Ultrastructural features of eosinophilic oesophagitis: impact of treatment on desmosomes

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ABSTRACT

Aims A growing body of evidence suggests a role for altered epithelial barrier function in the pathophysiology of eosinophilic oesophagitis (EoE), but few have described the epithelial structure during inflammation. The purpose of this study was to define ultrastructural features of active, inactive EoE and control subject’s oesophageal epithelia.

Methods We prospectively enrolled patients undergoing diagnostic upper endoscopy for evaluation of EoE. Mucosal pinch biopsies were obtained from the distal oesophagus and processed for routine histology and electron microscopic assessment. Clinical features of enrolled subjects were analysed and subjects were divided into four groups: normal, gastroesophageal reflux disease (GERD), inactive EoE and active EoE. Representative photomicrographs of the basal and superficial epithelia were reviewed for abnormalities. Desmosomes were quantified on the surface of epithelia three to four prickle-cell layers above the basal layer.

Results Twenty-nine paediatric cases (ages 2–18 years) were enrolled in the study. We observed a significant decrease in the number of desmosomes per cell (DPC) of subjects with active EoE compared with inactive EoE, GERD and normal epithelia. With respect to DPC, no significant differences were found between inactive EoE compared with GERD or normal subjects. Additional ultrastructural features observed included epithelial microvilli and evidence of eosinophil transmigration, degranulation, and sombrero formation.

Conclusions Consistent with clinical and molecular findings, our ultrastructural data provide support for an altered oesophageal barrier in paediatric cases with active EoE, which may improve following treatment.

INTRODUCTION

Immunohistochemical and electron microscopic studies of oesophageal epithelium affected by eosinophilic oesophagitis (EoE) have focused on findings related to eosinophil activation and have identified a number of important characteristics including eosinophil degranulation, inverted granule protein cores and sombrero vesicle formations.1–4 While these findings document the impact of activated eosinophils as a functional endpoint, a growing body of evidence supports a role for eosinophils in disease processes. For numbered affiliations see end of article.

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As described previously, we enumerated desmosomes within the epithelium by examining one to nine individual epithelial cells per patient that were three to four cell layers above the basal layer. This region of interest was selected to maintain consistency in analysis. Desmosomes were identified and quantified as discrete, linear hyper-densities along the outer cellular membrane that make physical contact with an adjacent cellular membrane. Up to 43 cells were analysed within each of the four patient groups. Additional morphological features of the epithelium were identified independently by five investigators (EPW, KEC, JCM, SDF, GTF) who then reviewed their findings together.

Statistical significance was determined using a Kruskal–Wallis test and Dunn’s test for multiple comparisons. Statistical analysis was performed using GraphPad Prism V.6.0d (GraphPad Software, La Jolla, California, USA).

RESULTS

Twenty-nine persons were enrolled in the study and clinical features are shown in table 1.

Typical endoscopic features of EoE, including linear furrows and exudates, as well as histological findings such as inflammation and basal cell hyperplasia, are seen in figure 1. To minimise technical variation that may exist with assessing the stratified squamous epithelia in electron micrographs, we analysed the basal layer of the epithelia (figure 2A). The desmosome number was significantly reduced in epithelia with active EoE compared with normal epithelia (7.6±3.7 vs 14.1±5.9, active EoE vs normal, p=0.0001; figure 2B). Following standard EoE treatment, the desmosome number in inactive EoE (13.2±5.4) was virtually the same as normal epithelia. Additionally, desmosome numbers in epithelia of patients with GERD (12.0±4.0) were similar to inactive EoE and normal epithelia.

Further assessment of the intercellular space (ICS) revealed that the width of the ICS appears to be narrower in normal epithelia (7.8±2.7 μm) compared with normal epithelia (14.1±5.9 μm, active EoE vs normal, p=0.0001; figure 2B). Following standard EoE treatment, the desmosome number in inactive EoE (13.2±5.4) was virtually the same as normal epithelia. Additionally, desmosome numbers in epithelia of patients with GERD (12.0±4.0) were similar to inactive EoE and normal epithelia.

In addition, the desmosome number in subjects with active EoE is significantly reduced compared with inactive EoE, GERD and normal subjects.

DISCUSSION

Over the last decade, EoE has emerged as a leading cause of feeding dysfunction in young children and dysphagia and food impaction in teenagers and adults. Treatment, such as diet restrictions and topical steroids, are highly effective in inducing remission, and an increasing body of evidence identifies an underlying type-2 cytokine-mediated pathogenetic pathway. Recent studies suggest that the epithelial barrier may play a critical role in the initiation and/or perpetuation of this inflammatory disease. As such, we performed a prospective study to determine ultrastructural features of the epithelial surface affected by EoE. This study reports a number of new observations related to ultrastructural epithelial features in children with EoE. Most prominent of these is that the number of desmosomes in subjects with active EoE is significantly decreased compared with inactive EoE, GERD and normal subjects.

Previous studies have identified ultrastructural epithelial abnormalities associated with peptic oesophagitis and oesophageal cancers, but ours is the first to identify epithelial ultrastructural findings in a prospective fashion in paediatric cases with active and inactive EoE. Our findings bear particular relevance and support for recent studies that identify a role for epithelial barrier dysfunction in EoE. Through the use of electrical impedance, transepithelial resistance and fluorescent flux, van Rijn et al reported diminished epithelial barriers in adults with EoE compared with those with normal epithelia. Katzk et al demonstrated increased dilated ICS associated with decreased expression of filaggrin, zona ocludens-1 and claudin-1 in oesophageal biopsies from patients with untreated EoE. Sherrill et al examined molecular regulation of barrier dysfunction and found that desmoglein-1 expression was decreased via a type-2 cytokine microenvironment, and was associated with diminished barrier function in an ex vivo squamous epithelial model. In an electron micrograph, the number of desmosomes appears to be diminished, but enumeration was not presented. Here, we provide quantitative, morphological evidence that the oesophageal epithelial ultrastructure is indeed altered in EoE, a finding consistent with these functional and molecular studies. Additionally, we show that desmosome number returns to that found in the normal epithelium following treatment. This observation suggests that morphological evidence of barrier dysfunction occurs secondary to inflammation and not as a primary defect. Alternatively, treatments may repair underlying inherent epithelial barrier defects.

Our results also identify a number of interesting and important features related to relationships between the epithelium and eosinophils. First, while our findings identify the morphological...
disruption of the normal stratified epithelia, our assessment also reveals the inherent nature of the oesophageal epithelium to maintain an intact barrier. We show that the superficial epithelia associated with active and inactive EoE, similar to that found in GERD and normal subjects, maintain a robust pattern of interdigitating microplicae or intercellular ridges. This 'zipper-like' morphology emphasises the importance of maintaining a barrier, which appears different than the basal layer, and further illustrates the pleotropic nature of the oesophageal epithelium. Therefore, the superficial surface possesses an infrastructure comprised of flattened epithelial cells, microplicae, tight junctions and narrow ICS that creates a network to prevent luminal contents from penetrating the underlying immunomicromilieu. In EoE, the epithelia may limit exposure of the immune system to food allergens that initiate and perpetuate eosinophilic inflammation. Additionally, we provide evidence of the dynamic nature of eosinophil chemotaxis within the epithelia as seen in the figures demonstrating eosinophilic transmigration. Despite a
number of studies defining the chemotactic pathways associated with EoE, none have captured an eosinophil actively migrating between the stratified squamous epithelia in EoE. Lastly, our ultrastructural analysis confirms recent observations that report the presence of eosinophil degranulation, sombrero vesicle formation and reversal of core proteins in EoE. While this ultrastructural report provides a high level of detail, it is difficult to provide a more expansive quantitative assessment or deeper mechanistic understanding than can be obtained through routine histological or molecular analysis, respectively. For instance, since eosinophils are unevenly distributed within the epithelia, quantification of their features and patterns of distribution in such a small field of view may not truly represent the overall mucosal inflammatory state. Therefore, we were limited to observational assessments only.

Similarly, we did not report exact measurements of the width of the ICS due to the high degree of variability in cell membrane shapes and forms. Although enumeration of desmosomes within superficial squamous epithelia has been used in a number of previous studies to assess epithelial integrity and morphology, our enumeration focused on the deeper, basal epithelial cells primarily due to technical concerns about the integrity of the superficial mucosa. Because of mechanical stresses on the epithelial surface caused by the pinch biopsy procedure, we could not consistently assess the uppermost superficial surfaces of the epithelia. However, we felt confident that the basal layer could be identified by allowing the underlying lamina propria to serve as a landmark. Thus, the numbers of desmosomes may appear to be sparse in the basal layer (figure 3) when compared with
those in other more superficial parts of the epithelia (figure 4). These findings may indicate that the more superficial epithelia require a larger number of desmosomes than the more basal epithelia to maintain barrier function for reasons discussed above, but this speculation would require further studies. Notwithstanding the technical challenges, we feel that the present study using transmission electron microscopy provides unique ultrastructural insights into features of EoE that would not be permitted through either routine histological or molecular techniques.

In summary, we report that the basal layer of stratified squamous epithelia appears to be altered in paediatric subjects with EoE. Future studies using laser capture microscopy to determine the exact molecular patterns leading to this finding will provide more mechanistic insights and potential identification of novel therapeutic targets.

**Take home messages**

- Oesophageal epithelial barrier dysfunction may play a critical role in the initiation and/or perpetuation of eosinophilic oesophagitis (EoE), a chronic immune-mediated inflammatory condition.
- At the ultrastructural level, a significant decrease in the number of desmosomes on the surface of the oesophageal epithelia of paediatric subjects with active EoE is observed. This provides support for recent works identifying evidence of altered barrier function in EoE.
- Eosinophils actively transmigrate through the oesophageal epithelium where they intimately adjoin with squamous epithelial cells prior to degranulation.
REFERENCES


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