

Relative lack of T regulatory cells in adult eosinophilic esophagitis – no normalization after corticosteroid therapy

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Eosinophilic esophagitis (EoE) is a chronic-inflammatory, Th2-type allergic disease of the esophagus with a rapidly increasing prevalence. EoE is clinicopathologically characterized by symptoms attributed to esophageal dysfunction in combination with a dense esophageal eosinophilia, both of which are refractory to proton pump inhibitors (1). T regulatory cells represent a subset of T cells that play a central role in the prevention of immunopathology and might be decreased in either numbers or function in autoimmune and allergic diseases (2). Foxp3 represents a crucial transcription factor required for

In active eosinophilic esophagitis, the proportion of Foxp3 positive T cells is reduced by about 50%. The relative lack of T regulatory cells in this allergic disorder is not reversible by corticosteroid therapy, which is otherwise successful in bringing patients in clinical and histological remission.

the large majority of T regulatory cells (2).

We have recently reported that budesonide, in a clinical, randomized, double-blinded, placebo-controlled trial, significantly reduced both clinical symptoms and mean eosinophil numbers in the esophageal epithelial layer of adult EoE patients (3). Here, we report the results of T regulatory cell number measurements, which we additionally analyzed within this study (EoE patients, $n = 35$). As control group, we recruited 20 esophagus-healthy individuals. In the epithelium of the esophagus, numbers of total T cells and T regulatory cells were assessed by immunofluorescence and confocal microscopy. Indirect immunostainings were performed on four proximal and four distal biopsies of each patient and control individual, respectively, as previously described (3). Rabbit anti-CD3 (DakoCytomation, Glostrup, Denmark) and monoclonal anti-Foxp3 (clone PCH101; eBioscience, Frankfurt, Germany) were used as primary antibodies. Representative examples of the original immunofluorescence data are shown in Fig. 1A. Positive cells were counted in a blinded manner in 20 hpf (area of 1 hpf 0.0538 mm²) to obtain average numbers per 1 hpf.

Compared to control individuals, adult EoE patients had significantly increased numbers of infiltrating T cells within the epithelial layer of the esophagus (Fig. 1B, left panel), confirming previously published work (4). In contrast, the proportion of Foxp3-positive CD3-positive T cells was approximately 50% reduced in EoE compared to esophagus-healthy controls (Fig. 1B, right panel), suggesting a relative lack of T regulatory cells at the site of inflammation in adult EoE patients that might contribute to both immunopathology and chronic character of the disease. It

should be noted that in pediatric EoE, increased numbers of T regulatory cells were reported (5). The reason for this discrepancy between pediatric and adult EoE remains unclear at the moment.

Corticosteroids have been reported to increase numbers of T regulatory cells in asthma (6). Therefore, we analyzed the effect of short-term treatment with topical budesonide (3) on T regulatory cell numbers within the epithelial layer of the esophagus in EoE patients. Twice daily topical therapy with 1 mg budesonide over a time period of 15 days resulted in a significant reduction in total numbers of infiltrating T cells, whereas placebo had no effect (Fig. 1C, left panel). However, this treatment had no influence on the relative numbers of T regulatory cells that remained low compared with esophagus-healthy individuals (Fig. 1C, right panel). Therefore, corticosteroids, in spite of improving clinical symptoms and depleting eosinophils in the esophageal epithelium (3), seem to be unable to correct the relative lack of T regulatory cells in EoE, at least when applied for 15 days only.

Taken together, we report here that adult EoE is associated with a relative lack of T regulatory cells in the epithelial layer of the esophagus, and this abnormality is not corrected by short-term therapy with budesonide, which is highly effective in inducing a clinical and histological remission in EoE patients (3). Future work is required to answer the question whether the available T regulatory cells exhibit additional functional defects in EoE. Moreover, it remains unknown which other T-cell subpopulations are recruited into the esophageal mucosa under both physiological and pathological conditions.

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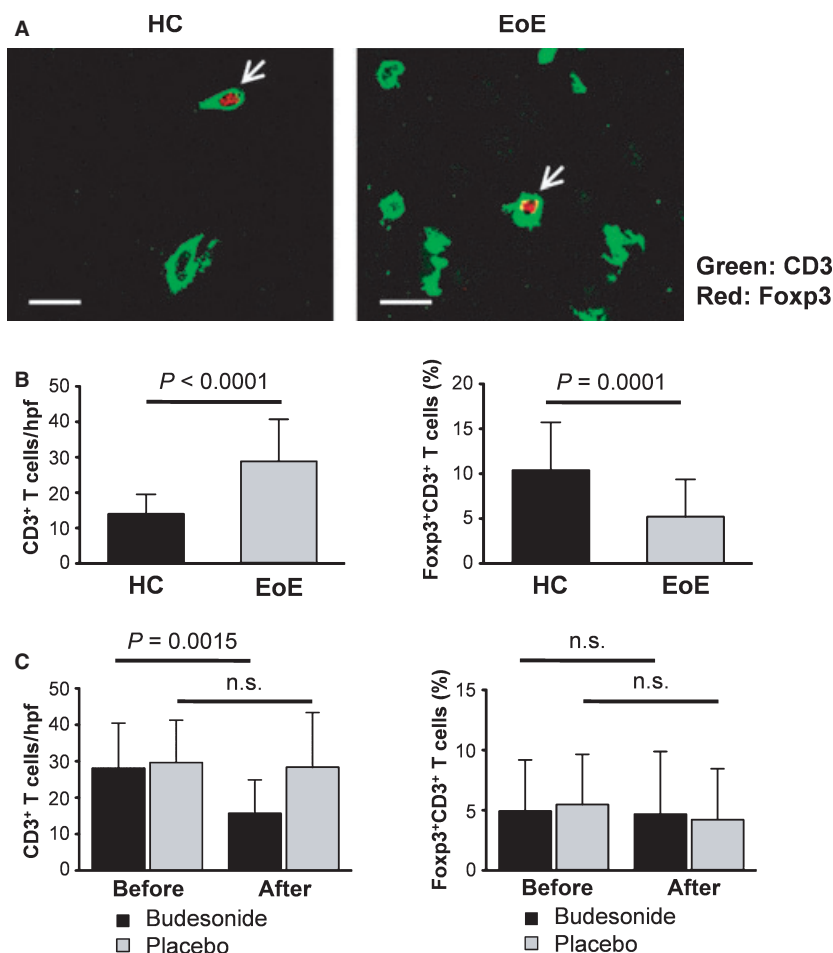


Figure 1 Steroid-resistant reduction of Foxp3-expressing T cells in the epithelial layer of the esophagus of adult EoE patients. (A) CD3-positive (green) and Foxp3-expressing (red) cells in healthy controls (HC) and EoE patients. Double-positive cells are indicated by arrows. The majority of the T cells were single CD3 positive, particularly in EoE. Bars, 10 μ m. (B) Total numbers of T cells per hpf (left) and percentage of T regulatory cells (right) in healthy controls (HC, $n = 20$) and EoE patients ($n = 35$). Values are mean levels $\pm$ SD. P -values are indicated. (C) Effect of placebo-controlled short-term budesonide therapy on total numbers of T cells per hpf (left) and percentage of T regulatory cells (right) in EoE patients (budesonide, $n = 18$; placebo, $n = 17$). Values are mean levels $\pm$ SD. P -values are indicated. n.s., not significant.

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