Effects of Topical Steroids on Tight Junction Proteins and Spongiosis in Esophageal Epithelia of Patients With Eosinophilic Esophagitis

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BACKGROUND & AIMS: **The allergic response associated with eosinophilic esophagitis (EoE) occurs when food antigens permeate tight junction–mediated epithelial dilated intercellular spaces. We assessed whether levels of tight junction proteins correlate with the dilation of intercellular spaces (spongiosis) and the effects of topical steroids on these parameters.**

METHODS: **We assessed esophageal biopsy samples from 10 patients with active EoE treated with topical fluticasone, 10 untreated patients, and 10 patients without esophageal disease (controls) for degree of spongiosis. Immunohistochemical assays were used to determine the levels of the tight junction proteins filaggrin, zonula occludens (ZO)-1, ZO-2, ZO-3, and claudin-1. Histology and immunohistochemistry results were assessed blindly, with levels of tight junction proteins and degree of spongiosis rated on scales of 0 to 3.**

RESULTS: **The mean degrees of spongiosis in untreated and treated patients with EoE were 1.3 and 0.4, respectively ($P = .016$). Esophageal epithelia did not stain significantly for ZO-1 or ZO-2. Filaggrin was observed in a predominant cytoplasmic pattern, compared with the cytoplasmic and membranous patterns of ZO-3 and claudin-1. In biopsy specimens from patients with active EoE, the mean staining intensities for filaggrin, ZO-3, and claudin-1 were 1.6, 1.4, and 0.7, respectively. In biopsy specimens from patients treated with fluticasone, levels of filaggrin, ZO-3, and claudin-1 were 2.8 ($P = .002$ compared with untreated patients), 1.7 ($P = .46$ compared with untreated patients), and 1.3 ($P = .25$ compared with untreated patients), respectively. The correlation between the level of filaggrin and the degree of spongiosis was $r = 0.23$, and between ZO-3 staining and the degree of spongiosis was $r = .016$ ($P = .001$ for filaggrin vs ZO-3 staining).**

CONCLUSIONS: **Filaggrin, ZO-3, and claudin-1 (but not ZO-1 or ZO-2) are detected in the esophageal mucosa of patients with EoE treated with steroids and individuals without esophageal disease. Without treatment, spongiosis increases, corresponding with reduced levels of filaggrin, ZO-3, and claudin-1. Loss of tight junction regulators and dilation of intercellular spaces appear to be involved in the pathophysiology of EoE and could be targets for treatment.**

Keywords: Esophagus; Inflammation; Therapy; Allergy.

Eosinophilic esophagitis (EoE) is an allergy-mediated disease in which food antigens in contact with esophageal mucosa generate a T-helper cell type 2 (Th-2) cascade leading to tissue eosinophilia, inflammation, and fibrosis.[1] The recognition of these antigens is believed to be mediated by dendritic cells that reside in the esophageal mucosa. The precise mechanism by which this interaction occurs is unknown.

For antigen penetration of the deeper layers of the mucosa to be achieved, paracellular transport must occur. This largely is regulated through tight junction regulators and proteins that can lead to widening of the gap between epithelial cells. This is referred to histologically as *dilated intercellular spaces* or *spongiosis*. Spongiosis has been well documented as a common histopathologic finding in patients with EoE. It is not clear, however, how well spongiosis responds to steroid

Abbreviations used in this paper: EoE, eosinophilic esophagitis; eos, eosinophils; HPF, high-power field; ZO, zonula occludens.

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treatment. Furthermore, there is little information on tight junction proteins in esophageal epithelium and their role in dilated intercellular spaces. In this study, we investigated the following: (1) which tight junction proteins are present in esophageal epithelia, (2) if staining density corresponds inversely to the presence of dilation of intercellular spaces in esophageal epithelium as measured by the histologic appearance of spongiosis, and (3) if with a steroid-induced reduction in the intercellular space (manifest by spongiosis) there is an accompanying increase in staining for tight junction protein.

Methods

Twenty patients with EoE were included in this study. The diagnosis of EoE was established by consensus guidelines.[1] In addition, all patients had at least one atopic characteristic (asthma, rhinitis, oral allergy syndrome, or atopic dermatitis). Ten of these patients had untreated EoE with more than 15 peak eosinophils (eos) per high-power field (HPF), a history of dysphagia, consistent endoscopic findings, and a lack of histologic response to an 8-week course of proton pump inhibitors or a negative ambulatory esophageal pH monitoring study. Biopsy specimens were analyzed from another 10 patients with proven pretreatment active EoE who were treated with 880 ug oral fluticasone twice daily for 2 months. Esophageal biopsy specimens showing a reduction of esophageal eosinophilia to fewer than 5 eos per HPF obtained at the end of treatment were used for analysis. Esophageal biopsy specimens from 10 control patients also were analyzed. These patients underwent endoscopy for clinical reasons unrelated to esophageal disease and all had a normal esophageal endoscopic appearance and were histologically normal. None of these patients had a history of gastroesophageal reflux disease. Neither patients nor controls were on acid-suppressing therapy at the time of the study.

Formalin-fixed, paraffin-embedded esophageal biopsy specimens were cut into 3-μm sections and the unstained sections were treated with a progressively concentrated xylene wash, and then incubated in a citrate buffer within a water bath heated to 85°F. After completion of this step to expose antigen-binding sites for one tight junction protein of interest (claudin-1, zonula occludens [ZO]-1, ZO-2, ZO-3, and filaggrin), the optimal dilution of the primary antibody for that protein was added to each slide (Abcam, Cambridge, MA) (Supplementary Table 1). Because there are little prior data studying these proteins in esophageal tissue, checkerboard titrations were used to determine the optimal dilutions of the primary antibody and other reagents. Skipping the primary antibody was used as a negative control and skin was used as a positive control. Specimens were left overnight at 4°C to maximize binding of the antibody. After a secondary antibody was added to enhance specificity, each tissue was treated with the ABC reagent kit (Vector Laboratories, Burlingame, CA) to attach an enzymatic substrate to each site. Samples subsequently underwent a colorimetric peroxidase reaction under timed conditions. Once microscopy was used to optimize the level of histologic staining, the reaction was quenched. Each slide then was washed and processed with a progressively dilute xylene for preservation of immunostaining. Finally, each sample was visualized under a microscope and a semiquantitative scale was applied to determine the location and concentration within each tissue specimen. Grading of tight junction staining was performed with a scale of 0 to 3+ based on the control patient biopsy specimens. On routine histologic analysis, the degree of spongiosis was graded on a scale of 0 to 3. All slides (both EoE and controls) were read blindly by one of the investigators (T.C.S.). Grading of spongiosis and tight junction staining was determined by calculating the average finding over all the biopsy specimens in each paraffin block to account for possible patchiness in the distribution of findings.

Statistical Analysis

Analysis of variance with Tukey's test were used to compare results from the 3 groups. The Spearman coefficient was used to determine correlation of spongiosis to tight junction staining.

Approval was obtained for this study through the Mayo Clinic Institutional Review Board.

Results

The clinical and histologic profiles of the 20 patients used in this study are shown in Table 1. Nineteen of 20 patients included in this study had typical endoscopic features of EoE including rings, linear furrowing, white plaques, or mucosal friability. In the treated group, 6 patients had no eos present and 8 patients had fewer

Table 1. Patient Group Characteristics

EoE groups	Age, *y* (range)	Male	Peak eos/HPF (range)	Dysphagia	Characteristic EGD EoE, findings/mean EREF score[19]
Untreated	40 (9–51)	9	57 (20–120)	10	10/3.2
Fluticasone	39 (7–53)	9	37 (15–100)	10	9/3.5

EREF, Endoscopic Reference Score.