywords

Eosinophilic gastritis; EG transcriptome; IL-13; eosinophils; regulatory T cells; mast cells; CCL26

Introduction

Eosinophilic gastrointestinal disorders (EGID) constitute a diverse group of disorders that have increased numbers of eosinophils in one or more parts of the gastrointestinal (GI) tract in the absence of known causes of eosinophilia (e.g. secondary infection) or an underlying systemic inflammatory disease (e.g. inflammatory bowel disease [IBD])^{1–6}. The most studied EGID is eosinophilic esophagitis (EoE), partly because it has well-defined, consensus diagnostic criteria⁷. Diagnostic criteria for other EGID, including eosinophilic gastritis (EG), are less well-defined. Consensus diagnostic criteria currently do not exist for EG, but an average density of 127 eosinophils/mm² in at least five separate fields (30 peak eosinophils/400X high-power field [HPF]) in gastric biopsies in the absence of known causes of eosinophilia has been proposed for “histological EG”⁸. Additional EG criteria focus on the abnormal eosinophil localization in the surface or foveolar epithelium, muscularis mucosae, or submucosa; mucosal damage (e.g. foveolar hyperplasia); architectural distortion; and the presence of significant chronic or active inflammation^{9, 10}. Although EG is commonly considered to be specific to the stomach, it has not yet been determined whether EG has eosinophilic involvement of other GI tract segments. Investigating this aspect is especially relevant as the relationships among the diverse EGID have not been determined.

The molecular pathogenesis of EoE involves an immune/antigen-driven, type 2 helper T cell (Th2)-associated process^{11, 12}. In particular, the affected tissue of EoE patients exhibits a unique gene expression pattern including upregulation of interleukin (IL) 13^{13, 14}. This Th2 cytokine promotes disease pathogenesis partially through its effects on epithelial cells, including upregulation of eotaxin-3 (CCL26), an eosinophil-specific chemoattractant and activating factor^{13–16}. Although less is known about the molecular pathogenesis of EG, peripheral blood T cells of EG patients secrete higher levels of IL-4 and IL-5 but less interferon (IFN) γ upon mitogenic stimulation than those of control patients; in addition,

gastric *IL5* mRNA had been shown to be elevated in EG compared with control patients¹⁷. In a murine model of allergen-induced EGID, eosinophils were responsible for GI pathology, including gastric dysmotility¹⁸.

We report seminal observations concerning the cellular and molecular characteristics of EG, including evidence that EG is typically part of a more generalized EGID and rarely exists in isolation; peripheral blood eosinophilia is prominent and correlative with peak gastric eosinophil numbers; FOXP3⁺ cells (likely regulatory T cells [Treg]), mast cells, and proliferating cells are increased; EG has a prominent and conserved transcriptome that has minimal overlap with the EoE transcriptome; and Th2 cytokines (e.g. *IL4*, *IL5*, *IL13*) and the eosinophil-related chemokine eotaxin-3 (*CCL26*) are upregulated.

Methods

Entry criteria and patient characteristics

Patients were defined to have active EG if they had gastric biopsies that met the criteria for histological EG (≥ 30 peak eosinophils/HPF in five separate HPF⁸) and that also showed architectural abnormalities, including excessively branched and/or coiled glands. Controls were patients without EGID who otherwise met entry criteria and were sex- and age-matched to EG patients. See the Methods section in this article's Online Repository for more details. This study was approved by the Cincinnati Children's Hospital Medical Center (CCHMC) Institutional Review Board. Individual patient characteristics and atopic condition histories are provided (Table E1 and E2, respectively, in this article's Online Repository).

Immunohistochemistry

Antibody information and procedures are provided (Table E3, A, in this article's Online Repository).

Quantitative microscopy

Multiple levels of gastric biopsies were surveyed, the areas containing the greatest concentration of eosinophils were identified, and eosinophils were counted in five separate HPF (0.3 mm²). A peak eosinophil count was obtained for other GI tract site biopsies if eosinophils appeared excessive. See the Methods section in this article's Online Repository for more details.

RNA isolation

Biopsy specimens collected during the index endoscopy were stored in RNAlater until subjected to RNA isolation using the miRNeasy kit (Qiagen, Valencia, CA) per the manufacturer's instructions.

Microarray analysis

RNA labeling and hybridization to the GeneChip Human Genome U133 Plus 2.0 Array (Affymetrix, Santa Clara, CA) was performed as reported¹³ by the CCHMC Gene Expression Microarray Core.

Real-time polymerase chain reaction analysis

Complementary DNA was synthesized from total RNA (500 ng) using Superscript II Reverse Transcriptase (Invitrogen, Carlsbad, CA) per the manufacturer's protocol. Real-time polymerase chain reaction (RT-PCR) was performed using the iQ5 system and SYBR green mix (BioRad, Hercules, CA). The value obtained for each primer set (Table E3, B) was normalized to the *GAPDH* value.

Gene ontology and pathway analysis

Gene lists were subjected to GOrilla (Gene Ontology enRIchment anaLysis and visuaLIzAtion tool) for gene ontology analysis¹⁹. Single experiment analysis was performed using Genespring 12.5 software (Agilent Technologies, Santa Clara, CA). Pathway analysis was done using Ingenuity Pathway Analysis (IPA) (Ingenuity Systems, Inc., Redwood City, CA).

Statistical analysis

Data are expressed as mean $\pm$ standard deviation (SD) unless otherwise noted. Statistical significance was determined using the unpaired *t* test (2 groups, normal distribution, equal variance) or Mann-Whitney test with Dunn's multiple comparison post-test (2 groups, nonparametric); binary data were analyzed using the Fisher's exact test (Prism 5.0 software; GraphPad Software, Inc., La Jolla, CA). Correlation analysis was performed using Prism 5.0. Microarray data (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54043>) were analyzed using Genespring GX 7.3 software (Agilent Technologies).

Results

Patient phenotypes

An enrichment of allergic diseases (e.g. asthma, food allergy, food anaphylaxis) was observed in EG (Table I, Table E2 in this article's Online Repository). Fourteen of the 15 EG patients were evaluated with skin prick tests, and 7 reacted to food and aeroallergens (Table E2). Nine EG patients had multiple positive radioallergosorbent (RAST) tests. The age of the study population was 11.1 ± 6.5 (range 1–30) years (y) with EG patients being older than controls.

Elevated blood eosinophils in EG

Blood eosinophil counts were increased in 12/15 EG patients and were significantly higher in EG compared with controls (EG $1.09 \pm 0.88 \times 10^3$ [K]/ μ l vs. control 0.09 ± 0.08 K/ μ l, $P = .0027$) (Fig 1, A; Table I; Table II, A; Table E2). Eosinophils comprised a normal percentage (4%) of white blood cells in control patients, whereas 13/15 EG patients had an elevated eosinophil percentage (EG $14.27 \pm 8.63\%$ vs. control $1.33 \pm 1.22\%$, $P = .00021$) (Fig 1, B; Table I; Table II, A; Table E2).

EG chronicity and association with other EGID

EG patients had disease involving gastric eosinophilic inflammation for a duration of 2.2 ± 2.9 y (range 0 – 10.9 y) prior to the index endoscopy. Of 15 EG patients, 13 (87%) had

extra-gastric eosinophilic inflammation in the GI tract either at the index ($n = 9/15$) or prior endoscopies ($n = 11/15$) (Table I, Table E1). Of these 13 EG patients, 3 were diagnosed with another form of EGID prior to being diagnosed with EG (mean time of EGID before EG diagnosis = 3.3 ± 1.3 y). One patient was diagnosed with EG prior to being diagnosed with another form of EGID (time of EG prior to other EGID diagnosis = 0.4 y). Nine patients were diagnosed with EG and another EGID concurrently. At the index endoscopy, 9 patients had eosinophilic inflammation present in the esophagus ($n = 7$), duodenum ($n = 1$), and/or jejunum ($n = 2$) at numbers sufficient for histologic diagnosis of EoE, eosinophilic duodenitis (ED), and/or eosinophilic jejunitis (EJ), respectively. Prior to the index endoscopy, 11 patients had eosinophilic inflammation present in the esophagus ($n = 9$), duodenum ($n = 3$), and/or jejunum ($n = 1$) at numbers sufficient for histologic diagnosis of EoE, ED, and/or EJ, respectively.

Endoscopic and gastric biopsy histopathology

Endoscopic appearance of the stomach of EG patients—All EG patients for which endoscopic appearance was reported had endoscopic abnormalities (Table E1), with gastric mucosal nodularity observed in 10/15 EG patients (Fig 1, C; Fig E1, A and B, in this article's Online Repository). Three EG patients (#17, 25, 26) had gastric polyps (Fig E1, C, in this article's Online Repository); one patient (#25) had co-existing phosphatase and tensin homolog (PTEN) hamartoma tumor syndrome²⁰. The gastric mucosa appeared to be normal in all control patients.

Marked eosinophilic inflammation in EG—In all EG cases, the eosinophilic inflammation was nonuniform, varying among and within biopsies (Fig 1, D). In addition to the abnormal eosinophil quantity, the eosinophil distribution in EG biopsies was different from control biopsies. Eosinophils in EG biopsies spanned the depth of the mucosa and often appeared concentrated in the superficial lamina propria (Fig E2, A, in this article's Online Repository, arrow). In contrast, eosinophils in control biopsies were confined to the deep lamina propria (Fig E2, B and C, arrows). Numerous intraepithelial eosinophils were seen in glands in EG biopsies (Fig E2, D and E, arrows) but not in control biopsies. Submucosal eosinophils were present in all EG specimens with visible submucosa, but there were fewer eosinophils in the submucosa than in the lamina propria. The peak and average eosinophil counts were significantly increased in EG biopsies compared to control biopsies (peak: EG 282.7 ± 163.9 [range 68–664]/HPF vs. control 11.0 ± 8.5 [range 3–37]/HPF, $P = 6.1 \times 10^{-7}$; Fig 1, E; Table II, A; Table E4 in this article's Online Repository; average: EG 202.4 ± 109.4 /HPF vs. control 6.1 ± 4.7 /HPF, $P = 1.5 \times 10^{-7}$; Fig E3, A, in this article's Online Repository; Table II, A; Table E4). Gastric biopsy peak eosinophil counts correlated with absolute peripheral blood eosinophil counts (Pearson $r^2 = .8102$, $P < .0001$; Fig 1, F; Fig E3, B).

Architectural changes in EG biopsies included elongated and excessively branched or coiled glands (Fig E2, E). Lamina propria fibrosis was observed in 3 EG (Fig E2, A, arrowheads) and no control cases. In EG biopsies, chronic inflammation, including numerous plasma cells, was sometimes seen in microscopic fields in which eosinophils were not numerous. In one EG case, acute inflammatory cells were seen in the epithelium of a few glands (Fig E2,

F, arrowheads). *Helicobacter pylori* organisms were not seen in gastric biopsies by H&E or anti-*H. pylori* stains.

Increased cell proliferation in EG—The number of gastric epithelial and lamina propria cells that stained positively for MIB-1, a marker of cell proliferation, was greater in EG than control biopsies (epithelial: EG 551.2 ± 135.3 /HPF vs. control 288.4 ± 178.5 /HPF, $P = .03$; lamina propria: EG 65.6 ± 43.7 /HPF vs. control 14.6 ± 2.9 /HPF, $P = .03$; Fig E4, A and B, in this article's Online Repository; Table II, B; Table E5 in this article's Online Repository). In several EG cases, there was expansion of the proliferative zone to include continuous staining of surface epithelial cells, a pattern not seen in control cases (Fig 2, A and B).

Increased FOXP3⁺ cells in EG—FOXP3⁺ lymphocytes were most numerous in and immediately adjacent to lymphoid aggregates. Since lymphoid aggregates were not found in all biopsies, only cells in the lamina propria that were not associated with lymphoid aggregates were quantified. The number of such FOXP3⁺ cells was significantly increased in EG compared to control biopsies (EG 18.4 ± 11.0 /HPF vs. control 4.2 ± 2.6 /HPF, $P = .02$) (Fig 2, C and D; Fig E4, C; Table II, B; Table E5).

Global gene expression in EG patient gastric tissue

We performed global transcript analysis on mRNA isolated from the gastric antrum biopsies of control or EG patients ($n = 5$ each; Patients #1–5 and 16–20, respectively, Table E1). Statistical analysis (analysis of variance) and fold-change filters were applied to these data to generate lists of transcripts differentially regulated between control and EG gastric tissue (Table E6, A, in this article's Online Repository). Volcano plot analysis showed that the least stringent criteria identified 1709 differentially regulated transcripts, representing ~8% of the genome ($P < .05$, 1.5 fold; hereafter designated the “EG transcriptome”); more stringent criteria identified 393 ($P < .05$, 2 fold) and 104 ($P < .01$, 2 fold) (Fig 3, A). The 1709 differentially regulated transcripts (Table E6, B) were graphed by their fold change; transcripts having 5-fold change in EG are listed (Fig 3, B). The expression of the 104 transcripts ($P < .01$, 2 fold) for individual patients is shown (Fig 3, C). The most highly upregulated transcripts were *CCL26* (eotaxin-3, 24.7 fold), *DUOXA2* (dual oxidase maturation factor 2, 13.2 fold), *CDH26* (cadherin 26, 12.3 fold), *CLC* (Charcot-Leyden crystal protein, 9.6 fold), *ITLN1* (intelectin 1, 9.1 fold), and *CCL18* (chemokine [C-C motif] ligand 18, 8.8 fold), a ligand for the Th2 cell chemokine receptor CCR8²¹.

We compared the EG gastric transcriptome with the EoE esophageal transcriptome. Of the 1709 transcripts in the EG transcriptome, 41 overlapped with the EoE transcriptome (Table E7 in this article's Online Repository, ¹³). Of these 41 common transcripts, 31 were regulated in similar manners in EG and EoE (i.e. upregulated or downregulated in both) (Fig 3, D; Table E7). Comparing the most upregulated (Fig 3, E) and downregulated (Fig 3, F) gastric transcripts in EG to esophageal transcripts differentially regulated in EoE indicated that 16 of the 30 most upregulated transcripts in EG were also upregulated in EoE and that only 1 of the 30 most downregulated transcripts in EG was also downregulated in EoE.

Also differentially regulated in EG were 28/354 transcripts previously identified as a “mast cell transcriptome” in EoE²² (Table E8 in this article’s Online Repository). Transcripts characteristic of mast cells were upregulated in EG, including *CPA3* (carboxypeptidase A3, 5.0 fold, $P = .0571$), *TPSAB1/TPSB2* (tryptase alpha/beta 1 / tryptase beta 2, 2.2 fold), and *HPGDS* (hematopoietic prostaglandin D2 synthase, 5.6 fold). Consistent with mast cell involvement, CD117⁺ cells were more numerous (EG $93.8 \pm 33.1/\text{HPF}$ vs. control $51.0 \pm 3.8/\text{HPF}$, $P = .02$; Fig E5, A–C, in this article’s Online Repository; Table II, B; Table E5) and more frequently localized to the superficial lamina propria in EG compared to control biopsies. In control biopsies, CD117⁺ cells were most numerous in the deep lamina propria but were also in the muscularis mucosa.

Gene ontology (GO) analysis of the EG transcriptome using GOrilla revealed significant enrichment of GO terms, including several basic metabolic processes and also inflammatory processes; significantly enriched function-related GO terms included chemokine activity, cytokine activity, and chemokine receptor binding (Table E9, A–C, in this article’s Online Repository). Single experiment analysis revealed “glycolysis and gluconeogenesis” as the most significant pathway and identified several cytokine signaling pathways (IL-6, IL-2, IL-3, IL-4, tumor necrosis factor [TNF]- α , IL-5, transforming growth factor [TGF]- β , IL-11, IFN- γ) and other pathways (ErbB signaling, classical complement activation, coagulation cascade, angiogenesis, Wnt signaling and pluripotency) (Table E10 in this article’s Online Repository). By IPA, the top canonical pathways identified included glycolysis and gluconeogenesis, but other significant pathways identified included ErbB, GM-CSF, and cholecystokinin/gastrin-mediated signaling (Table E11, A, in this article’s Online Repository). Upstream regulator analysis suggested activation of IL-4, IL-5, and IL-13 pathways (Table E11, B).

Cytokine and chemokine expression in EG patient gastric tissue

A significant increase in *IL4*, *IL5*, and *IL17* and a significant decrease in *IL33* were observed in EG compared to control tissue (Fig 4, A–D). Furthermore, *IL13* was highly upregulated in EG compared to control gastric tissue (Fig 5, A). *CCL26* expression was significantly increased in EG gastric tissue (Fig 5, B) and correlated significantly with *IL13* expression (Fig 5, C). *CCL26* expression significantly correlated with the average gastric tissue eosinophil count (Pearson $r^2 = .51$, $P = .02$) (Fig 5, D), but not with peak eosinophil count or with CD117⁺, FOXP3⁺, or MIB-1⁺ cell counts (Fig 5, E; Fig E6, A–D, in this article’s Online Repository).

Discussion

Herein, we report the fundamental features of EG and define EG as a systemic Th2-associated disease based on gastric tissue molecular profiling and significant elevations in circulating eosinophil counts. Circulating eosinophil numbers correlated with tissue pathology, particularly gastric eosinophil numbers. Thus, blood eosinophil numbers may represent a non-invasive biomarker for this disease in most patients. Notably, we demonstrate that EG rarely occurs as an isolated EGID but more often involves other GI segments, which has clinical implications for monitoring and treating patients. By defining

the EG transcriptome, we identified key and potential operational pathways (e.g. IL-13–driven Th2 immunity; IL-17–, ErbB–, and Wnt-dependent pathways). In terms of the underlying immunoeiology, EG is mechanistically similar to EoE. However, the respective disease transcriptomes are >90% divergent, providing rationale for shared and unique therapeutic intervention strategies. Importantly for the understudied EG, we define multiple parameters that have potential utility as diagnostic criteria, including molecular transcripts and the increased presence of mast cells and Treg/activated T cells (FOXP3+ cells).

Although specific clinicopathologic consensus diagnostic criteria have not been established for EG as they have for EoE, our study suggests common clinical, histopathological, and molecular features of EG. In addition to exceeding the tissue eosinophil density value suggested by Lwin et al.⁸, we also observed that all EG biopsies in this study had changes suggesting chronic damage and had intraepithelial eosinophils. In addition, we demonstrate that gastric mastocytosis (increased CD117+ cell numbers and mast cell transcriptome expression) and increased FOXP3+ cell numbers are characteristics of EG and may have value as diagnostic criteria. Notably, specific gene expression in the stomach (e.g. profoundly elevated *CDH26* and *CCL26*) may also have diagnostic merit. It is notable that although blood eosinophilia and a history of atopy are present in most EG patients, changes in some transcripts are universally present (e.g. upregulation of *CCL26*), providing molecular criteria for the disease that has previously been diagnosed strictly on the basis of tissue histology. However, in the absence of determining such molecular criteria, we suggest that strong consideration be given for EG diagnosis if patients have a history of atopy, endoscopic abnormalities of the stomach, particularly with nodules, and/or peripheral blood eosinophilia.

The incidence of EG as an isolated form of EGID versus EG co-occurring with other forms of EGID is not known. We found that only 2/15 (13%) EG patients had a history of exclusively gastric involvement. To examine the generalizability of this finding, we expanded the EG cohort across the EGID database at CCHMC, resulting in the identification of 128 EG patients. Notably, 13 (10%) had a history of only EG and no other EGID (Table E12 in this article's Online Repository). Our data suggest that EG is typically part of a spectrum of EGID rather than an isolated event and thus underscore the importance of obtaining biopsies from multiple GI tract sites in EGID patients who previously had single-site disease, especially if new peripheral eosinophilia develops.

Interestingly, our EG cohort (n = 15) is on average older than control (n = 15) subjects (Table I). Since both groups were systematically selected using the same criteria, it is likely that EG presents at an older age compared with the typical pediatric patient undergoing endoscopy for similar indication, although only a prospective study would prove this. Supporting this observation, the mean age at time of endoscopy of all subjects in the EGID database was 9.6 ± 6.1 y for controls (n = 179) and 12.7 ± 8.8 y for EG patients (n = 135) ($P < .0001$). Despite the age difference observed in these larger patient cohorts, no significant age difference was observed between the cohort of patients (n = 5 EG and n = 5 control) subjected to microarray analysis (EG 12.6 ± 6.2 y vs. control 9.0 ± 7.3 y, $P = .425$). Furthermore, of all transcripts that exhibited a significant correlation with age (Pearson correlation $r > .80$, n = 473 transcripts; Spearman correlation $r > .80$, n = 478 transcripts),

none overlapped with those present in the EG transcriptome. Taken together, this suggests that age difference between control and EG patients does not account for the observed EG transcriptome.

The reported EG transcriptome is representative of histological EG despite treatment status. Therefore, the treatments being taken by the patients are not effective in eradicating the disease, and thus key molecular pathways involved in causing the disease are likely still active, at least to some extent. We speculate that the majority of the altered gene expression between control and EG patients is due to the factor of non-disease vs. active histological EG rather than common gene expression changes due to treatments, particularly since the treatments are variable among the EG patients. It is notable that despite ongoing treatment, there are still common gene expression differences, both at the global and candidate cytokine level, between patients with active histological EG and control patients. EG transcriptome analysis showed evidence of immunological-related pathway activation that could account in part for eosinophil recruitment to the gastric tissue. The EG transcriptome was enriched in GO terms including inflammatory processes and cytokine and chemokine activity. Singular enrichment analysis identified Th2 cytokine signaling pathways including IL-4 and IL-5. IPA suggested activation of the IL-4, IL-5, and IL-13 pathways, and significant increases in *IL4*, *IL5*, and *IL13* were observed. Increased local Th2 cytokine expression has also been observed in EoE and in allergic lung and skin inflammation with predominant eosinophilic inflammation. Although neither *CCL11* (eotaxin-1) nor *CCL24* (eotaxin-2) expression was significantly elevated in EG, *CCL26* (eotaxin-3), a known target of IL-4 and IL-13 signaling, was highly upregulated in EG compared to control tissue and exhibited significant, positive correlation with average gastric tissue eosinophil counts. Taken together, our data suggest IL-13-driven Th2 immunity is locally operational in EG.

Our data additionally uncovered evidence of activation of immune-related pathways previously not directly associated with EGID. Interestingly, we observed *IL17* upregulation, which has not been reported in EGID (e.g. EoE), although increased IL-17 levels have been observed in chronic allergic inflammatory diseases such as allergic asthma and atopic eczema^{23,24,25}. IL-17 signaling results in secretion of pro-inflammatory cytokines and chemokines including IL-8, IL-6, CXCL1, CXCL3, CXCL5, and CXCL6²⁶. Although IL-17 signaling was not directly identified by bioinformatics analysis of EG, evidence of IL-6 pathway activation was detected. Pathways potentially upstream of IL-17 were also predicted to be activated, including IL-1 and IL-1 β . Furthermore, a prominent histological feature in EG is acute and chronic inflammation; IL-17 could promote neutrophil and macrophage recruitment and activation in EG.

Despite identifying common patterns of gene expression between EoE and EG, most (93%) differentially regulated transcripts did not overlap between these two diseases. Several possibilities could account for this finding, such as differences in tissue composition (e.g. resident cell types) or distinct disease mechanisms (e.g. differential cell recruitment or altered gene expression program of resident cells). The dissimilarities in differentially regulated transcripts in EoE and EG may at least in part arise from the distinct structural cells and immunocytes present in these tissues at baseline.

Transcripts present in the glycolysis and gluconeogenesis pathway were enriched in the EG but not the EoE transcriptome. Notably, transcripts encoding nearly all enzymes in this pathway are upregulated in EG. Concerted upregulation of glycolytic enzymes at the transcriptional level occurs under hypoxic conditions and during certain states related to inflammation. For example, lipopolysaccharide-activated macrophages and dendritic cells, M1 macrophages, and type 17 helper T cells (Th17) exhibit a metabolic shift toward glycolysis²⁷. Moreover, effector CD4⁺ T cells (Th1, Th2, Th17) have increased glycolytic activity compared to naïve CD4⁺ T cells²⁸. Additionally, IL-4 induces glucose utilization in a STAT6-dependent manner in B lymphocytes²⁹. Future studies may elucidate which cells and processes contribute to EG's increased transcription of glycolytic enzymes.

Although divergent gene expression patterns were detected between the EG and EoE transcriptomes, several common patterns of cellular involvement emerged between these diseases according to our observations. An increased number of proliferating (MIB-1⁺) cells was observed both in the gastric epithelium and lamina propria of EG patients. These findings are similar to those in EoE, in which expansion of the basal epithelium and increased cell proliferation (MIB-1⁺ cells) have been documented³⁰. The observed enrichment of transcripts involved in basic metabolic processes including nucleotide metabolism and energy production may relate to increased proliferation in EG.

Our findings substantiate mast cell involvement in EG. We observed that CD117⁺ cells were significantly increased in EG biopsies, consistent with increased levels of *CPA3* and other mast cell-associated transcripts. However, the number of tryptase⁺ cells was not different between EG and control populations (Table E5). These data are similar to those of Chehade et al.³¹, in which no significant difference in tryptase⁺ cells was observed in the gastric antrum tissue of allergic eosinophilic gastroenteritis patients compared to control patients. Notably, although tryptase gene expression was increased in our EG cohort, the effect was modest. It is likely that the gastric mast cell involved in EG may have unique characteristics, being CPA^{high}, consistent with mast cell phenotypes in EoE^{22, 32}.

Treg affect the development and progression of allergic diseases by impacting the sensitization and effector phases of such conditions³³. In EG, we observed increased numbers of cells stained positively for FOXP3⁺, a critical transcription factor that promotes development and function of Treg³⁴. However, it is also possible that the FOXP3⁺ cells in the gastric tissue represent activated T cells rather than or in addition to Tregs³⁵. Although the function of FOXP3⁺ cells in EGID is not known, they are increased in esophageal tissue of EoE patients compared to that of control and gastroesophageal reflux disease (GERD) patients^{36, 37}. It is possible that the observed FOXP3⁺ cells are Treg since Treg have also been implicated in other allergic diseases. In atopic individuals, CD4⁺CD25⁺ cells have less suppressive capacity compared to those from control individuals^{38,39}, and peripheral blood Treg from allergic asthma patients exhibit diminished chemotactic responses⁴⁰. In tissue, pulmonary CD4⁺CD25^{hi} cells from pediatric asthmatic patients displayed impaired suppression of Th2 cytokine production by T cells⁴¹. Similar to other human diseases (e.g. asthma, EoE), Treg may be recruited in EG to counter-regulate inflammatory processes despite this capacity not being evident at the time point surveyed in the active disease process. Interestingly, human atopy and EGID have been associated with defects in TGF-β

signaling, which normally promotes Treg differentiation. For example, patients with Loeys-Dietz syndrome, who have mutations in *TGFBR1* or *TGFBR2*, have a high prevalence of allergic phenotypes including EGID⁴², and patients with connective tissue disorders have a high prevalence of EoE⁴³, suggesting alternate pathways of Treg differentiation in EoE and EG. Alternatively, given the increased level of Th2 cytokines present locally in the gastric tissue of EG patients, it is interesting to speculate that the observed FOXP3⁺ cells may be activated Th2 cells.

In conclusion, we have expanded observations of “histological EG” by examining other inflammatory cells in EG and defined the fundamental clinical and molecular parameters of EG. In addition to eosinophil-predominant inflammation, the affected tissue of EG patients has a conserved transcriptome and increased numbers of mast cells, FOXP3⁺ cells (Treg or activated T cells), and proliferating cells, which could potentially be used as biomarkers for this disease. We propose that the diagnostic criteria of EG be expanded from simply “histological EG” to include upregulation of a subset of these genes (e.g. *CCL26*). Collectively, these data substantiate EG as a systemic disorder involving profound blood and GI tract (gastric and extra-gastric) eosinophilia and a Th2 (IL-4, IL-5, IL-13)-associated gastric transcriptome that readily differentiates EG from EoE.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

CCHMC	Cincinnati Children’s Hospital Medical Center
CCED	Cincinnati Center for Eosinophilic Disorders
cDNA	complementary DNA
EC	eosinophilic colitis
ED	eosinophilic duodenitis
EG	eosinophilic gastritis
EGID	eosinophilic gastrointestinal disorder
EJ	eosinophilic jejunitis
EoE	eosinophilic esophagitis

GERD	gastroesophageal reflux disease
GI	gastrointestinal
GO	gene ontology
GOrilla	gene ontology enrichment analysis and visualization tool
<i>H. pylori</i>	<i>Helicobacter pylori</i>
HPF	400X high-power field
IBD	inflammatory bowel disease
IFN	interferon
IPA	Ingenuity pathway analysis
IL	interleukin
K	x1000
PAS	Periodic acid-Schiff
PTEN	phosphatase and tensin homolog
RAST	radioallergosorbent test
RT-PCR	real-time polymerase chain reaction
SD	standard deviation from the mean
SEM	standard error of the mean
TGF-β	transforming growth factor beta
Th2	Type 2 helper T cell
Th17	Type 17 helper T cell
TNF-α	tumor necrosis factor alpha
Treg	Regulatory T cell

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Clinical implications

EG is a systemic Th2 disorder commonly associated with extra-gastric eosinophil involvement, and blood eosinophil numbers are a good biomarker for the degree of gastric eosinophilia.

FIG 1.

Gastric tissue of patients with eosinophilic gastritis (EG) displays marked eosinophilic inflammation that correlates with peripheral blood eosinophil counts. **(A)** Peripheral eosinophil count (K/μl) and **(B)** percentage of eosinophils within peripheral blood leukocytes (control, n = 9; EG, n = 15). **(C)** Example of gastric nodules in an EG patient. **(D)** H&E-stained gastric antrum biopsy specimen of an EG patient (Patient 19; magnification = 200X). Eosinophils densely populate the lamina propria forming sheets (arrows). **(E)** Peak gastric antrum tissue eosinophil counts (control, n = 15; EG, n = 15). **(F)** Peak gastric tissue eosinophil count was correlated with the absolute peripheral eosinophil number (K/μL) (Patients 1–30, if available [n = 9 control, n = 15 EG]). Pearson $r^2 = .8102$, $P < .0001$. For **(A–B)** and **(E)**, data are expressed as mean $\pm$ standard error of the mean (SEM)

and were analyzed using the unpaired *t* test. ***P* < .01, ****P* < .001. HPF, 400X high-power field; K, x1000.

FIG 2.

Eosinophilic gastritis (EG) patient gastric tissue exhibits increased levels of proliferating cells and FOXP3⁺ cells. **(A–D)** Gastric antrum sections obtained during the index endoscopy (Patients 1–5 and 16–20; defined in Table E1) were stained with antibodies against the indicated protein. Representative photograph (magnification = 200X) of **(A–B)** MIB-1-stained and **(C–D)** FOXP3-stained gastric tissue from control and EG patients.

FIG 3.

Gastric tissue of eosinophilic gastritis (EG) patients exhibits a conserved pattern of gene expression. **(A)** Microarray data (Patients 1–5 [control] and 16–20 [EG]) were analyzed by volcano plot. Transcripts of interest are denoted (top panel). **(B)** The number of transcripts that exhibit the indicated minimum fold difference in EG versus control patients is graphed. Transcripts ($n = 32$) with ≥ 5 -fold change (i.e. ≥ 5 or $\leq .2$) are listed. **(C)** Expression values for individual patients ($n = 104$ transcripts; $P < .01$ and ≥ 2 -fold change). Patient numbers correspond to those in Table E1. **(D)** The 41 transcripts common to the EG transcriptome (1709 transcripts) and the eosinophilic esophagitis (EoE) transcriptome are shown. **(E–F)**

The 30 most **(E)** upregulated and **(F)** downregulated transcripts in the EG transcriptome are listed along with the corresponding relative fold change in expression in EoE vs. control tissue. Affy probe ID, Affymetrix probeset ID number; CTL, control.

FIG 4.

Gastric tissue of eosinophilic gastritis (EG) patients exhibits a conserved pattern of cytokine gene expression. **(A–D)** Relative gastric antrum cytokine transcript levels (mean ± standard error of the mean [SEM]) **(A)** *IL4*, **(B)** *IL5*, **(C)** *IL17*, and **(D)** *IL33* were determined for Patients 6–15 (control) and 21–30 (EG). * $P < .05$, ** $P < .01$, *** $P < .001$.

FIG 5.

IL13 and *CCL26* are elevated in eosinophilic gastritis (EG) patient gastric tissue. **(A–B)** Relative gastric antrum transcript levels (mean $\pm$ SEM) of **(A)** *IL13* (Patients 1–5 [control] and 16–20 [EG]) and **(B)** *CCL26* (Patients 6–15 [control] and 21–30 [EG]). **(C)** Normalized *CCL26* transcript levels were correlated with normalized *IL13* levels (Patients 6–15 [control] and 21–30 [EG]). **(D–E)** Normalized *CCL26* transcript levels (Patients 1–5 [control] and 16–20 [EG]) were correlated with **(D)** average gastric tissue eosinophil count and **(E)** peak gastric tissue eosinophil count. For **(C–E)**, Pearson r^2 and P values are in bold; Spearman r and P values are in italics. * $P < .05$, *** $P < .001$. HPF, 400X high-power field; NS, not significant.

Table 1

Summary of patient characteristics^a

	CTL (n = 15)	EG (n = 15)	P value ^b
Age (y, mean ± SD, [range])	8.7 ± 5.5 (1–17)	13.5 ± 6.7 (3–30)	.0419
Male (n)	8	8	1.0000
Female (n)	7	7	1.0000
History of allergy (n)	4	13	.0013
History of food anaphylaxis (n)	0	4	.0498
History of asthma (n)	1	5	.1686
Elevated (>0.35 K/ μ l) absolute peripheral blood eosinophil count (n [%], mean count [range])	0/9 (0%), 0.09 (0.0–0.2)	12/15 (80%), 1.09 (0.2–3.0)	.0002
Elevated (>4%) peripheral blood eosinophil percentage of total leukocytes (n [%], mean percentage [range])	0/9 (0%), 1.33 (0–4)	13/15 (87%), 14.27 (3–35)	<.0001
Extra-gastric EGID at index endoscopy (n [%])	0 (0%)	9 (60%)	.0007
EoE	0 (0%)	7 (47%)	.0063
ED	0 (0%)	1 (7%)	1.0000
EJ	0 (0%)	2 (13%)	.4828
EC	0 (0%)	0 (0%)	n/a

CTL, control; EC, eosinophilic colitis; ED, eosinophilic duodenitis; EG, eosinophilic gastritis; EGID, eosinophilic gastrointestinal disorder; EJ, eosinophilic jejunitis; EoE, eosinophilic esophagitis; K, x1000; n/a, not applicable; SD, standard deviation from the mean; y, years

^a Individual patient values can be found in Tables E1 and E2.

^b P value for parameter comparison between control and EG groups. For age, unpaired *t* test was used to determine significance; for other characteristics, Fisher's exact test was used to evaluate the statistical significance of the difference in frequency.

Table II

Quantitative evaluation of tissue, blood, inflammatory, and proliferating cells

A. Tissue and blood eosinophils^a				
	Mean tissue eosinophil ^{b, c}	Peak tissue eosinophil ^b	Mean absolute eosinophil blood ^b	Mean % eosinophil blood ^b
CTL	6.1 ± 4.7	11.0 ± 8.5	0.09 ± 0.08	1.33 ± 1.22
EG	202.4 ± 109.4	282.7 ± 163.9	1.09 ± 0.88	14.27 ± 8.63
P value^d	1.5×10^{-7}	6.1×10^{-7}	.0027	.00021

B. Inflammatory and proliferating cells^a				
	MIB-1 ⁺ epithelial cells ^{b, e}	MIB-1 ⁺ lamina propria cells ^{b, e}	CD117 ⁺ cells ^{b, e}	FOXP3 ⁺ cells ^{b, e}
CTL	288.4 ± 178.5	14.6 ± 2.9	51.0 ± 3.8	4.2 ± 2.6
EG	551.2 ± 135.3	65.6 ± 43.7	93.8 ± 33.1	18.4 ± 11.0
P value^d	.03	.03	.02	.02

CTL, control; EG, eosinophilic gastritis

^a Individual patient values can be found in Tables E2, E4, and E5.

^b Values shown are mean ± standard deviation.

^c Mean of the eosinophil count of five separate HPF (400X)

^d P value for parameter comparison between control and EG groups. Data were compared using the unpaired *t* test.

^e Peak counts/HPF (mean ± standard deviation) in the gastric tissue for cells stained positively with antibodies against the indicated proteins