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Serum autoantibodies against epithelial cell adhesion molecules as disease biomarkers of eosinophilic esophagitis

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To the Editor

Eosinophilic esophagitis is an allergic/immune-mediated disease where a Th2 response is triggered by food or environmental allergens.^{1,2} The incidence and prevalence of EoE have been increasing over the past two decades.³ Because endoscopy and biopsy are required for diagnosis and monitoring, health care costs related to EoE are significant. There has been intense research in the identification of minimally or non-invasive diagnostic techniques or biomarkers for this condition, but as of yet no blood, stool, urine, saliva, or breath-based tests are available for clinical use.⁴

When considering candidate biomarkers, two recent observations about EoE are relevant. First, EoE has been associated with a range of autoimmune conditions.⁵ Second, defects in esophageal epithelial barrier structure and function, as well as increased fibrogenesis, are important in EoE pathogenesis. In addition, the pathogenic role of the autoantibodies in autoantibody-mediated autoimmune skin diseases is well established.⁶ Desmosomes and hemidesmosomes are adhesion organelles present in keratinocytes and other squamous epithelial cells, and abnormal alterations of these adhesions result in autoimmune skin diseases, such as pemphigus foliaceus (PF), pemphigus vulgaris (PV), and bullous pemphigoid (BP). These autoimmune skin conditions are associated with elevation in specific serum autoantibodies.⁶ Given that these adhesion molecules are present on gastrointestinal epithelial cells as well, it is possible that EoE is associated with

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autoantibodies derived against these adhesion molecules. However, the role of serum autoantibodies in EoE has not yet been explored.

The aims of this study were to determine whether selected autoantibodies directed at epithelial adhesion molecules were present in the serum of EoE patients in different concentrations than in non-EoE controls, and if present, whether they decreased after successful EoE treatment. Given similarities in the ultrastructure of the skin and the esophageal mucosa, and the immune-mediated nature of EoE, we hypothesized that we would be able to detect these autoantibodies in higher levels in EoE cases than controls, and that these levels would decrease after treatment.

We conducted a secondary analysis of specimens collected during a prospective cohort study at University of North Carolina (UNC) (UNC IRB #15-2851; see Supplemental Materials for full details of methods and analysis). For this study, we assessed epithelial-specific autoantibodies (IgG1 and IgG4) to desmoglein 1 and 3 (DSG1; DSG3) and to collagen XVII (NC16A). These autoantibodies had previously been examined in dermatologic conditions such as pemphigus and bullous pemphigoid,⁷ but had not been studied in EoE.

A total of 24 EoE cases and 24 non-EoE controls were included in this study (Patient characteristics are summarized in Table S1). The investigators who measured the antibody levels were blind to clinical details, and the investigators who classified clinical details were blind to the antibody levels. Compared to controls, EoE cases had similar median levels of anti-DSG1 IgG4, but higher levels of anti-DSG3 IgG4 (0.10, interquartile range [IQR] 0.07–0.13 vs 0.07, 0.06–0.10; $p=0.02$; all levels in optical density units) (Table 1). Anti-NC16A IgG4 was also significantly higher in cases (0.34, IQR 0.15–0.72 vs 0.10, 0.08–0.14; $p<0.001$) (Figure 1A). In the EoE cases, the Spearman correlation coefficient between the esophageal peak eosinophil count at baseline and the anti-NC16A IgG4 was 0.54 ($p<0.001$). There were few differences in anti-DSG1, -DSG3, or -NC16A IgG1, though anti-DSG1 IgG1 was lower in cases than in controls (Table 1). Given the prominent difference in anti-NC16A IgG4 levels between cases and controls (which also persisted after individually controlling for age, race, and presence of esophageal stricture), we performed ROC analysis on this autoantibody in isolation, and the AUC was 0.82 for predicting EoE case status. Using all three IgG4 markers (anti-DSG1, -DSG3, and -NC16A), the AUC was 0.86.

In the EoE cases with pre- and post-treatment samples available ($n=18$), we focused on NC16A IgG4 autoantibodies. There was no difference in baseline levels between responders and non-responders (Table 1). There was a trend ($p=0.09$) for lower levels in responders compared with non-responders after treatment. However, within the responder group, anti-NC16A IgG4 median levels were significantly lower after successful treatment compared with baseline (0.1, IQR 0.07–0.14 vs 0.10, 0.07–0.13; $p=0.04$).

Given the costs, burden to patients, and potential risks of repeated endoscopic examinations, there is a pressing need for less invasive techniques for diagnosis and monitoring EoE disease activity. In particular, identifying peripheral blood biomarkers would be a major breakthrough in the field. There is an emerging association between EoE and autoimmune diseases,⁵ suggesting that production of autoantibodies may be a common phenomenon in

both patient populations. Since there are similar structural properties between the skin and the esophageal mucosa, we evaluated a panel of serum-based epithelial autoantibodies in EoE cases and non-EoE controls. We also made an assessment before and after treatment in EoE cases. Our results show, for the first time, that autoantibodies are not only associated with EoE but might track with histologic response to treatment. In particular, anti-NC16A IgG4 distinguished EoE cases from controls, suggesting a high degree of diagnostic utility, and also was significantly lower in histologic responders to treatment, suggesting a possible role for monitoring of disease.

There has been extensive effort to identify biomarkers for EoE.⁴ While a number of potential markers have been described in the esophagus, use of tissue-based markers does not obviate the need for an invasive test such as endoscopy, or a less invasive test for obtaining a tissue sample. Preliminary work has been done in the saliva and stool, and a potential urinary biomarker has been reported, but these remain in early development for clinical use. Results of blood biomarkers have been largely disappointing,⁸ and while there are preliminary data with eosinophil granule proteins, the vast majority of cytokines and other inflammatory mediators do not appear to be amenable to detection in the blood in EoE for either diagnosis or monitoring. It is therefore somewhat difficult to contextualize our results, as this is the first study examining a novel serum-based autoimmune antibody, with the exception of data presented in abstract form showing no elevations in anti-DSG1 or 3 in a small sample of EoE patients. While we observed mild differences between cases and controls with anti-DSG3 IgG4, and it has previously been noted that desmoglein is markedly decreased in active EoE and is involved with a compromised epithelial barrier, NC16A has not been extensively explored in this condition. NC16A is a stretch of the ectodomain of collagen XVII (also known as BP180) and comprises almost the entire antigenic target of the autoantibodies in bullous pemphigoid (see Supplemental Materials). The detection of an IgG4 to a collagen protein raises the question of this autoantibody in the pathogenesis of EoE, and this should be a future area of investigation. This is particularly notable given that the IgG4 class of antibodies has been recently recognized to be important in EoE.^{9, 10}

It is important to note potential limitations of our study. These include a relatively small number of patients from a single referral center with post-treatment samples not available from all cases, which could lead to selection bias. However, the clinical characteristics of the EoE cases are reflective of a typical EoE population, and in a sensitivity analysis there were no clinical differences between the EoE cases with and without post-treatment samples. Additionally, even with the included sample of patients we were able to detect significant differences between the groups. Another issue related to the sample size is that we cannot stratify our population to examine autoantibody levels in different EoE phenotypes, particularly fibrostenotic vs inflammatory, to fully examine for all potential confounders, to characterize severity or activity of concomitant atopic diseases, or to control for baseline autoantibody levels, and these are future directions for research. With the case-control study design, we are not able to demonstrate causality. This is less important for a serum biomarker of diagnosis, as the strength of the association is of paramount importance. The fact that one of the biomarkers, anti-NC16A IgG4 autoantibodies, decreases after treatment, however, is suggestive that these autoantibodies may be involved in EoE pathogenesis, but this is also a future direction for investigation.

There are also a number of strengths to this study. The samples were collected with standard protocols during a rigorously conducted prospective study. The study design allowed comparison of cases to controls at baseline, in order to assess diagnostic utility, as well as follow-up of cases, in order to assess disease monitoring. Lab techniques and autoantibody measurements were also conducted in blinded fashion to minimize potential bias. Because of these strengths, we feel that the data and results are valid.

In conclusion, this analysis of samples from a prospective cohort of EoE cases and controls, showed for the first time that anti-NC16A and -DSG3 IgG4 autoantibodies were strongly associated with EoE. Anti-NC16A IgG4 not only had a high diagnostic utility for EoE, but levels decreased significantly in EoE cases with a histologic response after topical corticosteroid treatment. Given these findings, further evaluation of the diagnostic and monitoring utility of anti-NC16A IgG4 in EoE is warranted, as is investigation of the role of this autoantibody in EoE pathogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(A) Anti-NC16A IgG4 levels in non-EoE controls compared to EoE cases. Medians for each group are indicated by the horizontal bar and are compared with Wilcoxon rank-sum. (B) Anti-NC16A IgG4 levels in EoE before and after treatment for responders (peak eosinophil count < 15 eos/hpf) and non-responders. Medians for each group are indicated by the horizontal bar and are compared with Wilcoxon rank-sum

Table 1

Autoantibody levels in in EoE cases and non-EoE controls, and in EoE cases after treatment

Baseline antibody level comparison			
	Controls (n = 24)	EoE cases (n = 24)	p[*]
IgG4 median levels (IQR)			
DSG1	0.13 (0.09–0.16)	0.17 (0.08–0.22)	0.14
DSG3	0.07 (0.06–0.10)	0.10 (0.07–0.13)	0.02
NC16A	0.10 (0.08–0.14)	0.34 (0.15–0.72)	< 0.001
IgG1 median levels (IQR)			
DSG1	0.23 (0.14–1.1)	0.16 (0.12–0.23)	0.03
DSG3	0.12 (0.10–0.17)	0.12 (0.10–0.16)	0.74
NC16A	0.17 (0.13–0.21)	0.16 (0.15–0.24)	0.98
Anti-NC16A IgG4 levels in EoE patients before and after treatment			
	Non-responders^{**} (n = 8)	Responders^{**} (n = 10)	p[†]
Pre-treatment anti-NC16A IgG4 (median; IQR)	0.14 (0.12–0.25)	0.125 (0.07–0.14)	0.21
Post-treatment anti NC16A IgG4 (median; IQR)	0.15 (0.12–0.21)	0.10 (0.07–0.13)	0.09
p [‡]	0.48	0.04	

* Medians are compared with Wilcoxon rank-sum; values are reported in optical density units

** Responders had < 15 eos/hpf post treatment; non-responders had ≥ 15 eos/hpf post-treatment

† This p value compares medians between non-responders and responders before or after treatment with the Wilcoxon rank-sum test.

‡ This p value compares medians within either non-responders or responders before and after treatment with the Wilcoxon signed-rank test