

Atopic and non-atopic eosinophilic oesophagitis are distinguished by immunoglobulin E-bearing intraepithelial mast cells

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Aims: Eosinophilic oesophagitis (EoE) occurs in atopic individuals and features eosinophils and mast cells, but differences in the inflammatory cell density between the epithelium and lamina propria (LP) are not fully understood. The aim of this study was to determine if numbers of eosinophils, B lymphocytes and immunoglobulin E (IgE)-bearing mast cells are increased in the mucosa of EoE patients with and without concurrent atopy.

Methods and results: Oesophageal biopsies containing ≥ 4 high-power fields (HPF) of epithelium and LP were identified for normal ($n = 9$), gastroesophageal reflux disease (GERD) ($n = 5$) and EoE ($n = 25$) patients. Patients were classified as atopic or not by clinical history. Immunohistochemistry identified mast cells, B lymphocytes and eosinophils. Eosinophil density was

increased in the LP in EoE. Intraepithelial eosinophil density correlated with eosinophils/HPF, CD20⁺ B lymphocyte density and tryptase⁺ IgE⁺ mast cell density. Increased intraepithelial IgE⁺ cell density in EoE was associated with mast cells and not B lymphocytes. Intraepithelial IgE⁺ mast cell densities were significantly higher in biopsies from the subgroup of EoE patients with atopy.

Conclusions: EoE diagnosis using maximal eosinophil count/HPF correlates with average counts/mm², and intraepithelial eosinophil densities are higher in children than adults with EoE. In EoE, numbers of eosinophils and mast cells are increased in the LP. IgE-bearing mast cells are increased in atopic EoE patients but not in non-atopic EoE patients.

Keywords: B lymphocytes, eosinophilic oesophagitis, immunoglobulin E, mast cells, oesophagus

Abbreviations: EDN, eosinophil-derived neurotoxin; EoE, eosinophilic oesophagitis; GERD, gastroesophageal reflux disease; HPF, high-power field; HPS, haematoxylin–phloxine–safranin; IgE, immunoglobulin E

Introduction

Eosinophilic oesophagitis (EoE) is an emerging disorder of the oesophagus. Patients present with a variety of signs and symptoms of oesophagitis and, by definition, have high numbers of intraepithelial eosinophils in their oesophageal mucosa. The patient population is

heterogeneous, but consists mainly of adolescent and young adult males with a history of atopy. Allergy testing has revealed that the majority of EoE patients have identifiable hypersensitivity to both food and aeroallergens, although approximately 20% have no detectable sensitization.¹ Thus, the majority of EoE cases may be related to classic immunoglobulin E (IgE)-mediated type I hypersensitivity.

The role of B lymphocytes and IgE in EoE is not well understood. One study found no change in B lymphocyte (CD20-positive) numbers in biopsies from normal, gastroesophageal reflux disease (GERD), or untreated or

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corticosteroid-treated EoE patients.² In that study, intraepithelial IgE-positive cells were found only in untreated EoE patients and were not detected in healthy controls, GERD controls or corticosteroid-treated EoE patients. A second study described a significant increase in B lymphocytes (CD20-positive) in both the epithelium and the lamina propria of patients with EoE when compared to healthy controls.³ The same study found evidence of local B lymphocyte class-switching to IgE expression and a significant correlation between intraepithelial B lymphocytes and mast cells ($r = 0.744$).³ It is possible that the discrepancy in these studies is due to differences in the counting methods or the demographics of the patient populations. Furthermore, atopic individuals may have altered cellular infiltrate in tissue, even in the absence of disease.

Mast cells reside in the lamina propria and in the basal epithelial layer of the oesophagus in normal human biopsies. Tryptase immunostaining has identified increased numbers of mast cells in the oesophageal mucosa as a feature of EoE.^{4,5} Treatment for 3 months with topical fluticasone propionate did not decrease mast cell infiltration significantly.² In a guinea pig model of EoE, where aerosolized ovalbumin was used to induce disease, mast cells preceded eosinophils into the mucosa.⁶ These findings support a role for mast cells, rather than eosinophils, in the aetiology of EoE. Several groups have examined the presence of IgE-positive cells in oesophageal epithelium.^{2,4,5} Vicario *et al.*³ showed that a subgroup of IgE-positive cells that were CD117-positive and probably mast cells were present in EoE and some control patients. Mast cells are activated by cross-linking of surface-bound IgE by antigen, but it is not known if this process occurs in the oesophageal mucosa.

The lamina propria of the oesophagus is not always present in endoscopic biopsies,⁷ but it may contribute to the pathogenesis of EoE. Little is known about the role of immune cells in the lamina propria, and this has not been studied systematically.

In previous studies, the methods of staining and counting cells from EoE biopsies have varied widely. The present study methodology evaluates all the available biopsy tissue, and includes both epithelium and lamina propria, thus minimizing selection bias and enabling a more universal assessment of cell density.

In this study, the density of mast cells and IgE-bearing cells in the epithelium and lamina propria of the oesophageal mucosa was investigated. Biopsies from control patients without upper gastrointestinal (GI) pathology, patients with GERD and patients with

EoE were compared. Atopic status was assessed for each individual to determine if this played a role in differences in histological findings. We hypothesized that IgE-bearing mast cells are increased in the oesophageal epithelium in EoE (in general), and particularly in EoE patients with concurrent atopy.

Materials and methods

PATIENTS

Study patients (Table 1) were identified from our database of oesophageal mucosal biopsies from 1997 to 2010. Biopsies routinely stained using haematoxylin–phloxine–safron (HPS) that met the study criteria of ≥ 4 high-power fields (HPFs, 400 $\times$ magnification) of both epithelium and lamina propria were included. Patients were diagnosed as having EoE ($n = 25$) by the accepted original clinical criteria.⁸ Criteria for the diagnosis of EoE were: (i) presentation with symptoms and signs of oesophagitis, (ii) the possibility of GERD excluded and (iii) an oesophageal biopsy containing at least one HPF with ≥ 15 intraepithelial eosinophils. The patients had normal eosinophil counts in stomach and small bowel biopsies. GERD patients ($n = 5$) were identified initially by symptoms and endoscopic features of inflammation. The diagnosis of GERD was confirmed by histopathological features of acid reflux (inflammatory cell infiltration, basal epithelial hyperplasia and elongated vascular papillae), and < 15 eosinophils per maximal HPF.⁹ To minimize potential overlap between the EoE and GERD groups, we included only GERD patients who had ≤ 5 eosinophils per maximal HPF. Normal control patients ($n = 9$) had endoscopy and biopsies for upper GI symptoms, but had no specific oesophageal symptoms and no macro- or microscopic signs of inflammation in the oesophagus. Atopy was defined as a clinical history of type I hypersensitivity, including positive skin prick tests, positive IgE radioallergosorbent test, anaphylaxis or diagnosis by an allergist of asthma, allergic rhinitis or food allergy. The Queen's University Health Sciences Research Ethics Board approved this study.

SINGLE-STAIN IMMUNOHISTOCHEMISTRY

Mucosal biopsies obtained for routine histopathological analysis during upper endoscopy were placed into 10% neutral buffered formalin and then embedded in paraffin. Slides were prepared with two 4- μ m tissue sections, one for immunostaining and one to act as a negative control, where the primary antibody was

Table 1. Patient demographics and histopathological characteristics

Gender	Age	Diagnosis	Symptoms	Endoscopic findings	Atopy	Eos/HPF
F	8	NL	None	Hiatal hernia	NA	0
M	3	NL	None	None	NA	0
F	11	NL	Chest pain	None	NA	0
M	57	NL	None	None	A	0
M	14	NL	Vomiting	None	NA	0
M	2	NL	Feeding aversion	None	A	0
F	15	NL	Nausea, abdominal pain	None	NA	0
M	65	NL	None	None	NA	0
F	13	NL	Abdominal pain	None	UK	0
M	13	GERD	Retrosternal pain	Nodularity, furrowing	NA	1
F	15	GERD	Abdominal pain, vomiting	None	A	2
F	13	GERD	Abdominal pain, heartburn	None	NA	3
F	9	GERD	Heartburn	Erosions	NA	3
M	14	GERD	Heartburn	Hiatal hernia, furrowing, rings	NA	4
F	27	EoE	Dysphagia	None	A	19
M	18	EoE	Gas/bloating	None	NA	19
M	13	EoE	Dysphagia	White plaques, linear furrowing, friability	A	22
M	50	EoE	Dysphagia	Food impaction, trachealization	NA	24
M	31	EoE	Dysphagia, past food impactions	Food impactions	NA	30
M	2	EoE	Vomiting	Furrowing, oedema, hiatal hernia	A	36
M	6	EoE	Dysphagia	None	A	39
M	2	EoE	Feeding aversion, failure to thrive	Furrowing, trachealization	A	46
M	32	EoE	Heartburn	Hiatal hernia, LA class I oesophagitis	NA	47
M	21	EoE	Dysphagia, history of food impactions	Furrowing, trachealization	A	59
F	11	EoE	Abdominal pain	White plaques	A	63
M	17	EoE	Heartburn, nausea	Furrowing	A	63
M	14	EoE	Dysphagia	Trachealization	A	66

Table 1. (Continued)

Gender	Age	Diagnosis	Symptoms	Endoscopic findings	Atopy	Eos/HPF
M	10	EoE	Epigastric pain	Furrowing	NA	69
M	14	EoE	Heartburn	Furrowing	NA	73
M	14	EoE	Dysphagia, past food impaction	Hiatal hernia, furrowing, nodularity, erosions	A	76
M	15	EoE	Dysphagia	Furrowing	A	82
M	10	EoE	Dysphagia, heartburn	White papules, furrowing	A	82
F	7	EoE	Vomiting	White plaques, trachealization	NA	83
F	17	EoE	Retrosternal pain, dysphagia	Furrowing	A	102
M	27	EoE	Dysphagia, weight loss	Hiatal hernia, LA class I oesophagitis	A	103
M	17	EoE	Abdominal pain	Furrowing	A	111
M	18	EoE	Dysphagia, heartburn	Trachealization	A	111
M	23	EoE	Dysphagia	Trachealization, furrows, white papules	A	225
M	39	EoE	Dysphagia	Trachealization	NA	231

A, atopic; EoE, eosinophilic oesophagitis; F, female; GERD, gastroesophageal reflux disease; LA class, Los Angeles class; M, male; NA, non-atopic; Eos/HPF, eosinophils/high-power field; UK, unknown.

replaced with mouse IgG (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

The tissue was rehydrated and endogenous peroxidase activity quenched by immersion in 3% H₂O₂ for 10 min. Non-specific binding was blocked with 30 min of 5% goat serum, 0.02% azide and 0.02% Tween-20 in phosphate-buffered saline (PBS). Primary antibody was applied overnight and consisted of mouse anti-human mast cell tryptase (1:200; Dako-Cytomation, Glostrup, Denmark), mouse anti-human CD20 (1:100; AbD Serotec, Oxford, UK) or mouse anti-human IgE (1:200; Abcam, Cambridge, MA, USA). Next, biotinylated goat anti-mouse antibody (Invitrogen, Carlsbad, CA, USA) was added to all sections for 40 min. Staining was completed using the avidin–biotin–peroxidase complex method with 3,3'-diaminobenzidine substrate and 1 min of haematoxylin counterstain. Slides were coverslipped and mounted in Permount (Thermo Fisher Scientific, Hampton, NH, USA). Immunostaining was visualized (Figure 1) on a Nikon Eclipse TE-2000U microscope and pictures were taken with a Nikon DXM 1200C digital camera (Nikon, Mississauga, ON, Canada) and

NIS-Elements BR version 2.30 software. Positive (brown) stained cells were counted as either intraepithelial or within the lamina propria.

DUAL-LABEL IMMUNOHISTOCHEMISTRY

For dual-label immunohistochemistry, biopsies were processed and prepared in the same way as specified above for single-stain immunohistochemistry. Tissue sections were blocked with a solution of 5% goat serum, 5% donkey serum, 0.02% azide and 0.02% Tween-20 in PBS for 30 min. A cocktail of primary antibodies was applied overnight, as was a preparation of each individual antibody and a negative control tissue section. Primary antibodies were either a cocktail of mouse anti-human mast cell tryptase (1:200; DakoCytomation) and rabbit anti-human IgE (undiluted; Abcam), or a combination of mouse anti-human IgE (1:200; Abcam) and rabbit anti-human eosinophil-derived neurotoxin (EDN) (1:200; Abcam). For negative control sections, the primary antibodies were replaced with normal mouse IgG. A cocktail of fluorochrome-conjugated secondary antibodies was then

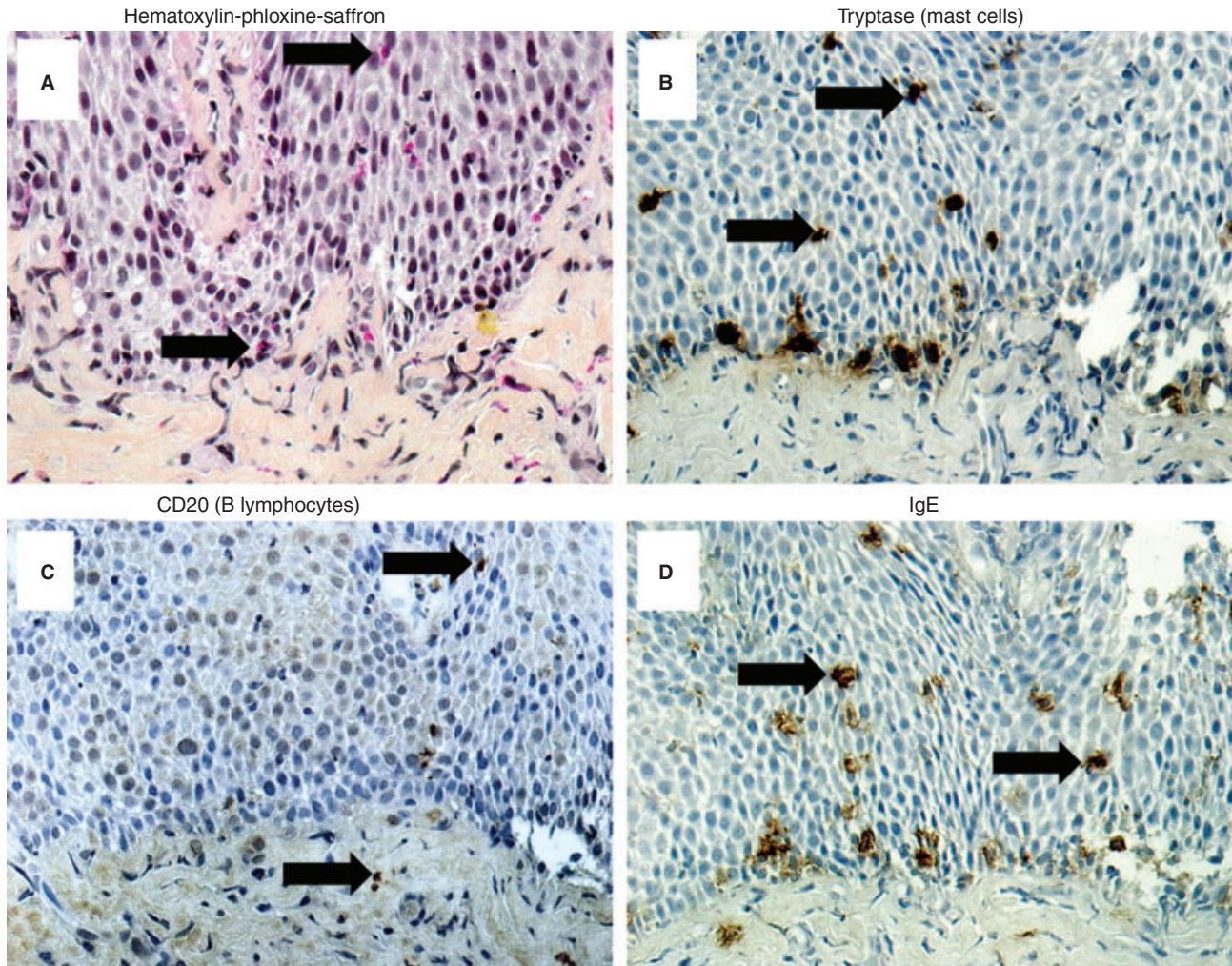


Figure 1. Representative histological images of stains used on oesophageal mucosal biopsies in this study. A representative site from the same patient with eosinophilic oesophagitis is shown in each panel. A, Intraepithelial eosinophils visible with routine haematoxylin-phloxine-saffron stain; B, immunohistochemistry (IHC) showing intraepithelial tryptase-positive mast cells; C, CD20 IHC labels B lymphocytes within both the oesophageal epithelium and lamina propria; and D, IHC staining for immunoglobulin E-positive cells.

applied for 40 min. Secondary antibodies used were donkey anti-mouse IgG conjugated to DyLight 488 (1:50; Abcam) and goat anti-rabbit IgG conjugated to cyanine 5 (Cy5) (1:200; Abcam). Slides were then counterstained with Hoechst dye (8 ng/ml) for 10 min. Slides were mounted in a 1:1 solution of PBS and glycerol and visualized and photographed with the microscope described above.

IMMUNOSTAINED CELL DENSITY ANALYSIS

The tissue area was determined by area analysis of images taken with Nikon NIS-Elements version 2.30 software (Nikon, Mississauga, ON, Canada). The boundaries of the tissue compartment were defined manually with the cursor and the areas obtained

from adjacent images were combined. The entire tissue compartment area was added by combining the areas (in mm²) from all the images of a slide. Cell density was calculated by counting all the immunostained cells in the epithelium and lamina propria of the tissue and dividing by the total area of that particular tissue compartment (epithelium or lamina propria). The area within vascular papillae was considered to be part of the lamina propria.

STATISTICAL ANALYSIS

The Kruskal–Wallis one-way analysis of variance test with Dunn's post-test was used to compare cell densities from EoE, GERD and normal patient samples. The Mann–Whitney *U*-test was used to compare cell

densities from atopic and non-atopic patient samples. Dependence was tested by linear regression and represented by Spearman's rank correlation coefficient. Statistical significance was considered to be a P -value <0.05 ; 'not significant' (NS) is indicated if the P -value was ≥ 0.05 .

Results

EOSINOPHIL DENSITY

As expected, intraepithelial eosinophil density was significantly higher in biopsies from patients with EoE when compared to biopsies from patients with GERD and normal patients (Figure 2A). Lamina propria eosinophil density was also significantly greater in

biopsies from EoE patients when compared to GERD and normal patient biopsies (Figure 2B); however, lamina propria eosinophil density did not correlate with intraepithelial eosinophilia. No significant difference was found in intraepithelial or lamina propria eosinophil density when comparing atopic to non-atopic EoE patients (Figure 2C,D).

To evaluate the utility of counting eosinophils per maximal HPF ($\times 400$ field; the currently accepted diagnostic practice), these values were correlated with the entire tissue biopsy eosinophil density (per mm^2) for each patient sample. Overall eosinophil density correlated significantly with eosinophil counts per maximal HPF in the epithelium and total mucosa (Figure 3A,C). Lamina propria eosinophil density did not correlate with lamina propria eosinophil count per

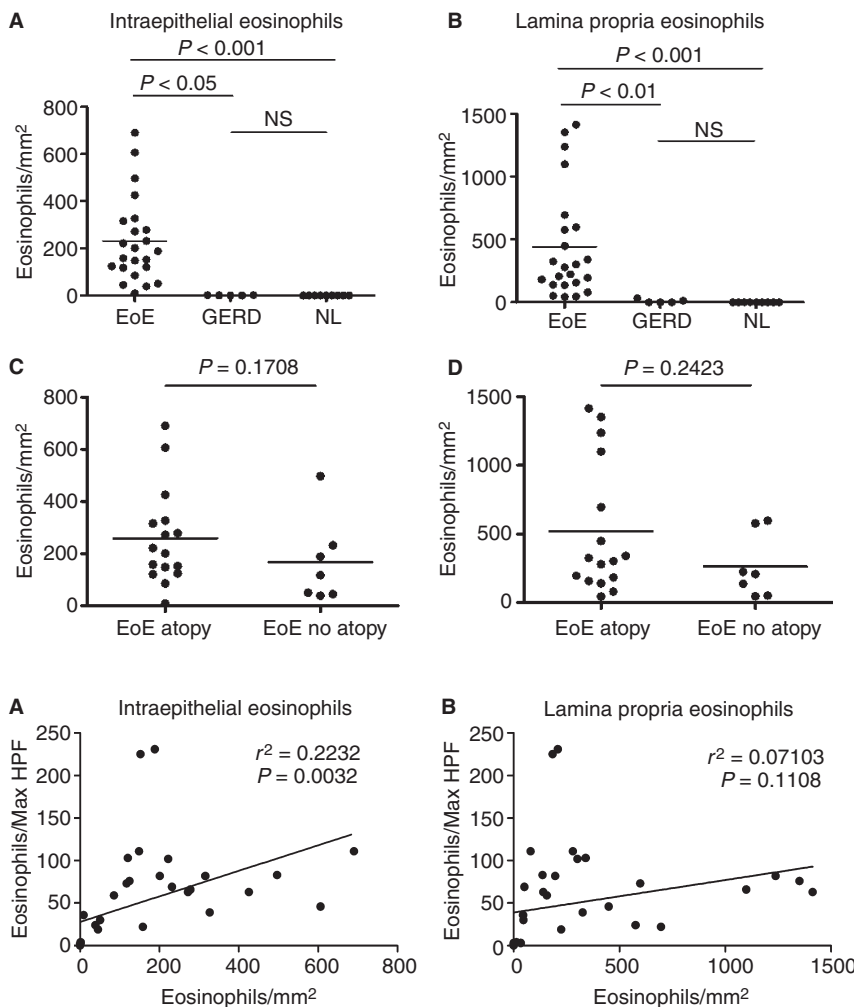


Figure 2. Comparison of eosinophil density in (A) the epithelium and (B) the lamina propria of oesophageal mucosal biopsies. In patients with eosinophilic oesophagitis (EoE), the presence of atopy did not alter significantly the density of eosinophils in (C) the epithelium or (D) the lamina propria.

Figure 3. Correlation of peak eosinophil counts per maximal high-power field with eosinophil density in (A) the epithelium, (B) the lamina propria and (C) the entire mucosa (epithelium and lamina propria). Peak eosinophil count correlated with intraepithelial eosinophil density and total eosinophil density, but not lamina propria density.

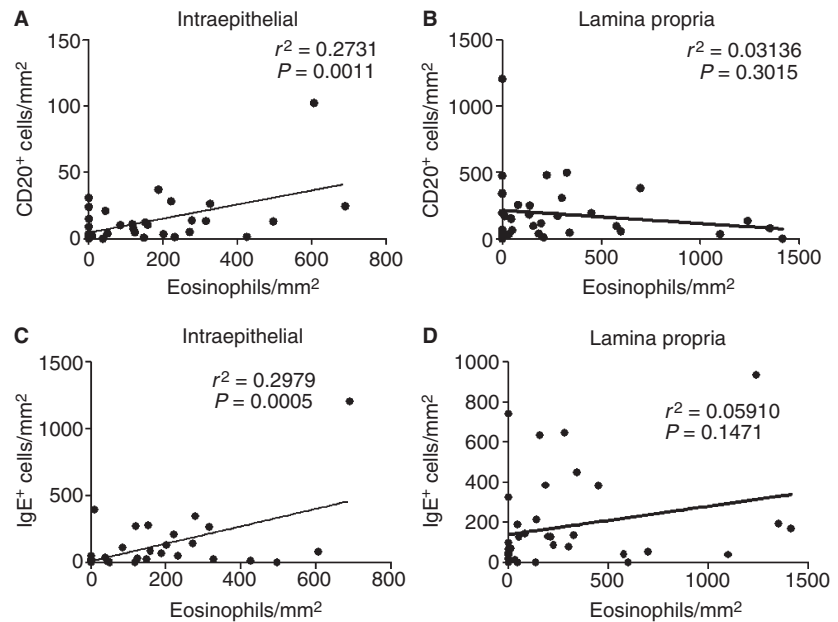


Figure 4. A significant correlation exists between (A) intraepithelial B lymphocyte and eosinophil density, but not (B) lamina propria B lymphocyte and eosinophil density. Similarly, a significant correlation exists between (C) intraepithelial immunoglobulin E (IgE)-positive cell and eosinophil density, but not (D) lamina propria IgE-positive cell and eosinophil density.

maximal HPF (Figure 3B). In general, maximal intraepithelial HPF counts appeared to underestimate eosinophil density.

Intraepithelial eosinophil density also correlated with B lymphocyte (Figure 4A) and IgE-positive cell (Figure 4C) density. Lamina propria B lymphocyte (Figure 4B) and IgE-positive cell (Figure 4D) density did not correlate with lamina propria eosinophil density. Mast cell density did not correlate with eosinophil density in either the epithelium or lamina propria.

MAST CELL DENSITY

Intraepithelial mast cell density was increased significantly in biopsies from patients with EoE when compared to GERD ($P < 0.01$) and normal ($P < 0.001$) patient biopsies (Figure 5A). Lamina propria mast cell density was not significantly different between EoE, GERD and normal patients (Figure 5B). When EoE patients were stratified by atopic status, overall mast cell density tended to be greater in atopic EoE patients, but this did not reach statistical significance (Figure 5C,D).

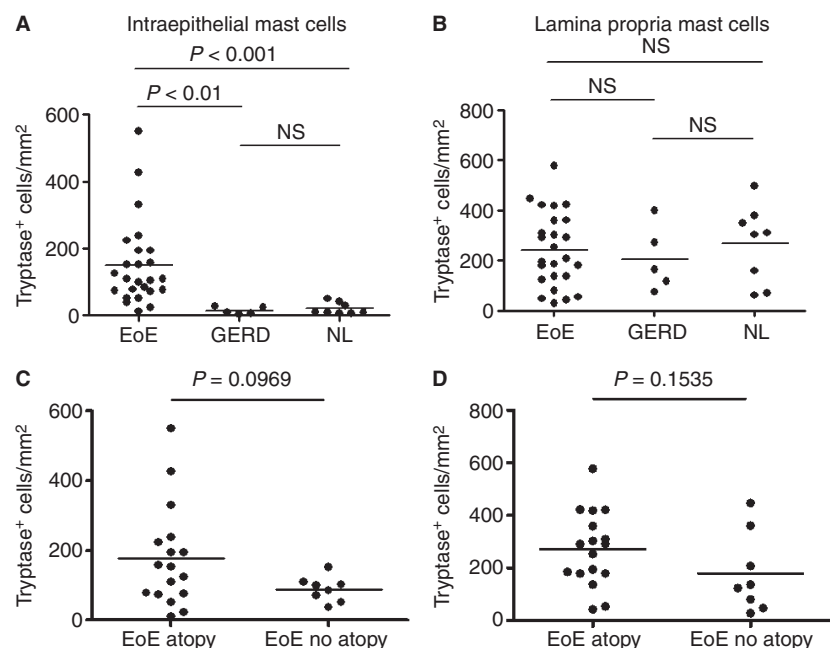


Figure 5. A, Tryptase mast cell density is significantly higher in the epithelium of eosinophilic oesophagitis (EoE) patients. There is no significant difference in (B) lamina propria mast cell density in EoE, gastroesophageal reflux disease (GERD) or normal patients. Among EoE patients, intraepithelial (C) and lamina propria (D) mast cell density is not significantly different in atopic versus non-atopic individuals.

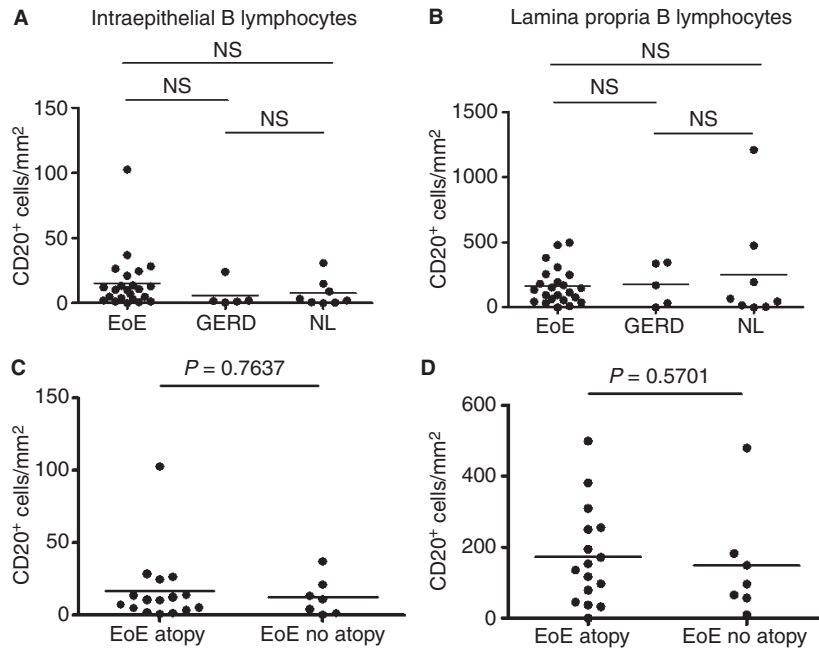


Figure 6. There was no difference in the density of (A) intraepithelial or (B) lamina propria B lymphocytes in eosinophilic oesophagitis (EoE), gastroesophageal reflux disease (GERD) and normal patient biopsies, or when dividing EoE patients with atopy from those without atopy on the basis of B lymphocyte density in the (C) epithelium or (D) lamina propria.

B LYMPHOCYTE DENSITY

When comparing EoE, GERD and normal patient biopsies, there was no significant difference in B lymphocyte density in either the epithelium (Figure 6A) or the lamina propria (Figure 6B). B lymphocyte density was not altered significantly in EoE patients with atopy when compared to those without atopy, in either the epithelium (Figure 6C) or lamina propria (Figure 6D).

CORRELATION OF OESOPHAGEAL MUCOSAL B LYMPHOCYTES WITH MAST CELLS AND IGE-POSITIVE CELLS

There was no significant correlation between the density of B lymphocytes and mast cells within the oesophageal epithelium (Figure 7A) or lamina propria (Figure 7B), or between the density of B lymphocytes and IgE-positive cells within the oesophageal epithelium (Figure 7C) or lamina propria (Figure 7D).

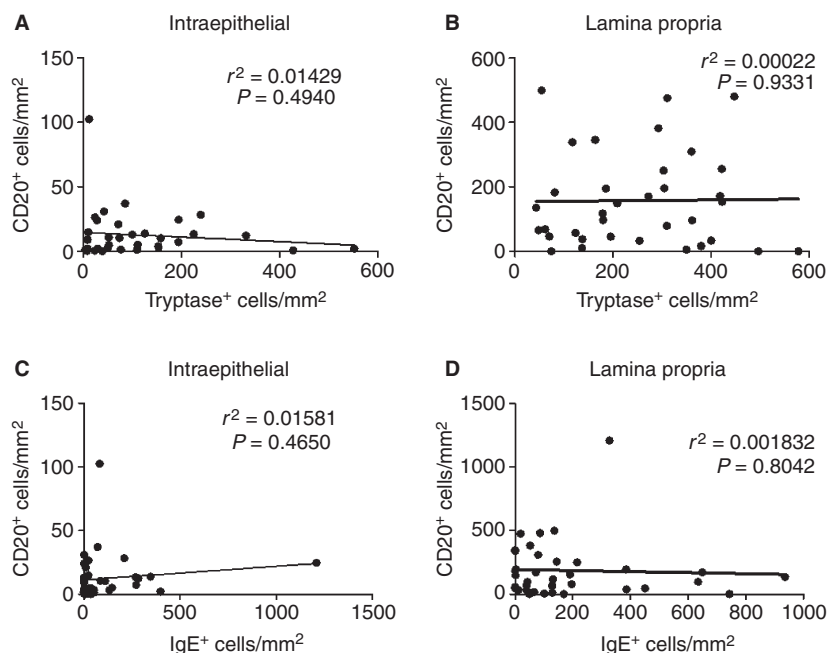


Figure 7. No significant correlation was found between B lymphocyte and mast cell density in (A) the epithelium or (B) the lamina propria of the oesophageal mucosal biopsies analysed. Similarly, there was no significant correlation between the densities of B lymphocytes and immunoglobulin E-positive cells in either the (C) epithelium or (D) lamina propria.

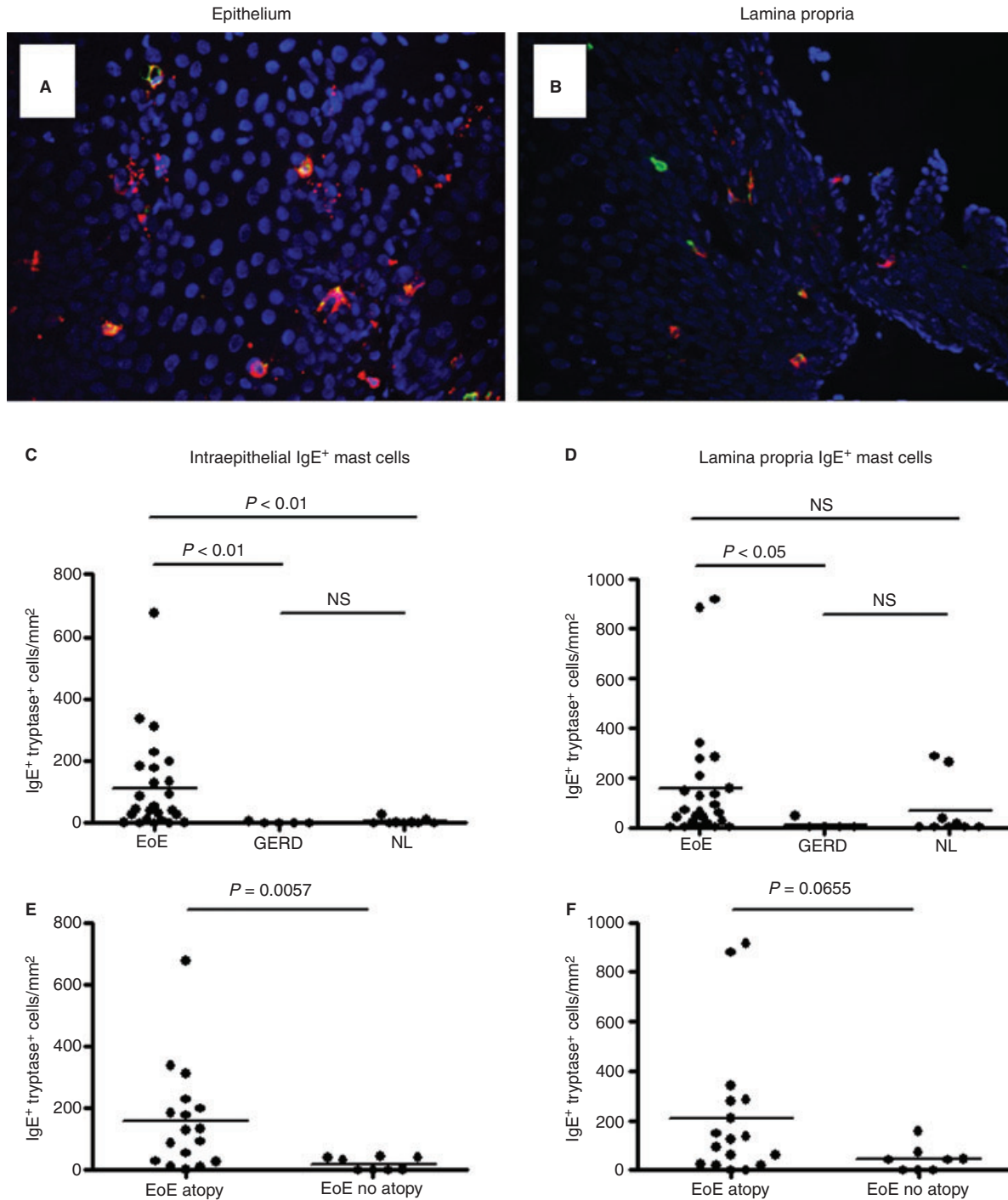


Figure 8. Dual immunostaining for mast cell tryptase (green) and immunoglobulin E (IgE) (red) revealed (A) intraepithelial and (B) lamina propria IgE-bearing mast cells (combination of green and red = yellow). C, Intraepithelial IgE-positive mast cells were increased in eosinophilic oesophagitis (EoE) when compared to both gastroesophageal reflux disease (GERD) and normal control patient biopsies. D, Lamina propria IgE-positive mast cell density was significantly higher in EoE patients when compared to GERD controls, but there was no significant difference between lamina propria EoE and normal patient IgE-positive mast cell density. E, Biopsies from EoE patients diagnosed with atopy had a significantly higher intraepithelial density of IgE-positive mast cells when compared to EoE patients without a diagnosis of atopy. F, There was no significant difference between atopic and non-atopic patient lamina propria IgE-positive mast cell density.

IgE-POSITIVE MAST CELL DENSITY

Dual-label immunostaining for mast cell tryptase and IgE revealed IgE-bearing mast cells in the epithelium (Figure 8A) and lamina propria (Figure 8B). IgE-bearing mast cell density was increased significantly in the epithelium of EoE patient biopsies versus GERD and normal patient biopsies (Figure 8C; $P < 0.01$ for both comparisons). In the lamina propria, IgE-bearing mast cell density was significantly greater in EoE when compared to GERD patient biopsies ($P < 0.05$), but differences from normal patient biopsies did not reach statistical significance (Figure 8D). In EoE patient biopsies, IgE-bearing mast cell density was increased significantly in patients with a history of atopy, but only in the epithelium (Figure 8E; $P = 0.0057$) and not in the lamina propria (Figure 8F).

IgE-POSITIVE EOSINOPHIL DENSITY

The presence of IgE-bearing eosinophils was assessed using dual-label immunostaining for EDN and IgE. Regardless of disease state, no IgE-bearing eosinophils were identified in either the epithelium (Figure 9A) or the lamina propria (Figure 9B) of the patient biopsies evaluated.

GENDER AND AGE DIFFERENCES IN IMMUNE CELL DENSITY

Infiltrating immune cell density, as analysed by single immunostaining for mast cells, B lymphocytes and IgE-positive cells, was not influenced significantly by gender. Similarly, eosinophil density as analysed by routine HPS staining was not influenced significantly by gender (data not shown).

In EoE patient biopsies, eosinophil density in the epithelium correlated negatively with the patient's age (Figure 10A); that is, eosinophil density was greater in younger patients. No significant correlation was found between EoE patient biopsy eosinophil density within the lamina propria and age (Figure 10B). No significant correlation was found between age and mast cell density within the epithelium (Figure 10C) or lamina propria (Figure 10D) of EoE patient biopsies.

Discussion

Biopsies containing a substantial amount of lamina propria were selected for this study. The present method of evaluating all the available biopsy material, including the lamina propria from all sites, is novel. Previous studies have differed in the methods

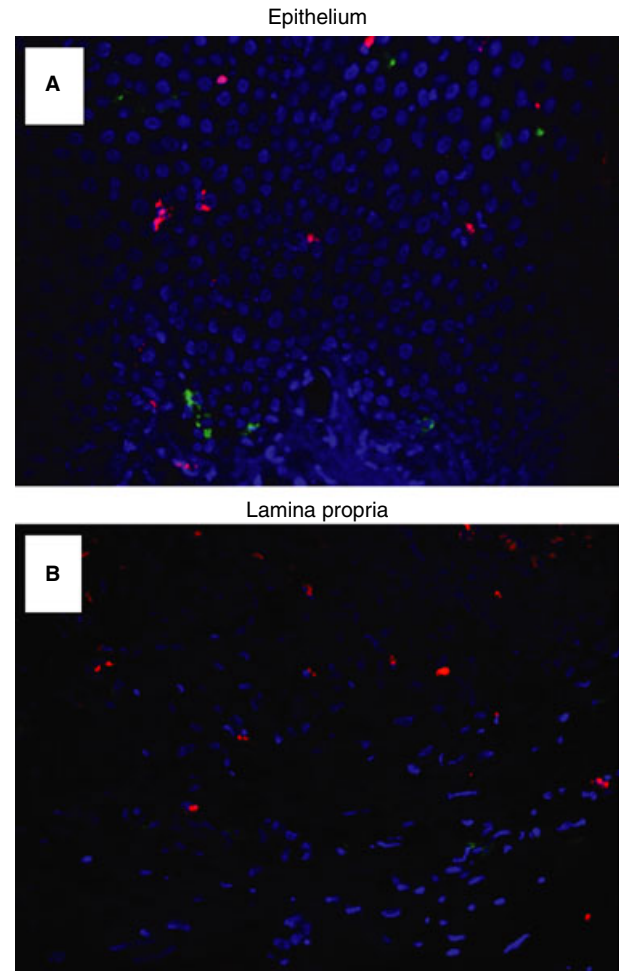


Figure 9. Immunoglobulin E (green fluorochrome) was not found on eosinophils (red, labelled by anti-eosinophil-derived neurotoxin) within the (A) epithelium or (B) lamina propria of any oesophageal mucosal biopsy, regardless of disease state.

of enumerating cell types, making comparisons difficult.

It was discovered that the density of intraepithelial oesophageal IgE-bearing mast cells was increased in EoE patients as a whole, and specifically in EoE patients with a history of atopy. A subgroup of patients, those non-atopic patients with significantly lower IgE-positive mast cells, appear to be phenotypically distinct. This highlights the concept that detailed immunohistochemical studies identify heterogeneity in patients with EoE, and that underlying atopy is one factor that may account for these differences.

In general, mast cells mediate the early stages of type I hypersensitivity reactions.¹⁰ The connection between intraepithelial mast cell density, IgE and atopy in EoE patients may mean that, in some patients, an acute

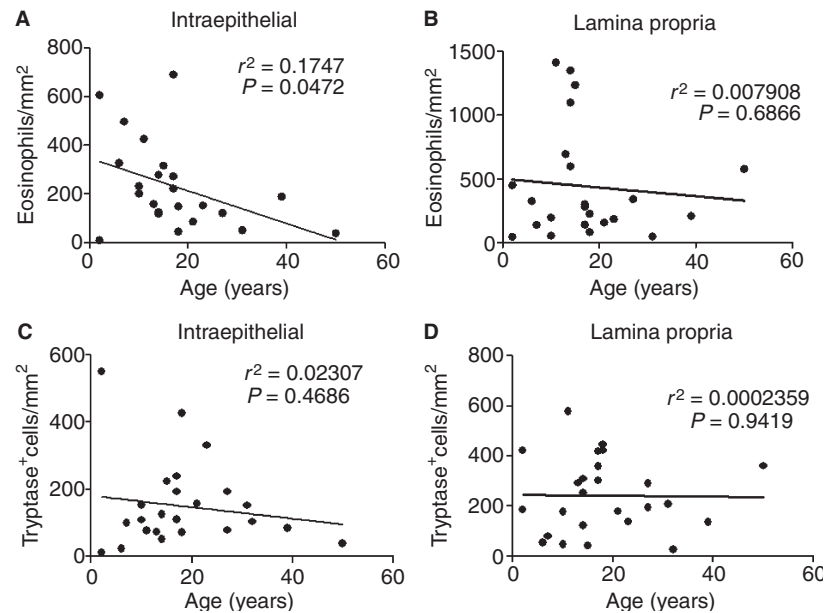


Figure 10. In eosinophilic oesophagitis patients, eosinophil density in (A) the epithelial compartment but not (B) the lamina propria correlated negatively with age. Mast cell density in (C) the epithelium or (D) the lamina propria was not correlated with age.

response to antigen in the oesophagus triggers local eosinophilia. It was also found that the variation in both total and IgE-bearing mast cell density is limited to the epithelial compartment of the oesophageal mucosa. This implies indirectly that oesophageal mast cell activity, in so far as it is related to the pathophysiology of EoE, may be localized to the epithelium. Previous studies have found that the epithelial cells themselves are involved in the process that attracts eosinophils to the oesophagus.¹¹ Other studies have identified that epithelial hyperplasia is related to both mast cell and eosinophil products.^{11,12} Taken together, these findings support the evolving concept that eosinophilic oesophagitis is involved primarily in the epithelial compartment of the mucosa, and that its aetiology may be related directly to mast cells within the epithelium.

Mast cells are known to be associated with inflammation in the oesophagus, including the mucosa^{4,13} and smooth muscle¹⁴ in EoE, but also in other oesophageal diseases, including GERD-induced oesophagitis in mice¹⁵ and oesophageal carcinoma.¹⁶ Blanchard *et al.*¹¹ have demonstrated that oesophageal mast cell numbers correlate with eotaxin-3 level ($r^2 = 0.18$), maximum eosinophil numbers ($r^2 = 0.18$) and basal zone hyperplasia ($r^2 = 0.51$). It is also known that a unique mast cell-associated transcriptome is expressed in the oesophagus in EoE, which includes carboxypeptidase A3 and KIT-ligand.¹⁷ Studies of a guinea pig model of EoE found that mast cells preceded eosinophils into the oesophageal mucosa following administration of aerosolized ovalbumin,⁶ suggesting that mast cells may be more important to the underlying pathophysiology than thought

previously. The effect of corticosteroids on mast cells in EoE treatment trials varies. Three months of fluticasone therapy in adult EoE patients² did not reduce mast cell numbers significantly, while a similar study in children¹⁸ found a significant decrease in mast cells after treatment. Vicario *et al.*³ found that mast cell numbers correlated significantly with B lymphocyte numbers in the oesophageal epithelium ($r^2 = 0.55$). However, in the present study, a significant correlation was not found between intraepithelial mast cells and B lymphocytes ($r^2 = 0.014$). Some of these incongruences may be due to differences in methodology.

The rationale for evaluation of lamina propria eosinophils and other inflammatory cell types was based on the findings in the murine model of EoE, where eosinophil infiltration is concentrated primarily in the lamina propria.¹⁹ Eosinophil density was not influenced by atopic status in EoE patients. This supports the theory that EoE may not be related to atopy in all patients. Intraepithelial eosinophil density was lower in older patients, but mast cells did not correlate with age. While mast cell density is increased in EoE overall, the present study did not find a statistical correlation between mast cells and eosinophils. These findings are further evidence that mast cells and eosinophils may act independently in the oesophagus.

For the dual immunostaining in this study, fluorescence was used to identify positive staining. When investigating eosinophils by immunofluorescence, detection is limited by the autofluorescence of eosinophils.²⁰ By using individual negative controls for each stain, the exposure time for our detection system at

which eosinophil autofluorescence appeared was identified (approximately 800 ms in both the green and red channels). All images were taken with an exposure time well below this threshold.

The density of B lymphocytes in the oesophagus was not affected by disease state or atopy and did not correlate with mast cell or IgE-positive cell density. These findings are in keeping with previous studies that did not find a difference in B lymphocyte numbers between EoE and GERD patient biopsies.^{2,4} In a murine model of EoE, mice lacking B lymphocytes had similar numbers of eosinophils in the oesophagus.²¹ These findings imply that B lymphocytes do not effect oesophageal eosinophil numbers directly, at least in an intranasal *Aspergillus fumigatus*-induced murine model of EoE. However, IgE is increased in EoE patient biopsies and IgE is produced exclusively by B lymphocytes. One limitation of the present study is that B lymphocytes were identified by immunostaining for CD20, which is expressed on almost all B lymphocyte subtypes, although not on mature plasma cells.²² The IgE-positive cell count was increased in atopic patients with EoE, although there was no concurrent change in local B lymphocyte density, as most of the IgE-positive cells detected were mast cells.

One of the difficulties in human EoE studies is that the diagnosis of EoE is based on expert consensus without a single diagnostic gold standard test.^{8,23} Overlap may occur with GERD, and some patients may have both disorders. The mucosal immune system of atopic individuals may behave differently to non-atopic individuals in the face of GERD or EoE, further confounding the picture. Although these diagnostic difficulties exist, we attempted to study some of these differences by segregating patients by atopic status, not only in EoE patients who have a high prevalence of atopy, but also in the control groups.

In summary, IgE-bearing mast cells in the epithelium of the oesophagus are increased in atopic but not in non-atopic individuals with EoE. By examining immune cells other than eosinophils, a pattern of patient heterogeneity begins to emerge. EoE may not be the same disease in all individuals, and identifying phenotypically unique patient groups will advance the understanding of the pathophysiology of EoE.

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Competing interests

No conflicts of interest to declare.

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