Esophageal type 2 cytokine expression heterogeneity in eosinophilic esophagitis in a multi-site cohort

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Abstract

Background: There is strong evidence for a role of type 2 cytokines in the pathogenesis of eosinophilic esophagitis (EoE); however, heterogeneity in type 2 gene expression has not been examined.

Objective: We examined type 2 immunity-associated gene expression in esophageal biopsies aiming to determine the degree of cytokine heterogeneity and its potential clinical significance.

Methods: Patients (n=312) were recruited from 10 sites associated with the Consortium of Eosinophilic Gastrointestinal Disease Researchers (CEGIR). In addition to histologic and endoscopic assessment, esophageal biopsies were examined for expression of 96 genes within the EoE Diagnostic Panel (EDP).

Results: Five subgroups of patients with active EoE were identified by unsupervised clustering based on expression of *IL4*, *IL5*, *IL13*, *CCL26*, *TSLP*, *CLC*, *CCR3*, and *CPA3*. These groups differed in age ($P < 0.02$) and EDP score ($P < 1.08E-30$) but not eosinophil levels. Group V patients had the highest expression of *IL5*, *TSLP*, *CCL26*, and genes associated with tissue remodeling such as *COL8A1*, *ACTG2* and *TSPAN12*. *IL5* and *IL13* were highly expressed in group IV; however, groups IV and V differed in age (34 vs. 14 years, $P < 0.001$). Groups II and III, which exhibited intermediate expression of *IL5* and *CPA3*, were differentiated by high *TSLP* and *IL13* in group III.

Conclusion: We observed heterogeneous type 2 gene expression among patients with active EoE. Type 2 gene overexpression was not directly proportional to disease features; this was especially true for tissue remodeling events. These findings highlight a clinical opportunity for leveraging molecular endotypes to implement personalized medicine in EoE.

Graphical Abstract

CEGIR, Consortium of Eosinophilic Gastrointestinal Disease Researchers; EoE, Eosinophilic Esophagitis; EDP, EoE Diagnostic Panel; EREFS, Endoscopic Reference Score.
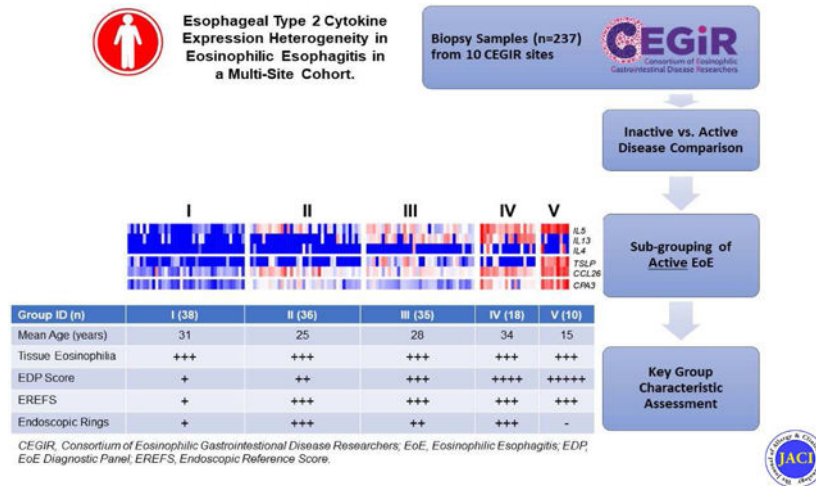
Capsule Summary

This study demonstrates heterogeneity of type 2 immunity in eosinophilic esophagitis independent of esophageal eosinophil levels. These data substantiate the presence of clinical endotypes and suggest heterogeneity of responses to anti-type 2 therapy.

Keywords

INTRODUCTION

Eosinophilic esophagitis (EoE) is a chronic inflammatory disease triggered by delayed immune hypersensitivity to foods and aeroallergens resulting in the accumulation of excessive eosinophils in the esophagus.[1-3] Children and adult patients can present with vomiting, dysphagia and food impaction.[4]

Substantial evidence is accumulating supporting that EoE is driven by a type 2 immune response, associated with IL-4, IL-5 and IL-13.[5-15] In particular, mRNA expression of *IL5* and *IL13* (and *IL4* to a lesser extent) are increased in the esophagus of EoE patients compared with controls.[6, 16, 17] IL-5 is required for induction of experimental EoE in mice, and overexpression leads to esophageal inflammation and remodeling.[10] IL-5 induces integrin ligation, activation, and survival of eosinophils, all of which can be inhibited in vivo in humans with a neutralizing antibody against IL-5, which is now approved for the treatment of eosinophilic asthma.[18, 19] IL-13 appears to be particularly important in EoE as overexpression in mice induces cardinal features of the disease, including induction of esophageal eotaxin expression, eosinophil accumulation, epithelial hyperplasia, angiogenesis and fibrosis, processes that occur in the human disease.[15] In addition, IL-13 induces impaired barrier function of esophageal epithelial cells partially via upregulation of

calpain-14 (*CAPN14*), which is encoded by a gene located at the site of one of the chief EoE susceptibility loci, 2p23.[12, 15, 20-22] Genetic studies have also identified genome wide susceptibility at 5q22, the thymic stromal lymphopoietin (*TSLP*) locus. TSLP-activated dendritic cells cause T cell polarization to Th2 cells that serve as sources of IL-5 and IL-13 in EoE.[7, 23, 24] Antibodies against IL-5 and IL-13, including its receptor IL4Rα (also a receptor for IL-4) reduce esophageal eosinophils and improve endoscopic, histologic and/or esophageal transcriptomic abnormalities in early clinical studies, but the effects are variable.[5, 11, 13, 14, 25, 26] Despite strong evidence for a role of type 2 cytokines in EoE pathogenesis, implementation of targeted therapeutics is hampered, in part, by the inability to predict responders and non-responders and the heterogeneity of treatment response.

Levels of esophageal eosinophils correlate with the severity of endoscopic findings.[27] Yet, disease severity associates with multiple histological parameters better than esophageal eosinophil counts alone.[28] Transcript expression profiling of esophageal biopsies has identified unique disease specific transcripts, notably rich in genes that encode type 2 cytokines or products induced by these cytokines (e.g. IL-13 induced eotaxin-3).[6, 17, 29-31] An EoE diagnostic panel (EDP), composed of 96 genes involved in diverse functions including inflammation, remodeling, and ion transport, effectively differentiates patients with active EoE from control individuals including EoE patients in remission.[17] Molecular profiling with the EDP has identified substantial molecular heterogeneity associated with distinct histologic and endoscopic features which lead to the identification of three distinct disease endotypes.[16] Herein, we examine 237 adult and pediatric EoE patient biopsies and clinical features from 10 sites associated with the Consortium of Eosinophilic Gastrointestinal Disease Researchers (CEGIR).[32, 33] Esophageal biopsies were examined for the expression of type 2 immunity associated gene products, aiming to test for the presence of patient heterogeneity, based on type 2 immune responses, and its significance.

METHODS

Patient Recruitment

This study was conducted within the context of CEGIR, a national collaborative network of 16 academic centers caring for adults and children with eosinophilic gastrointestinal disorders (Supplemental Table 1). The CEGIR clinical trial, Outcomes Measures in Eosinophilic Gastrointestinal disorders across the Ages (OMEGA), is a longitudinal cohort study aimed at understanding the natural history of EoE, eosinophilic gastritis, and eosinophilic colitis during routine clinical care.[16, 32-34] With this aim, demographic, clinical, endoscopic and histologic data, as well as gastrointestinal and blood samples have been prospectively collected from 2015 through 2018. Ten sites provided the EoE patient and sample data for this study; samples without peak eosinophil counts were excluded from the current analysis (Fig. 1; Supplemental Table 2). Clinical features of subjects were determined during standard of care evaluation with intake and follow up forms. All subject data were stored at the Data Management and Coordinating Center (DMCC) at the University of South Florida in Tampa, FL and were systematically extracted from the database. We defined patients with active EoE as having symptomatic esophageal dysfunction and a peak count of 15 or more eosinophils per high-power field (HPF); samples

with no documented eosinophil count at the time of biopsy were excluded from the current study. Specimens obtained from patients with confirmed EoE with fewer than 15 peak eosinophils per HPF were classified as inactive. Pediatric subjects were defined as those less than 18 years of age. Atopy was defined based on self-report of allergic rhinitis, dermatitis, asthma, or food allergy. The definition of "response to steroid" was determined using a positive/negative response to whether swallowed topical steroids had been effective on the basis of symptom and pathology. These data were captured across 10 sites by the CEGIR questionnaire, as reported.[16, 35] This study was approved by the institutional review boards of the participating institutions via a central institutional review board at Cincinnati Children's Hospital Medical Center (CCHMC). Participants provided written informed consent.

Biopsy acquisition

We obtained distal esophageal biopsy specimens during endoscopy; 2-3 specimens were taken from the area 2-4 cm above the lower esophageal sphincter.[30] Gene expression in unfixed biopsy specimens was analyzed by qPCR with the EDP as previously described. [16, 17] The EoE transcriptome as measured by the EDP is enriched for epithelial genes as reported although it also has embedded markers of T cells, mast cells and eosinophils.[17] Changes in cycle thresholds (ΔCt) for all genes of interest was calculated by subtracting the Ct of GAPDH. In order to control for the potential effect of variable GAPDH detection on ΔCt values for samples with Ct = 40 (the lower limit of detection), ΔCt values associated with Ct=40 were removed, the minimum expression of each gene was identified, and ΔCt values associated with Ct = 40 were replaced with (minimum-1) values. The EDP score (previously described as EoE score) was calculated by summing ΔCT values of the 76 highly dysregulated genes ($\Sigma\Delta$CT) in the panel, as described previously.[16, 17] In order to reflect the disease-specific expression signature and disease severity, the CT sums were separately derived for the upregulated and downregulated genes, and then their two sums were combined. Of note, the EDE score is inversely correlated with disease severity. The assignment of the subject's EoE endotype based on the expression of 8 genes in the panel of genes was established by a method comprising linear discriminant analysis determining a probability distance using Mahalanobis distance.[16] By using the same algorithm, probabilities and predicted EoE endotypes were calculated based on the highest predicted probability.

Data analysis

Unsupervised clustering was used to identify groups of patients with active EoE based on expression of Th2-associated genes previously shown to be upregulated in EoE; *IL4*, *IL5*, *IL13*, *CXCL8*, *CCR3*, *CCL26*, *TSLP*, as well as markers of eosinophils (*CLC*), and mast cells (*CPA3*). The selection of K=5 for patient clustering was based on obtaining a sufficient fit, the results of a sensitivity analysis, and also to limit depletion of cluster size. Gene expression, EDP score, and age were compared between these groups using one-way ANOVA with Bonferroni post-test, and significance with respect to inactive patients was assessed with Dunnett's post-test. Values from the histologic scoring system (HSS),[28] endoscopic reference scoring system (EREFS),[27, 36] and peak eosinophil count were compared with the non-parametric Kruskal-Wallis test and Dunn's post-test. Features of the

HSS were scored for grade (0-3) and stage (0-3), which were combined and the sums normalized to a range of 0-1 for the total scores. Lamina propria fibrosis could not be assessed in over 30% of samples, therefore these measurements were excluded from the current analysis. Features of the EREFS were scored (0-2) and features were added to obtain total scores in the distal and proximal esophagus; proximal and distal scores were, in turn, added to obtain a final score.

To characterize groups of patients with active EoE, we examined the distribution of previously described EoE endotypes. To assign endotypes, an algorithm with discriminant analysis was performed based on expression of 8 genes expression previously described.[16] Data compilation, filtration and K-means clustering were performed in R Studio (version 3.5.1) and statistical analyses were completed in GraphPad Prism (version 8.0.1); statistical significance was assigned at p-values less than 0.01. Morpheus (https://software.broadinstitute.org/morpheus/) was used to generate heatmaps.

RESULTS

EoE Patient Characteristics

Patient age ranged from 4 to 71 years with mean age of 26 years (Table 1). A majority of the patients were male (67%) (Table 1). A subset of patients were on proton-pump inhibitor, dietary elimination and topical steroid therapy (Table 1). Patients with active EoE had mean value of 50 eosinophils/HPF, whereas patients with inactive EoE had 3 eosinophils/HPF (Table 1). The EDP total score differed in active and inactive group (123 vs 332, $P < 0.0001$; Table 1). Consistent with the increase in esophageal eosinophil levels, expression of esophageal *CLC* mRNA was increased in active EoE compared with inactive EoE (Ct = 5.45, $P < 0.0001$; Fig. 2). Levels of *CPA3*, a mast cell specific transcript, were increased in biopsies from active EoE patients compared with patients with inactive EoE (Ct = 2.84, $P < 0.0001$; Fig. 2).

Type 2 cytokine profiles in EoE

Of the upregulated genes in active vs. inactive disease, we noted numerous type 2 immunity associated genes, such as *IL5*, *TSLP*, and *IL13* (Fig. 2). These included increases in several IL-13-inducible genes including *CCL26* (Ct = 5.12, $P < 0.0001$), *SLC26A4* (Ct = 4.88, $P < 0.0001$), *MMP12* (Ct = 4.32, $P < 0.0001$), *LRRC31* (Ct = 8.21, $P < 0.0001$) and *ALOX15* (Ct = 7.47, $P < 0.0001$). Focusing on type 2 associated cytokines, we observed increased *IL5* (Ct = 4.02, $P < 0.0001$) and *IL13* (Ct = 2.15, $P < 0.0001$). By contrast we observed only a modest increase in *TSLP* (Ct = 0.09, $P < 0.05$) and no significant change in *TGFB1*.

Expression of type 2 inflammatory genes distinguishes subgroups of active EoE

We set out to identify unique subgroups of patients with active EoE based on differential expression of type 2 cytokines (*IL4*, *IL5*, *TSLP* and *IL13*) and the allergic inflammatory cell specific markers *CLC* and *CPA3*. Using unsupervised clustering, we identified five groups of patients with low (Group I), intermediate (Groups II and III), and high (Groups IV and V) expression of type 2 cytokine genes, which were ordered according to expression of *IL5*

(Fig. 3, A). *IL5* and *CPA3* were not significantly increased in Group I with respect to patients with inactive EoE (Fig. 3, B). Eosinophil levels were comparable among all 5 subgroups (Fig. 3, C). Whereas *IL13* was significantly increased in Groups III-V, the increase in Groups III and IV was most dramatic. *TSLP* was increased specifically in Groups III and V. Of note, *IL4* was universally low; it was undetectable in 95 of 97 patients with inactive EoE, and in all Group I patients; conversely, *IL4* expression was detectable in approximately 20% of *IL5*-high patients with active EoE (Groups IV and V; Fig. 3, A).

Groups I-V differed significantly in total EDP score, both compared to one-another and compared to patients with inactive EoE (Fig. 3, C). Groups that did not significantly differ in EDP score were Groups II and III, the *IL5*-intermediate groups, as well as Groups IV and V, the *IL5*-high groups (Fig. 3, C). While there was no difference in age between patients with active or inactive EoE (Table 1), patients in Group IV were significantly older than patients with inactive EoE ($P < 0.05$; Fig. 3, C). Moreover, treatment status did not account for patient grouping, as chi-square analysis did not reveal significant enrichment of PPI, steroid usage, or ongoing diet therapy in any of the five patient groups (Supplemental Table 4).

Cytokine heterogeneity associates with histologic and endoscopic phenotypes as well as molecular endotypes

We aimed to determine if the identified patient subgroups associated with histological and endoscopic features. In patients in Group I with low *IL5* expression, we observed that HSS grade and stage scores were higher than in the inactive group corroborating their assignment as active EoE; however, we observed no significant increase in total endoscopic scores (EREFS) with respect to inactive EoE (Fig. 4, A-B). Peak eosinophil counts did not differ among the active groups; however, significant differences between active and inactive disease patients and significant intergroup differences occurred among the individual features of the HSS (Fig. 4, C; Supplemental Table 3). The most frequent abnormalities were eosinophilic inflammation (EI), basal zone hyperplasia (BZH) and dilated intercellular spaces (DIS); EI and BZH were significantly increased in all groups compared to inactive EoE, BZH was significantly increased in Group V compared to Group I, and DIS was significantly increased in Groups II-V compared to inactive EoE. Surface epithelial alteration (SEA) was significantly increased in Group V compared to inactive patients. Eosinophil abscesses (EA), eosinophil surface layering (ESL) and dyskeratotic epithelial cells (DEC) were less common, and significant increases were not found. ESL was most common in Group III and DEC in Group V. Group V had the highest scores for EI, BZH, DIS, SEA and DEC.

Focusing on endoscopic features, the identified patient subgroups had unique associations. Group I did not have a significant increase in rings or edema, whereas edema was elevated across Groups II-IV (Fig. 4, B). Exudates were elevated across Groups II-V. Although furrowing was observed in all patient groups with respect to inactive disease, the most severe furrowing was observed in Groups II, III and V, which were significantly greater than Group I. Of note, rings (or trachealization) were highest in Groups II, III and IV and were absent in Group V.

We aimed to understand the relationship between the identified cytokine heterogeneity and previously described EoE endotypes. Absence of rings in Group V patients led us to question whether these biopsies shared characteristics with the inflammatory EoE endotype (EoEe2), which also exhibited a notable lack of rings.[16] Whereas Group V overlapped entirely with EoEe2, Group IV represented a combination of EoEe2 and the fibrostenotic endotype, EoEe3 (Fig. 4, D). Groups II and III were primarily represented by EoEe3, while the remaining patients conssited of the normal-like endotype, EoEe1, as well as EoEe2. Finally, Group I consisted primarily of patients assigned to EoEe1, while the remainder of patients consisted of EoEe3 and only 1 individual from EoEe2. Collectively, our data substantiate distinct EoE endotypes, independent of esophageal eosinophil levels in active biopsies, based on cytokine expression.

Group-specific gene dysregulation corroborates endotype membership

We aimed to determine which genes and/or pathways associated with the unique *IL5*-related endotypes. By enumerating the 10 genes that were the most highly upregulated in each group, we identified several genes that were upregulated in all five groups including *ALOX15*, *CCL26*, and *LRRC31*, which are induced by IL-13, as well as *CLC* and *PMCH* (Fig. 5, B). *MMP12*, *SLC26A4*, and *TNFAIP* were among the most induced genes of Groups II-V. In Group III, there was notable upregulation of structural remodeling genes *MMP12* and *COL8A2* paired with a decrease in genes associated with epithelial integrity and differentiation such as *FLG*, *DSG1*, and *SPINK7* –this result corroborates the observation that Group III is largely congruent with the fibrostenotic endotype (EoEe3; Fig. 5, A *)*.[16] Whereas *IL13* was preferentially expressed in Group IV, several IL-13-dependent transcripts were preferentially expressed in Group V, including *LRRC31* and *SLC26A4*. Similarly, we observed that both groups preferentially expressed specific genes associated with structural remodeling, specifically *COL8A2* in Group IV and *ACTG2* as well as *MMP12* in Group V.

DISCUSSION

Herein, we have examined the relationship between type 2 gene expression and EoE related disease features. In this study, we examined heterogeneous expression of the type 2 cytokines *IL5* and *IL13* because of their known roles in promoting eosinophil survival and activation through both direct and indirect mechanisms and the emerging set of therapies that target these cytokines and/or their receptors.[5, 11, 13, 14, 18] We have identified subgroups of patients with active EoE that have low, intermediate, and high expression of *IL5*, and furthermore demonstrated concomitant upregulation of genes associated with fibrotic tissue remodeling in *IL5*-intermediate patient groups (Fig. 6, A). Treatment status did not account for differential gene expression that determines patient grouping, as chi-square analysis did not reveal significant enrichment of PPI, steroid usage, or ongoing diet therapy in any of the five patient groups. Importantly, of the two groups with high *IL5* expression, *IL13* expression was elevated in only one of them, suggesting the existence of a mechanism by which regulation of *IL5* and *IL13* gene expression is discordant either spatially or temporally, as group IV patients were markedly older in age than group V patients We have demonstrated a positive relationship between high *IL5* expression and increased disease

severity as assessed by the total EDP score and endoscopic parameters. Specifically, we found that patients with active EoE but low expression of *IL5* did not exhibit a significant global increase in endoscopic disease severity scoring, although they did exhibit higher scores compared to inactive patients via histologic scoring. These findings support the practice of obtaining biopsies from patients suspected of having EoE even if the endoscopic appearance is normal.

Previous studies have reported a correlation between eosinophil counts by histology and expression of inflammatory genes including *IL5*, *IL13*, and *CCL26*[6, 17]. In contrast, by focusing specifically on patients with >15 eosinophils/HPF, we describe differential gene expression in groups of patients with no difference in esophageal eosinophil counts. However, we observed discordance between *CLC* transcript and eosinophil levels. This result may be due to the presence of extracellular vesicles containing *CLC* mRNA, which may be indicative of eosinophil activation. Further investigation will be needed to establish a relationship between *CLC* transcript, intact eosinophils, and extracellular eosinophil-derived vesicles in biopsy tissue.

A recent single cell RNA sequencing study of esophageal residing CD3^{+} T cells[23] has identified the cellular source of IL-5 and IL-13 to an enriched population of activated effector memory T cells (designated the T8 cell population), consistent with prior reports. [37, 38] The single cell RNA sequencing paper was limited to 17 subjects; whereas, the current study examines over 200 subjects, thus allowing a deeper analysis of patient heterogeneity, as well as phenotypic associations. On a single cell level, only a minority of pathogenic effector Th2 cells produce only IL-5, whereas most produce IL-13. Collectively, our data substantiate patient specific differences in the presence of CD3^{+} T cells based on IL-5 and IL-13 expression, and that this heterogeneity associates and is likely causal, at least in part, with distinct disease features.

Heterogeneity of disease presentation may be indicative of chronological stages of disease or distinct disease mechanisms. Recently, we have reported the identification of three molecular endotypes of active EoE with distinctive histologic and endoscopic features and presented evidence that patients transition from a normal-like endotype (EoEe1) to an inflammatory (EoEe2) and finally a fibrostenotic (EoEe3) endotype.[16] Although samples in this report represent a single timepoint, the data substantiate previous reports that EoE progression is not a simple linear pathway from low to high Th2 cytokine expression.[39] By comparing the current findings with endotype assignments, these collective results support a disease mechanism that transitions from *IL5*-low/normal to *IL5*-high/inflammatory and finally to *IL5*-intermediate/fibrostenotic.

Whereas expression of type 2 cytokines *IL13* and *TSLP* were detected at low levels in patients with low *IL5* expression or with inactive EoE, *IL4* transcript was undetectable in these patients and was only expressed at a low level in patients with active EoE and high *IL5* expression. It is possible that active protein production and signaling is not adequately accounted for by mRNA quantification. We have recently reported differential cytokine expression in T-cells from patients with EoE, and observed that IL-4 was readily detected by flow cytometry but not in RNA-sequencing data, indicating that its regulation may occur

post-translationally and that biologically significant changes in IL-4 signaling may not be detected at the level of mRNA quantification.[23] Moreover, significant evidence exists that although IL-4 and IL-13 share receptor components, due to differential receptor subunit usage, IL-4 exerts biological activity at substantially lower concentrations than IL-13;[40-42] it is therefore possible that IL-4 signaling is a critical component of the signaling events in patients from Groups IV and V, since low expression may be sufficient to skew the inflammatory environment. Furthermore, as cytokines largely exert their activity in local tissue microenvironments and immunological synapses, mRNA quantification in bulk tissue may fail to detect bioactive quantities of IL-4 expression.

High-throughput EoE genetic studies used to identify common genetic susceptibility elements (single nucleotide polymorphisms; SNPs) and altered gene expression have implicated several genes associated with type 2 inflammation. Thymic stromal lymphopoietin (TSLP), which acts on DCs to promote Th2 polarization, was identified by GWAS as the site of multiple SNPs that are associated with EoE.[7, 43, 44] We have shown that upregulation of TSLP expression in EoE patients is affected by the presence of a risk allele in the 5q22 locus.[43] Whether patient genotype at the TSLP locus accounts for patients with high *IL5* expression but low *TSLP* expression, as in Group IV, will be the subject of future study.

Improved understanding of the relationship between type 2 gene expression and clinical progression may enable selection of therapeutic interventions that target IL-13, IL-4Rα and/or IL-5. Anti-IL-13 leads to improvement in clinical outcomes in EoE and suggests the presence of responders and non-responders.[9, 13] Trials using anti-IL-5 in EoE have shown significant improvement in some patients evidenced by decreased eosinophilia and tissue remodeling.[5, 11, 14, 25, 26] Anti-IL-5 (mepolizumab and reslizumab) has utility in asthma but only in the eosinophilic endotype[8]; as such, it is reasonable to expect that anti-IL-5 may be useful for EoE in patients with specific endotypes. Our data supports the importance of studying anti-IL-5 therapeutics in patients from groups IV and V. Moreover, a recent report suggests that factors aside from IL-5 signaling may drive eosinophil survival in allergic disease[45], therefore cytokine expression alone may not predict eosinophil survival and it would be beneficial to develop functional assays to assess eosinophil responsiveness to cytokines in inflammatory patients. It is interesting to speculate that anti-IL-4Ra and anti-IL-13 based therapeutics may be particularly effectively against subgroup III and IV, which harbor the highest levels of IL-13 mRNA.

Due to the nature of the CEGIR patient cohort, in this analysis we were able to retrospectively examine the relationship between inflammatory gene expression and patient characteristics in EoE; however, we are unable to make conclusive statements regarding the patient population as a whole or over time. We found that K-means clustering using 5 clusters resulted in reproducible groupings with biologically meaningful distinctions; however, the current study is not sufficiently powered to claim that these 5 groups are intrinsic to the EoE population or to propose diagnostic or prognostic value of these clusters. Future study will be necessary to evaluate patient outcomes in relation to type 2 gene expression. Moreover, this is one of several studies that suggest a progression from normal-like to inflammatory and finally to fibrostenotic phenotypes. A longitudinal analysis to

examine changes in patient gene expression and phenotype over time will be an important future step for the field. Future studies should also be designed to account for atopic status. Finally, whereas atopic status is reported for some patients in the CEGIR cohort, this information is not available for all patients and it therefore not feasible to observe associations between atopic status and inflammatory gene expression. It will be important for future studies to standardize the definition of atopic status and to ensure thorough collection of this information.

In conclusion, we have identified substantial heterogeneity of type 2 cytokine associated gene expression in EoE, resulting in the identification of five groups of active EoE patients, all of which do not differ significantly in esophageal eosinophil counts. Our findings support a non-linear model to explain EoE pathogenesis, where cytokine expression is heterogeneous and associated with distinct endoscopic and histological features, and that elevated *IL5* is not directly proportional to the degree of histological pathology and endoscopic abnormalities, esophageal eosinophilia, and tissue remodeling. Our data supports a disease model wherein a Th2 low response transitions to a Th2 high response associated with decreased barrier function, likely in response to allergic stimuli or food antigen exposure. Effective treatment reduces eosinophil numbers and is associated with decreased inflammatory gene expression, and we propose that under certain conditions this decrease in inflammatory gene expression results in patients reverting to a relatively normal-like gene expression signature; however, over time patients, gene expression adopts a fibrotic profile with intermediate inflammatory expression. Intermediate Th2 responses are observed in older patients and are associated with fibrostenotic remodeling; interestingly, eosinophil levels remain relatively stable throughout these phases. While this hypothesis needs to be validated by longitudinal studies, we present this pathogenic model for consideration (as summarized in Fig. 6, B). Our model is consistent with the finding that the development of fibrostenosis is proportional to the length of time that EoE is untreated[46]; however, we are not proposing that this is the only pathway to the fibrostenotic endotype. These findings provide a potential molecular basis for heterogeneity of patient responsiveness to therapeutic intervention and an emerging opportunity for personalized medicine approaches in the EoE field.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Abbreviations

ACTG2	Actin Gamma 2
ALOX15	Arachidonate 15-Lipoxygenase
ANOVA	Analysis of Variance
BZH	Basal Zone Hyperplasia
CCL26	C-C Motif Chemokine Ligand 26
CCR3	C-C Motif Chemokine Receptor 3
CEGIR	Consortium of Eosinophilic Gastrointestinal Disease Researchers
CLC	Charcot Leyden Crystal
COL8A2	Collagen Type 8 Alpha 2-Chain
DEC	Dyskeratotic Epithelial Cells
DIS	Dilated Intercellular Spaces
DSG1	Desmoglein1
EA	Eosinophil Abscesses
EDP	Eosinophilic Esophagitis Diagnostic Panel
EI	Eosinophil Infiltration
EoE	Eosinophilic Esophagitis
EoEe	Eosinophilic Esophagitis endotype
EREFS	Endoscopic Reference Score
FLG	Filaggrin
GWAS	Genome Wide Association Study
HPF	High Power Field
HSS	Histology Scoring System
IL	Interleukin
LRRC31	Leucine Rich Repeat Containing 31
MMP12	Matrix Metalloprotease 12
SEA	Surface Epithelial Alteration
SLC26A4	Solute carrier Family 26 Member 4
SPINK7	Serine Peptidase Inhibitor, Kazal Type 7

| **TGFB1** | Transforming Growth Factor Beta −1 |
TNFAIP	Tumor Necrosis Factor Alpha Induced Protein
TSLP	Thymic Stromal Lymphopoietin
TSPAN12	Tetraspanin 12

References

1. Simon D, Cianferoni A, Spergel JM, Aceves S, Holbreich M, Venter C, et al. Eosinophilic esophagitis is characterized by a non-IgE-mediated food hypersensitivity. Allergy 2016; 71:611–20. [PubMed: 26799684]

2. Lucendo AJ, Molina-Infante J, Arias A, von Arnim U, Bredenoord AJ, Bussmann C, et al. Guidelines on eosinophilic esophagitis: evidence-based statements and recommendations for diagnosis and management in children and adults. United European Gastroenterol J 2017; 5:335–58.

3. Dellon ES, Liacouras CA, Molina-Infante J, Furuta GT, Spergel JM, Zevit N, et al. Updated International Consensus Diagnostic Criteria for Eosinophilic Esophagitis: Proceedings of the AGREE Conference. Gastroenterology 2018; 155:1022–33 e10. [PubMed: 30009819]

4. Walsh SV, Antonioli DA, Goldman H, Fox VL, Bousvaros A, Leichtner AM, et al. Allergic esophagitis in children: a clinicopathological entity. Am J Surg Pathol 1999; 23:390–6. [PubMed: 10199468]

5. Assa'ad AH, Gupta SK, Collins MH, Thomson M, Heath AT, Smith DA, et al. An antibody against IL-5 reduces numbers of esophageal intraepithelial eosinophils in children with eosinophilic esophagitis. Gastroenterology 2011; 141:1593–604. [PubMed: 21835135]

6. Blanchard C, Stucke EM, Rodriguez-Jimenez B, Burwinkel K, Collins MH, Ahrens A, et al. A striking local esophageal cytokine expression profile in eosinophilic esophagitis. J Allergy Clin Immunol 2011; 127:208–17, 17 e1-7. [PubMed: 21211656]

7. Kottyan LC, Davis BP, Sherrill JD, Liu K, Rochman M, Kaufman K, et al. Genome-wide association analysis of eosinophilic esophagitis provides insight into the tissue specificity of this allergic disease. Nat Genet 2014; 46:895–900. [PubMed: 25017104]

8. Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, Sousa A, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. N Engl J Med 2009; 360:973–84. [PubMed: 19264686]

9. Hirano I, Collins MH, Assouline-Dayan Y, Evans L, Gupta S, Schoepfer AM, et al. RPC4046, a Monoclonal Antibody Against IL13, Reduces Histologic and Endoscopic Activity in Patients With Eosinophilic Esophagitis. Gastroenterology 2019; 156:592–603 e10. [PubMed: 30395812]

10. Mishra A, Wang M, Pemmaraju VR, Collins MH, Fulkerson PC, Abonia JP, et al. Esophageal remodeling develops as a consequence of tissue specific IL-5-induced eosinophilia. Gastroenterology 2008; 134:204–14. [PubMed: 18166354]

11. Otani IM, Anilkumar AA, Newbury RO, Bhagat M, Beppu LY, Dohil R, et al. Anti-IL-5 therapy reduces mast cell and IL-9 cell numbers in pediatric patients with eosinophilic esophagitis. J Allergy Clin Immunol 2013; 131:1576–82. [PubMed: 23623266]

12. Rochman M, Travers J, Abonia JP, Caldwell JM, Rothenberg ME. Synaptopodin is upregulated by IL-13 in eosinophilic esophagitis and regulates esophageal epithelial cell motility and barrier integrity. JCI Insight 2017; 2.

13. Rothenberg ME, Wen T, Greenberg A, Alpan O, Enav B, Hirano I, et al. Intravenous anti-IL-13 mAb QAX576 for the treatment of eosinophilic esophagitis. J Allergy Clin Immunol 2015; 135:500–7. [PubMed: 25226850]

14. Straumann A, Conus S, Grzonka P, Kita H, Kephart G, Bussmann C, et al. Anti-interleukin-5 antibody treatment (mepolizumab) in active eosinophilic oesophagitis: a randomised, placebo-controlled, double-blind trial. Gut 2010; 59:21–30. [PubMed: 19828470]

15. Zuo L, Fulkerson PC, Finkelman FD, Mingler M, Fischetti CA, Blanchard C, et al. IL-13 induces esophageal remodeling and gene expression by an eosinophil-independent, IL-13R alpha 2-inhibited pathway. J Immunol 2010; 185:660–9. [PubMed: 20543112]

16. Shoda T, Wen T, Aceves SS, Abonia JP, Atkins D, Bonis PA, et al. Eosinophilic oesophagitis endotype classification by molecular, clinical, and histopathological analyses: a cross-sectional study. The Lancet Gastroenterology & Hepatology 2018; 3:477–88. [PubMed: 29730081]

17. Wen T, Stucke EM, Grotjan TM, Kemme KA, Abonia JP, Putnam PE, et al. Molecular diagnosis of eosinophilic esophagitis by gene expression profiling. Gastroenterology 2013; 145:1289–99. [PubMed: 23978633]

18. Johansson MW, Gunderson KA, Kelly EA, Denlinger LC, Jarjour NN, Mosher DF. Anti-IL-5 attenuates activation and surface density of beta(2) -integrins on circulating eosinophils after segmental antigen challenge. Clin Exp Allergy 2013; 43:292–303. [PubMed: 23414537]

19. Fulkerson PC, Schollaert KL, Bouffi C, Rothenberg ME. IL-5 triggers a cooperative cytokine network that promotes eosinophil precursor maturation. J Immunol 2014; 193:4043–52. [PubMed: 25230753]

20. D'Mello RJ, Caldwell JM, Azouz NP, Wen T, Sherrill JD, Hogan SP, et al. LRRC31 is induced by IL-13 and regulates kallikrein expression and barrier function in the esophageal epithelium. Mucosal Immunol 2016; 9:744–56. [PubMed: 26462420]

21. Davis BP, Stucke EM, Khorki ME, Litosh VA, Rymer JK, Rochman M, et al. Eosinophilic esophagitis-linked calpain 14 is an IL-13-induced protease that mediates esophageal epithelial barrier impairment. JCI Insight 2016; 1:e86355. [PubMed: 27158675]

22. Wheeler JC, Vanoni S, Zeng C, Waggoner L, Yang Y, Wu D, et al. 17beta-Estradiol protects the esophageal epithelium from IL-13-induced barrier dysfunction and remodeling. J Allergy Clin Immunol 2019; 143:2131–46. [PubMed: 30578870]

23. Wen T, Aronow BJ, Rochman Y, Rochman M, Kc K, Dexheimer PJ, et al. Single-cell RNA sequencing identifies inflammatory tissue T cells in eosinophilic esophagitis. J Clin Invest 2019; 130:2014–28.

24. Kottyan LC, Rothenberg ME. Genetics of eosinophilic esophagitis. Mucosal Immunol 2017; 10:580–8. [PubMed: 28224995]

25. Straumann A Treatment of eosinophilic esophagitis: diet, drugs, or dilation? Gastroenterology 2012; 142:1409–11. [PubMed: 22542829]

26. Spergel JM, Rothenberg ME, Collins MH, Furuta GT, Markowitz JE, Fuchs G, 3rd, et al. Reslizumab in children and adolescents with eosinophilic esophagitis: results of a double-blind, randomized, placebo-controlled trial. J Allergy Clin Immunol 2012; 129:456–63, 63 e1-3. [PubMed: 22206777]

27. Hirano I, Moy N, Heckman MG, Thomas CS, Gonsalves N, Achem SR. Endoscopic assessment of the oesophageal features of eosinophilic oesophagitis: validation of a novel classification and grading system. Gut 2013; 62:489–95. [PubMed: 22619364]

28. Collins MH, Martin LJ, Alexander ES, Boyd JT, Sheridan R, He H, et al. Newly developed and validated eosinophilic esophagitis histology scoring system and evidence that it outperforms peak eosinophil count for disease diagnosis and monitoring. Dis Esophagus 2017; 30:1–8.

29. Wen T, Rothenberg ME. Clinical Applications of the Eosinophilic Esophagitis Diagnostic Panel. Front Med (Lausanne) 2017; 4:108. [PubMed: 28770203]

30. Dellon ES, Yellore V, Andreatta M, Stover J. A single biopsy is valid for genetic diagnosis of eosinophilic esophagitis regardless of tissue preservation or location in the esophagus. J Gastrointestin Liver Dis 2015; 24:151–7. [PubMed: 26114173]

31. Dellon ES, Veerappan R, Selitsky SR, Parker JS, Higgins LL, Beitia R, et al. A Gene Expression Panel is Accurate for Diagnosis and Monitoring Treatment of Eosinophilic Esophagitis in Adults. Clin Transl Gastroenterol 2017; 8:e74. [PubMed: 28181994]

32. Cheng K, Gupta SK, Kantor S, Kuhl JT, Aceves SS, Bonis PA, et al. Creating a multi-center rare disease consortium - the Consortium of Eosinophilic Gastrointestinal Disease Researchers (CEGIR). Transl Sci Rare Dis 2017; 2:141–55. [PubMed: 29333363]

33. Gupta SK, Falk GW, Aceves SS, Chehade M, Collins MH, Dellon ES, et al. Consortium of Eosinophilic Gastrointestinal Disease Researchers: Advancing the Field of Eosinophilic GI Disorders Through Collaboration. Gastroenterology 2019; 156:838–42. [PubMed: 30452923]

34. Aceves SS, King E, Collins MH, Yang GY, Capocelli KE, Abonia JP, et al. Alignment of parent- and child-reported outcomes and histology in eosinophilic esophagitis across multiple CEGIR sites. J Allergy Clin Immunol 2018; 142:130–8 e1. [PubMed: 29852258]

35. Shoda T, Wen T, Caldwell JM, Collins MH, Besse JA, Osswald GA, et al. Molecular, Endoscopic, Histologic and Circulating Biomarker-Based Diagnosis of Eosinophilic Gastritis: Multi-Site Study. J Allergy Clin Immunol 2019, 145(1): 255–269. [PubMed: 31738990]

36. Wechsler JB, Bolton SM, Amsden K, Wershil BK, Hirano I, Kagalwalla AF. Eosinophilic Esophagitis Reference Score Accurately Identifies Disease Activity and Treatment Effects in Children. Clin Gastroenterol Hepatol 2018; 16:1056–63. [PubMed: 29248734]

37. Wambre E, Bajzik V, DeLong JH, O'Brien K, Nguyen QA, Speake C, et al. A phenotypically and functionally distinct human TH2 cell subpopulation is associated with allergic disorders. Sci Transl Med 2017; 9.

38. Upadhyaya B, Yin Y, Hill BJ, Douek DC, Prussin C. Hierarchical IL-5 expression defines a subpopulation of highly differentiated human Th2 cells. J Immunol 2011; 187:3111–20. [PubMed: 21849680]

39. Dellon ES, Kim HP, Sperry SL, Rybnicek DA, Woosley JT, Shaheen NJ. A phenotypic analysis shows that eosinophilic esophagitis is a progressive fibrostenotic disease. Gastrointest Endosc 2014; 79:577–85 e4. [PubMed: 24275329]

40. Gour N, Wills-Karp M. IL-4 and IL-13 signaling in allergic airway disease. Cytokine 2015; 75:68–78. [PubMed: 26070934]

41. Perkins C, Wills-Karp M, Finkelman FD. IL-4 induces IL-13-independent allergic airway inflammation. J Allergy Clin Immunol 2006; 118:410–9. [PubMed: 16890766]

42. Finkelman FD, Yang M, Perkins C, Schleifer K, Sproles A, Santeliz J, et al. Suppressive effect of IL-4 on IL-13-induced genes in mouse lung. J Immunol 2005; 174:4630–8. [PubMed: 15814686]

43. Rothenberg ME, Spergel JM, Sherrill JD, Annaiah K, Martin LJ, Cianferoni A, et al. Common variants at 5q22 associate with pediatric eosinophilic esophagitis. Nat Genet 2010; 42:289–91. [PubMed: 20208534]

44. Sleiman PM, Wang ML, Cianferoni A, Aceves S, Gonsalves N, Nadeau K, et al. GWAS identifies four novel eosinophilic esophagitis loci. Nat Commun 2014; 5:5593. [PubMed: 25407941]

45. Geslewitz WE, Percopo CM, Rosenberg HF. Eosinophil persistence in vivo and sustained viability ex vivo in response to respiratory challenge with fungal allergens. Clin Exp Allergy 2018; 48:29–38. [PubMed: 29068539]

46. Schoepfer AM, Safroneeva E, Bussmann C, Kuchen T, Portmann S, Simon HU, et al. Delay in diagnosis of eosinophilic esophagitis increases risk for stricture formation in a time-dependent manner. Gastroenterology 2013; 145:1230–6 e1-2. [PubMed: 23954315]

Clinical Implications

In addition to illuminating distinct disease mechanisms, the heterogeneity of *IL5*, *IL13* and *TSLP* expression described herein is likely to impact patient response to biological agents that target these cytokines.
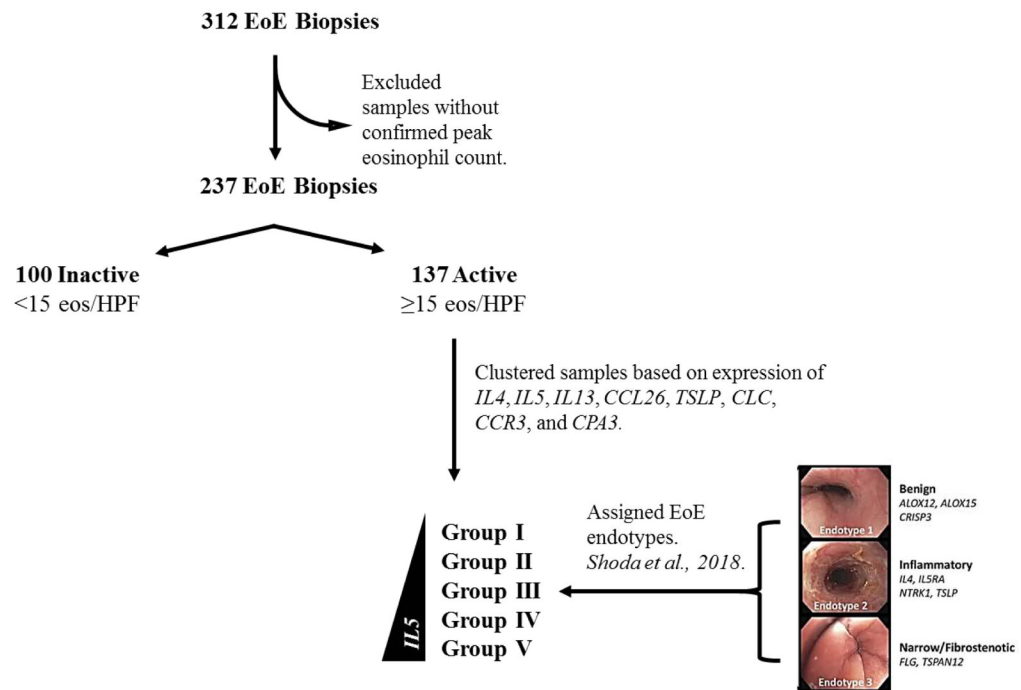
FIG 1:

Workflow diagram depicting sample inclusion and categorization criteria. Biopsies with confirmed active EoE were sorted into 5 groups by K-means clustering using inflammatory genes *IL4*, *IL5*, *IL13*, *CCL26*, *TSLP*, *CLC*, *CCR3*, and *CPA3*. Using a published algorithm[16], patients with active EoE were assigned to one of three EoE endotypes: benign (EoEe1), inflammatory (EoEe2), or fibrostenotic (EoEe3).
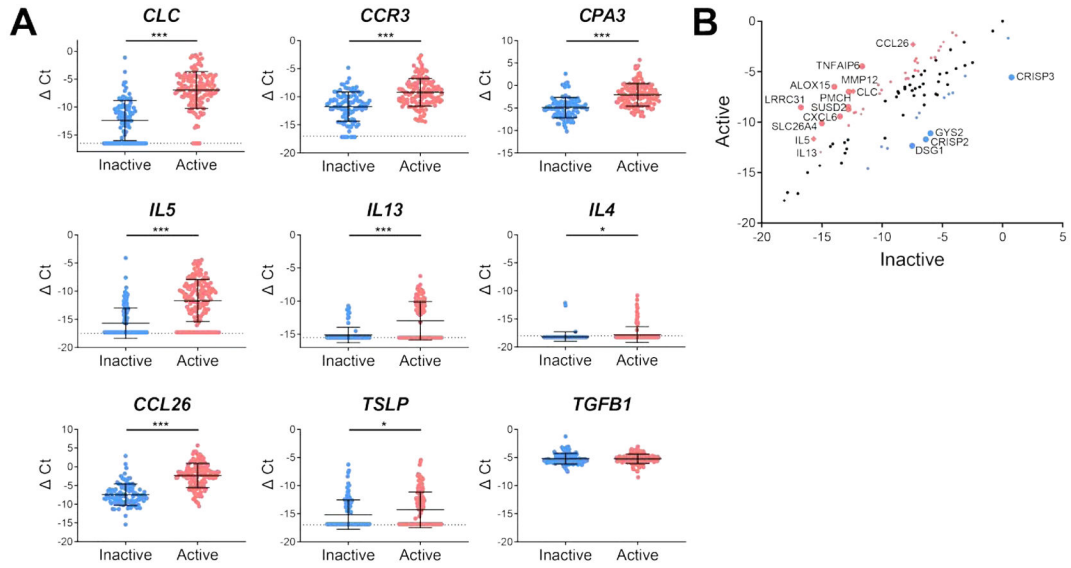
FIG 2:

Th2-associated gene expression in patients with active and inactive EoE (A) Expression of genes associated with eosinophils (*CLC* and *CCR3*) or mast cells (*CPA3*), Th2 activation (*IL5*, *IL13*, *IL4*, and *TSLP*), and eosinophil chemotaxis (*CCL26*) was measured and normalized by subtracting the Ct of *GAPDH* (ΔCt; active EoE n = 137, inactive EoE n = 100). Significance was assessed by Mann-Witney test. Dotted lines indicate limit of detection (B) Expression of genes in the EDP was averaged across active and inactive patients and differentially expressed genes were identified and annotated. Genes with |ΔCt| > 2 are indicated in blue if their expression was higher in inactive patients or in red if their expression was higher in active patients. * *P* < 0.05, *** *P* < 0.001. The data shown includes the cumulative results of all samples performed over 78 Custom TaqMan Gene Expression Array Cards. Experiments each supported the trends shown.
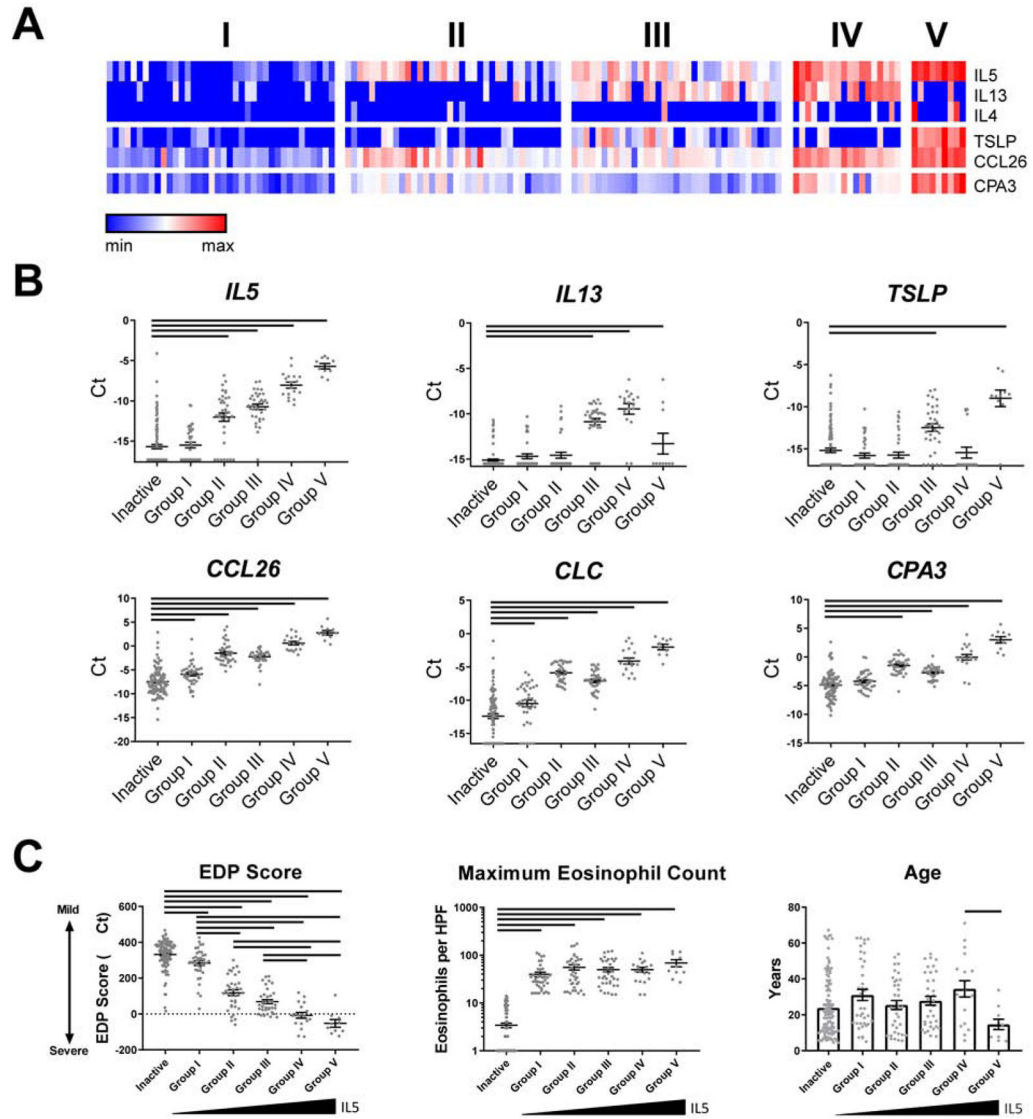
FIG 3:

Expression of Th2 genes in active EoE identifies distinct patient groups. (A) Five subgroups of active EoE were identified by K-means clustering based on expression of *IL4*, *IL5*, *IL13*, *TSLP*, *CCR3*, *CCL26*, *CLC*, and *CPA3*. (B) Normalized expression of Th2 genes was compared in inactive patients and 5 groups of active patients. Bars indicate statistical differences ($P < 0.05$) between inactive patients and specified groups, assessed by one-way ANOVA with Dunnett's post-test. (C) EDP score and patient age were compared between inactive patients and active groups I-V by one-way ANOVA with Tukey post-test. Maximum eosinophil count was compared via Kruskal-Wallis test with Dunn's post-test. Bars indicate $P < 0.05$. The data shown includes the cumulative results of all samples performed over 78 Custom TaqMan Gene Expression Array Cards.
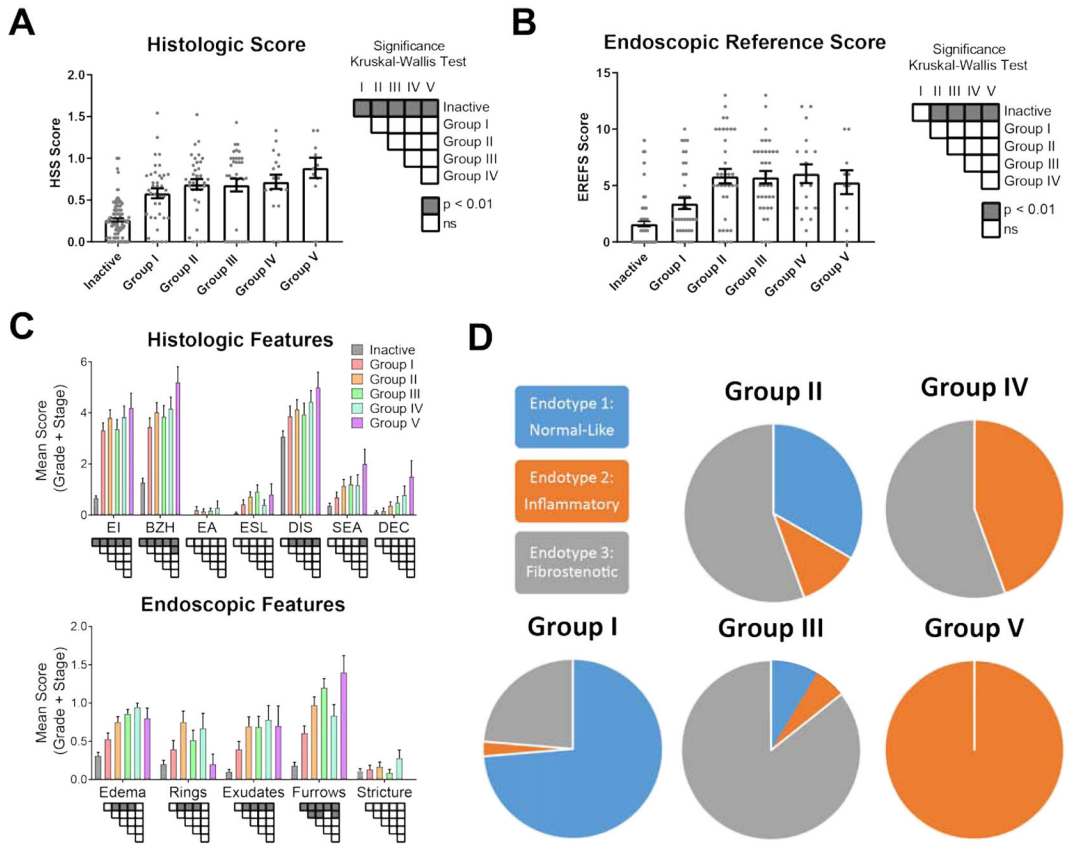
FIG 4:

Inflammatory and fibrostenotic phenotypes among IL5-high and intermediate groups. (A) Histologic and (B) endoscopic scoring of patients with inactive EoE or in Groups I-V. Statistical significance determined by Kruskal-Wallis post-test between individual groups is indicated as shading. (C) Features of the EoE histology scoring system (HSS) and EoE endoscopic reference score (EREFS) were scored for grade (0-3) and stage (0-3) across Groups I-V. Significance between active patient subgroups and inactive patients was calculated with two-way ANOVA and Tukey post-test (shading; $P < 0.01$). (D) EoE endotype assignment in groups I-V. Groups were found to be dependent on endotypes according to Chi Square Exact Test (p < 0.0001). Eosinophilic infiltrates (EI); basal zone hyperplasia (BZH); eosinophilic abscess (EA); eosinophilic surface layering (ESL); dilated intercellular spaces (DIS); surface epithelial alteration (SEA); dyskeratotic epithelial cells (DEC).
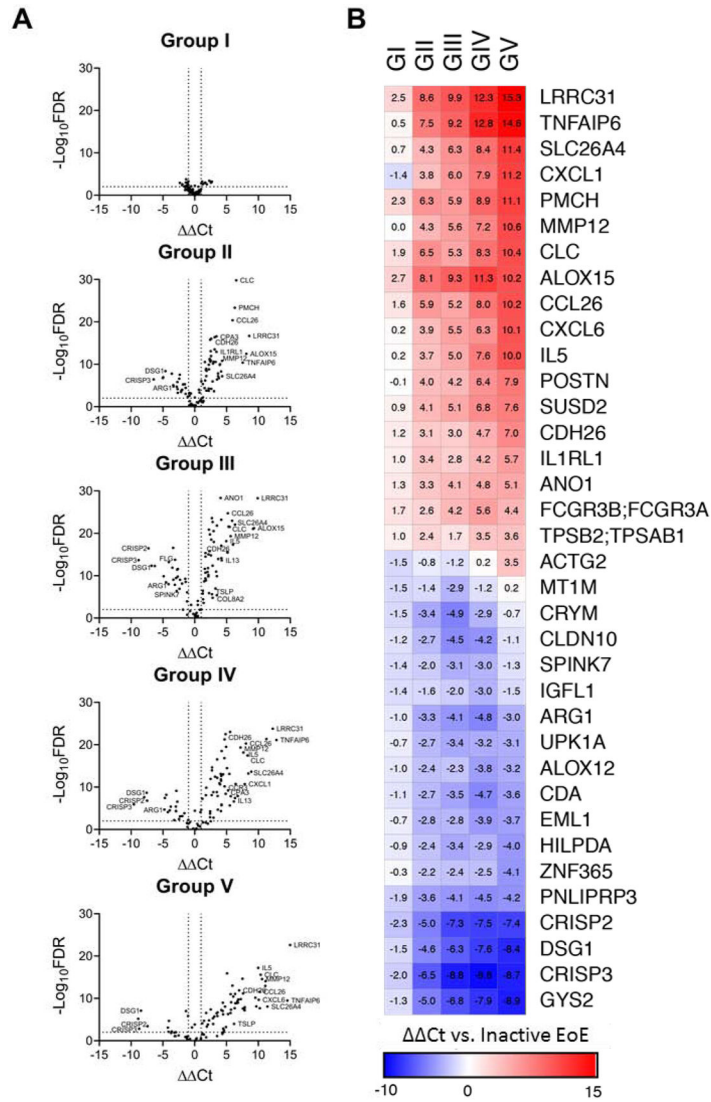
FIG 5:

Differential gene expression in Th2 subgroups. (A) The ΔΔCt values (ΔCt_GROUP − ΔCt_INACTIVE) and accompanying statistical significance (-log10FDR) were calculated for the entire EDP in each group with respect to patients with inactive EoE. Dotted lines indicate significance thresholds at – log10(FDR)>2 and | ΔΔCt| > 1. Genes of particular interest are annotated. (B) ΔΔCt values of the highest up- and downregulated genes in each group with respect to inactive EoE.
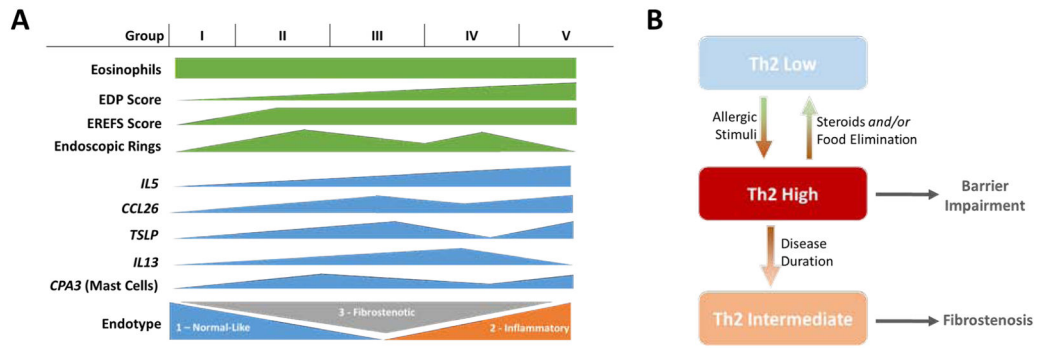
FIG 6:
Summary of study results and proposed disease mechanism. (A) Groups I-V as defined in this study were not statistically different on the basis of eosinophil count but exhibited increased disease severity as assessed by cumulative EDP scoring. Expression of cytokines and chemokines varied among the 5 groups, which also differed in terms of membership in EoE endotypes 1-3. (B) Model depicting patient progression from Th2-low phenotype (congruent with EoEe1) to a Th2-high phenotype (congruent with EoEe2) following allergic or inflammatory insult. Upon steroid treatment, food elimination, or biologic therapy the Th2-gene expression decreases and patients either resolve inflammation by reverting to a Th2-low phenotype or develop a fibrostenotic (EoEe3) signature.

TABLE 1:

Patient Demographics

	Inactive n = 100	Active n = 137	*P* value
Age (years ± SD; range)	23 ± 17 (4-67)	28 ± 17 (5-71)	0.07
Male (%)	64.0%	69.3%	
White (%)	95.0%	92.7%	
EDP Score *	332 ± 83	123 ± 136	< 0.0001
Peak Eosinophil Count (eosinophils/HPF)	3 ± 4	50 ± 34	< 0.0001
Atopic Status			
Allergic Rhinitis (%)	44.0%	38.7%	
Asthma (%)	24.0%	25.5%	
Eczema (%)	26.0%	24.1%	
Food Allergy (%)	42.0%	40.9%	
Ongoing Diet Therapy (%)	50.0%	51.1%	
Current PPI Usage (%)	34.0%	39.4%	
Current Oral Systemic Steroids (%)	0%	0%	
Current Swallowed Topical Steroids (%)	59.0%	47.4%	
History of Steroid Usage (%)	68.0%	71.5%	
Response to Steroid – Symptom (% Effective)	73.5%	63.3%	
Response to Steroid – Pathology (% Effective)	64.7%	51.0%	

*
EDP score calculated as the sum of delta Ct values.