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Immunophenotyping of Peripheral Eosinophils Demonstrates Activation in Eosinophilic Esophagitis

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Abstract

Background and Aim—Eosinophilic esophagitis (EoE) is a chronic inflammatory disorder characterized by upper gastrointestinal symptoms and the presence of high numbers of eosinophils in the esophagus. Although eosinophils in the esophagus have been found to be activated in subjects with EoE, detailed studies of intracellular signaling pathways involved in the mechanism of activation of eosinophils in EoE have heretofore been limited. The aim of the study was to assess whether any surface molecules or transcription factors are activated in peripheral eosinophils in subjects with EoE.

Methods—Eosinophils and CD3+ lymphocytes were identified directly from 50 μ L of whole blood of EoE and control subjects. Using Hi-FACS, levels of surface activation markers, including CD66b, and intracellular phosphoepitopes, including phosphorylated forms of signal transducer and activator of transcription (phospho-STAT) 1 and 6, were measured within each cell subset.

Results—Levels of surface CD66b as well as levels of intracellular phospho-STAT1 and phospho-STAT6 in peripheral blood eosinophils were significantly higher for untreated subjects with EoE vs healthy controls ($P < 0.05$). Levels of phospho-STAT1 and phospho-STAT6 in peripheral blood eosinophils were lower in subjects with EoE on therapy versus untreated subjects with EoE ($P < 0.05$).

Conclusions—Levels of phospho-STAT1 and phospho-STAT6, transcription factors involved in inflammatory processes, were both significantly higher in peripheral eosinophils from untreated (ie, newly diagnosed) subjects with EoE versus subjects with EoE on therapy, healthy controls. Blood-based measurements of CD66b and phospho-STAT levels in peripheral eosinophils may be beneficial for identifying EoE.

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Keywords

activation; atopy; phosphoepitope; signal transducer; activator of transcription

Eosinophilic esophagitis (EoE) is an inflammatory disorder characterized by an infiltration of high numbers of eosinophils into the esophagus, which is not resolved by antireflux therapy (1–5). Although the incidence of EoE is increasing (6), precise mechanisms of this disease remain largely unknown, and EoE appears to be associated with allergy (2,7). Esophagogastroduodenoscopy and histological examination of esophageal biopsy tissues are required for the diagnosis of EoE, and repeated procedures are often used for the assessment of response to therapy (8).

Eosinophilic activation has been implicated in the disease process of EoE. When eosinophils are activated, they secrete interleukin (IL) -5 and other mediators. This activation is thought to be mediated by FcεRI complexing with IgE as well as other mechanisms (1–5). Recently, researchers studying murine esophageal tissues reported IL-13-induced activation of eosinophils through the signal transducer and activator of transcription 6 (STAT6) pathway (9); however, little is known about what transcription factors mediate the downstream activation of eosinophils in humans after receptor/ligand binding. In addition, in a disease such as EoE, in which pathology is localized to the esophagus, little is known about whether eosinophils are initially activated in the periphery and whether this peripheral activation is involved in the migration and full activation of eosinophils in the local tissue of the esophagus. Previous work on subjects with allergic asthma has shown that blood eosinophils are activated following an ex vivo stimulation or challenge with an antigen, whereas they are not activated at baseline in the periphery (10,11).

In the present study, our objective was to assess whether any surface molecules or transcription factors were activated (ie, phosphorylated) in peripheral eosinophils in subjects with EoE. Although many studies on EoE have characterized local changes within esophageal tissues, our study is novel in that we are investigating surface markers and intracellular phosphoepitopes in peripheral eosinophils from whole blood. We hypothesized that early changes in peripheral eosinophils in EoE could be detected by analysis of surface activation markers (ie, CD66b) and intracellular transcriptional activation (12,13).

Previous work from our group on asthmatic subjects has shown that profiling levels of phosphorylated STAT (phospho-STAT) proteins in purified immune subsets can reflect ongoing immune polarization (14). Therefore, we focused on studying phospho-STAT proteins in peripheral eosinophils from subjects with EoE. In particular, because EoE appears to have a strong immunologic component, we looked at STAT1 and STAT6. STAT1 is involved in IL-5 signaling in eosinophils (15–17). IL-5 is a critical cytokine for eosinophil growth and function, and mouse models of EoE have demonstrated the importance of IL-5 for eosinophil trafficking to the esophagus (2,18–20). STAT6 mediates IL-4 and IL-13 signaling, which plays a role in IgE production (21,22), and patients with EoE tend to have high rates of IgE-mediated food and aeroallergen hypersensitivity (2,23). In certain cell types, STAT6 also mediates the expression of eotaxin, a major chemoattractant for eosinophils in EoE (24–26). Previous work from our group on subjects

with allergic asthma and/or food allergy has shown that CD66b, phospho-STAT1, and phospho-STAT6 levels were similar between allergic asthmatic subjects and controls (Supplemental Figure 1, <http://links.lww.com/MPG/A46>). We also have shown that STAT1 and STAT6 were not increased in peripheral blood cells from subjects with allergic rhinitis compared to control subjects (27).

Our data demonstrate that of the phosphoepitopes we tested, levels of both phospho-STAT1 and phospho-STAT6 are significantly higher in peripheral eosinophils of untreated subjects with EoE compared to subjects with EoE on therapy and healthy controls (HCs). Furthermore, levels of surface CD66b are significantly higher on peripheral eosinophils of untreated subjects with EoE.

METHODS

The supplementary materials contain detailed steps of the methods and procedures that were used.

Human Subjects

The study was approved by the Stanford Administrative Panel on Human Subjects in Medical Research. Patients who were to be evaluated by endoscopy were asked to participate in the study. All of the subjects signed informed consent forms. Blood and proximal, middle (in some cases), and distal esophageal tissues were obtained from subjects during endoscopy and biopsy. Subjects who had a negative pH probe result and whose histological sections contained >15 eosinophils per high-power field (hpf) were considered to have EoE as per standard and accepted guidelines (17,19). All subjects with EoE had failed a trial of proton pump inhibitor therapy before being diagnosed as having EoE. Treated subjects with EoE were grouped as “EoE subjects on therapy” and were confirmed with esophageal biopsies showing improvement of eosinophilia (fewer than 5–10 eosinophils/hpf) and demonstrated resolution of clinical symptoms at the time of biopsy. Subjects with eosinophilic gastritis, eosinophilic colitis, acute or chronic infections, autoimmune disease, and/or neoplasm were excluded from the study. All of the subjects with EoE in our study demonstrated either food allergies or allergic rhinitis. Six subjects with allergic asthma and/or food allergy but without EoE were included in the study as controls (allergic controls [ACs], and their characteristics are detailed in Supplemental Table 1, <http://links.lww.com/MPG/A46>). Subjects with HC were age matched and defined as those individuals who lacked any allergy and acute or chronic inflammatory disease of the gastrointestinal tract as defined by pathological biopsy analysis (Stanford Clinical Pathology, Stanford, CA) and by clinical history and laboratory tests.

Transcript expression analysis was performed on newly diagnosed subjects with EoE (n =35), subjects with EoE on therapy (n =7), and HCs (n =35). Of the 35 newly diagnosed subjects with EoE, surface activation marker and phosphoepitope studies were performed on 9 subjects, chosen at random based on the availability of fresh blood sampling at the time of endoscopy, along with the same subjects with EoE on therapy on which transcript analysis was performed (n =7) and HCs (n = 10) (Table 1).

Collection of Samples—Four milliliters of blood was obtained in a Vacutainer tube (containing 7.2 mg EDTA [ethylenediamine tetraacetic acid]; Becton Dickinson, Franklin Lakes, NJ) during diagnostic and follow-up blood draws. To limit artifactual activation of blood leukocytes, samples were placed on ice immediately upon collection; all of the subsequent staining steps were done at 4°C. Blood was first centrifuged (10 minutes, 400g) to remove plasma and resuspended to its original volume with phosphate-buffered saline (PBS) containing EDTA (2.5 mmol/L). Peripheral blood mononuclear cells (PBMCs) were removed and washed twice with PBS at 1800 rpm for 5 minutes. PBMCs were then resuspended as 1 million cells per milliliter in a solution of 90% fetal bovine serum and 10% dimethylsulfoxide.

Real-time Quantitative PCR (QT-PCR) RNA Analysis—RNA was isolated using a RNeasy Mini Kit (Qiagen, Valencia, CA), according to the manufacturer's instructions. For cDNA synthesis, 500 ng of total RNA was transcribed with cDNA transcription reagents (Applied Biosystems, Foster City, CA) using random hexamers, according to the manufacturer's instructions. Gene expression was measured in real time with the GeneAmp 7900 Sequence Detection System (Applied Biosystems) using primers and other reagents purchased from Applied Biosystems. Relative quantification was measured using the comparative CT (threshold cycle) method. The expression level of a gene in a given sample was represented as 2^{-CT} , where $CT = [CT_{(experimental)}] - [CT_{(medium)}]$ and $CT = [CT_{(experimental)}] - [CT_{(housekeeping)}]$ (28).

A total of 100 ng of total isolated RNA was submitted to reverse transcription (Invitrogen, Carlsbad, CA). Fifty nanograms of resulting cDNA was submitted to TaqMan PCR on an ABI Systems qPCR machine (Applied Biosystems) at the Stanford QT-PCR facility using gene-specific, fluorochrome-labeled probe/primer sets purchased from Applied Biosystems. Transcripts were normalized to the β -2-microglobulin gene expression. All of the PCR assays were performed in triplicate methods (28). Slopes between -3.3 and -3.5 were obtained for primer efficiency.

Surface Marker and Intracellular Phosphoepitope Profiling—Subsets of eosinophils, neutrophils, and CD3+ lymphocytes were identified from 50 μ L of whole blood without purification or stimulation, using previously described whole-blood staining methods (29). In brief, cells were stained with a live/dead probe (Invitrogen) and antibodies against surface determinants to identify eosinophils, including anti-CD3 (Invitrogen), anti-CD16, and anti-CD66b (BD Biosciences, San Jose, CA). Cells were then fixed with Lyse/Fix PhosFlow buffer (BD Biosciences). For intracellular phosphoepitope profiling, fixed cells were permeabilized with a methanol-based Perm Buffer III (BD Biosciences) at 4°C for 30 minutes in darkness. After washing cells twice in excess PBS containing EDTA, cells were stained with antibodies against the phosphoepitopes of interest, including anti-phospho-STAT1 and anti-phospho-STAT6 (BD Biosciences) at 4°C for 20 minutes in darkness. Cells were washed again with PBS containing EDTA and centrifuged (5 minutes, 490g).

Data Acquisition

Data for 100,000 cells per sample were acquired on a LSRII digital flow cytometer equipped with 4 lasers (535 nm, 488 nm, 633 nm, 405 nm), 2 light scatter detectors (yielding forward and side scatter data), and 18 fluorescent detectors (BD Biosciences), made available through the Stanford Shared FACS facility. To standardize signal output by the flow cytometer, before each session we ran a thorough calibration procedure using a standard set of multicolor fluorescence beads.

Statistical Analysis

Statistical analysis was performed with JMP8 software (SAS Institute, Cary, NC). The data distribution was assessed for normality using the Shapiro-Wilk test. Nonparametric tests were used because the data were not normally distributed. Between-group comparisons were made using the Wilcoxon rank-sum test. Correlation statistics were made using the nonparametric Spearman test. Differences were considered significant at $P \leq 0.05$.

RESULTS

Expression of STAT1, STAT6, and CD66b in Esophageal Tissues

First, QT-PCR studies were performed on local esophageal tissues and on PBMCs from untreated subjects with EoE ($n = 35$), subjects with EoE on therapy ($n = 7$), and HCs ($n = 35$). RNA purified from local esophageal biopsies at the same time blood samples were collected for each subject was used for the real-time PCR assays. In RNA derived from both esophageal tissues and PBMCs, transcript levels of STAT1, STAT6, and CD66b were significantly higher for the untreated EoE group compared with both the EoE on therapy and HC groups (Fig. 1). Interestingly, tissue expression of STAT1, STAT6, and CD66b were higher in esophageal tissues compared to that of PBMCs in subjects with EoE.

Surface CD66b Levels on Peripheral Eosinophils

As we found higher levels of CD66b transcripts in esophageal tissues of untreated subjects with EoE, we investigated whether this surface marker would also be elevated on peripheral eosinophils of untreated subjects with EoE compared with subjects with EoE on therapy and HCs by performing protein level expression analysis via flow cytometry. We measured the median fluorescence intensity (MFI) of CD66b expression for live eosinophils from whole blood (gated as live/dead-negative, CD3-negative, CD16-negative) (Fig. 2). Surface CD66b levels were significantly higher on peripheral eosinophils from untreated subjects with EoE ($n = 9$) compared with those of HCs ($n = 8$) (Fig. 3). No significant differences were observed between untreated subjects with EoE and subjects with EoE on therapy. As all subjects with EoE in our study demonstrated food allergy and/or allergic rhinitis, we also measured the level of surface CD66b on peripheral eosinophils from subjects with AC without EoE. No significant differences in the level of CD66b were observed between subjects with allergic asthma and/or food allergy without EoE ($n = 6$) and HCs ($n = 6$) (Supplemental Figure 1, <http://links.lww.com/MPG/A46>).

Phospho-STAT1 and Phospho-STAT6 Levels in Peripheral Eosinophils

To further assess the possible activation state of peripheral eosinophils in EoE, we also measured levels of intracellular phosphoepitopes in peripheral eosinophils of untreated subjects with EoE, subjects with EoE on therapy, and HCs. Based on our QT-PCR results, we chose to look at levels of phospho-STAT1 and phospho-STAT6 in peripheral eosinophils. MFIs of phospho-STATs were measured in live eosinophils from whole blood. We found that phospho-STAT1 and phospho-STAT6 levels were significantly higher for untreated subjects with EoE (n = 9) compared to subjects with EoE on therapy (n = 6) and HCs (n = 7) (Fig. 4A and B). In contrast, eosinophilic phospho-STAT1 and phospho-STAT6 levels were not elevated in subjects with AC without EoE (n = 6) compared to HCs (n = 6) (Supplemental Figure 2, <http://links.lww.com/MPG/A46>).

We were able to obtain fresh blood sampling for 2 subjects before and after they were started on therapy for EoE. In particular, subject 1 showed an approximate 11-fold decrease in eosinophilic phospho-STAT1 level after 5 weeks on an elemental diet (MFI of 149 before therapy vs 13 after therapy); subject 3 showed an approximate 9-fold decrease in eosinophilic phospho-STAT1 level after 2 months of swallowed budesonide (MFI of 360 vs 41) (Table 2). Subject 1 also showed an approximate 12-fold decrease in eosinophilic phospho-STAT6 level after therapy (MFI of 236 vs 20); subject 3 showed an approximate 14-fold decrease in eosinophilic phospho-STAT6 level after therapy (MFI 632 vs 45) (Table 2).

Phospho-STAT1 and Phospho-STAT6 levels in Other Cellular Subsets

For comparison, we tested whether phospho-STAT1 and phospho-STAT6 levels were elevated in peripheral T cells as well as eosinophils by performing simultaneous phosphoepitope analysis in T cells from whole blood. We found that intracellular phospho-STAT1 and phospho-STAT6 levels were also significantly higher in peripheral CD3 + T lymphocytes from untreated subjects with EoE (n = 9) compared with HCs (n = 7) (Figs. 2, 4C and 4D). Phospho-STAT1 and phospho-STAT6 in peripheral CD3 + T lymphocytes were similar between subjects with AC without EoE and HCs (data not shown). Blood neutrophils from subjects with EoE were found to have a significantly higher phospho-STAT6 level compared to HCs. Unlike with eosinophils, however, no significant differences were observed between phospho-STAT1 and phospho-STAT6 levels in peripheral neutrophils from untreated subjects with EoE versus subjects with EoE on therapy or between subjects with EoE on therapy versus HCs (Supplementary Figure 4, <http://links.lww.com/MPG/A46>).

Correlations

We tested whether cell-based measurements of phospho-STAT1 and phospho-STAT6 levels in peripheral eosinophils would be correlated with each other and with the number of eosinophils per high-power field in esophageal tissues, a key disease parameter of EoE. We found that among subjects with EoE, eosinophilic phospho-STAT1 levels correlated positively with phospho-STAT6 levels ($R = 0.87$). No correlations were observed between levels of phospho-STAT1 and phospho-STAT6 in peripheral eosinophils and the number of eosinophils per high-power field in esophageal tissues.

DISCUSSION

EoE is an inflammatory gastrointestinal disorder characterized by high numbers of eosinophils infiltrating into the esophagus (1–5). Although EoE has been shown to have a strong immunological component, precise mechanisms of this disease remain largely unknown. Eosinophilic activation has been implicated in the disease process of EoE (1–5), although little is known about whether eosinophils are initially activated in the periphery.

Our objective was to assess whether any transcription factors were activated (ie, phosphorylated) in peripheral eosinophils in subjects with EoE. We measured levels of activation markers, including surface molecules and intracellular transcription factors. Because CD66b was recently shown to regulate adhesion and activation of human eosinophils (12), we began by looking at levels of CD66b transcripts in esophageal tissues of subjects with EoE using QT-PCR. Because EoE seems to be associated with allergy, we also looked at levels of intracellular transcription factors STAT1 and STAT6 (2,15,18–22,24). We found that levels of CD66b, STAT1, and STAT6 transcripts were significantly higher in esophageal tissues of untreated subjects with EoE compared with subjects with EoE on therapy and HCs. These findings suggest that CD66b, STAT1, and STAT6 in esophageal tissues may play an important role in the immune pathology of EoE. Our finding is consistent with the role of STAT6 variants in allergy (30) and STAT6 differential gene expression reported recently in EoE (31). At this time, however, we are unable to directly assign the expression of phosphorylated STAT1 or STAT6 to eosinophils in the esophagus. The ability to detect phosphorylated STAT1 and STAT6 in esophageal tissues by immunohistochemistry was hindered by the paraffin-embedding process we used for pathological specimen processing; however, with the use of frozen tissue sections, it may be possible to detect co-staining of eosinophils and phosphorylated STAT1 and/or STAT6.

Based on our findings in esophageal tissues, we next surveyed protein expression in peripheral eosinophils of untreated subjects with EoE, subjects with EoE on therapy, and HCs. We showed that levels of surface CD66b were significantly higher on peripheral eosinophils of untreated subjects with EoE compared to HCs. Because our subjects with EoE also had other atopic conditions (eg, food allergy), we compared atopic subjects who did not have EoE versus HCs. We did not find significant differences in CD66b expression between these atopic controls versus HCs, which suggested that our observations on peripheral eosinophils from subjects with EoE were not the result of atopy alone. Additionally, we performed flow cytometry studies on a limited number of samples from subjects with gastroesophageal reflux disease (GERD). There appeared to be a trend of higher levels of CD66b on blood eosinophils of untreated subjects with EoE compared to subjects with GERD, although this result was not statistically significant (Supplementary Figure 5, <http://links.lww.com/MPG/A46>).

Yoon et al showed that CD66b is an activation marker for human granulocytes that is highly expressed on the surface of peripheral eosinophils, and that engagement of CD66b by monoclonal antibody or a natural ligand induces cellular adhesion, superoxide production, and degranulation of eosinophils (12). These authors concluded that CD66b molecules are involved in regulating adhesion and activation of eosinophils, possibly through their

localization in lipid rafts and interactions with other cell surface adhesion molecules, and that interactions between CD66b and its ligands may be important in the release of eosinophilic proinflammatory mediators (12). Our finding that CD66b is higher on peripheral eosinophils of untreated subjects with EoE suggests that peripheral eosinophils may be in an activated state in EoE. Although further research needs to be conducted, it is possible that interactions between surface CD66b molecules and endogenous ligands may play a role in the migration and full activation of eosinophils in the esophagus.

We also assessed levels of phosphorylated STAT1 and STAT6 in peripheral eosinophils of subjects with EoE and found that both phospho-STAT1 and phospho-STAT6 were significantly higher in peripheral eosinophils of untreated subjects with EoE compared to subjects with EoE on therapy and HCs. Previous work from our group has shown that intracellular phospho-STAT6 was increased in a purified immune subset (CD4+ CD161+ T cells, memory and effector T cells) in allergic asthmatic subjects, whereas phospho-STAT1 was increased in the same subset of cells in non-allergic asthmatic subjects (14). Neilsen et al have shown that in murine esophageal tissues, IL-13-induced activation of eosinophils is mediated through the STAT6 signaling pathway (9). Accordingly, our finding that intracellular phospho-STAT1 and phospho-STAT6 levels are significantly higher in peripheral eosinophils of untreated subjects with EoE compared with controls further suggests that peripheral eosinophils may be in an activated state in EoE. Conversely, phospho-STAT1 and phospho-STAT6 levels were similar in peripheral eosinophils of subjects with AC without EoE versus HCs. Importantly, in the group of subjects with GERD, phospho-STAT1 levels in eosinophils (but not phospho-STAT6 levels) were significantly higher compared to HCs (Supplementary Figure 6, <http://links.lww.com/MPG/A46>).

Our examination of peripheral CD3+ T cells, identified from the same blood samples from which eosinophils were identified, shows significantly higher levels of both phosphorylated STAT1 and STAT6 in untreated subjects with EoE compared with subjects with EoE on therapy and HCs. This is not seen in subjects with AC without EoE (data not shown). Recent studies on EoE provide evidence that this disease is a mixed-type immunological disorder involving both T_H1 and T_H2 responses (32–34). The family of STAT proteins mediates signaling by T_H1 and T_H2 cytokines and has been implicated in the development of inflammation and allergic diseases (15). STAT1 signaling involves the T_H1 cytokine IFN- γ , whereas STAT6 signaling involves the T_H2 cytokine IL-4 (21,35). Even though all of the subjects with EoE in the present study were diagnosed as having atopy (either allergic rhinitis and/or food allergies known to be associated with T_H2 activation), our finding of higher levels of both phosphorylated STAT1 and STAT6 in peripheral eosinophils and CD3+ T cells of untreated subjects with EoE further supports the understanding of EoE as a mixed-type immunological disorder. It is possible that activation of STAT1 and STAT6 signaling pathways in peripheral eosinophils and/or CD3+ T cells may play a role in T_H1 and T_H2 immune activation in EoE.

In summary, within the esophagus, expression of STAT1, STAT6, and CD66b were increased in untreated subjects with EoE compared with subjects with EoE on therapy and HCs. In the peripheral blood, eosinophils from untreated subjects with EoE exhibited higher

surface levels of CD66b compared with HCs. Furthermore, the surface levels of eosinophil CD66b was similar between the HCs and the ACs, which suggests that our findings are not characteristic of generalized atopic disease. Moreover, subjects with EoE have both phospho-STAT1 and phospho-STAT6 upregulated in blood eosinophils compared to subjects with EoE on therapy and HCs, which is different from what we observed in subjects with GERD (only phospho-STAT1 was higher in eosinophils) and what we published in allergic subjects (only phospho-STAT6 was higher in CD4+CD161+ T cells (14)). Therefore, the finding that both phospho-STAT1 and phospho-STAT6 levels are significantly higher in blood eosinophils appears to be unique to subjects with untreated EoE compared to subjects from other groups (EoE on therapy, GERD, HCs, and atopic controls). We conclude that together, phospho-STAT1 and phospho-STAT6 represent important pathophysiological insights into the molecular mechanisms of EoE. These results suggest that peripheral blood eosinophils from subjects with EoE may be in a preactivated state.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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FIGURE 1.

Real-time quantitative PCR transcript expression analysis, performed on purified PBMCs (A) and esophageal tissues (B). Expression of CD66b, STAT1, and STAT6 is significantly higher for untreated subjects with EoE vs subjects with EoE on therapy and HCs. Statistical significance was determined by Wilcoxon rank-sum nonparametric test. $P < 0.05$ was considered significant. Histograms represent the mean; bars represent the standard deviation. EoE =eosinophilic esophagitis; HC =healthy control; PBMC =peripheral blood mononuclear cell.

FIGURE 2.

Identification of eosinophils and CD3+ lymphocytes from whole blood. Viable cells are identified from whole blood using a fixable live/dead probe (A). Of the viable cells, lymphocytes are identified as being CD3+ (B), and eosinophils are identified as being CD16-negative, CD66b-positive (C). Example histograms of MFIs of CD66b, phospho-STAT1 (Ph-STAT1), and phospho-STAT6 (Ph-STAT6) are shown for eosinophil and CD3+ lymphocyte subsets (D,E). (F) Example of the value for phospho-STAT6. FMO =fluorescence minus one; MFIs = median fluorescence intensities.

FIGURE 3.

Levels of surface activation marker CD66b on peripheral eosinophils. Levels of CD66b on peripheral eosinophils are significantly higher for untreated EoE subjects vs HCs. No significant differences are observed for untreated EoE subjects vs EoE subjects on therapy. Each point represents a single sample. EoE = eosinophilic esophagitis; HC =healthy control; NS =not significant.

FIGURE 4.

Levels of intracellular phospho-STATs in peripheral eosinophils and CD3+ lymphocytes. Intracellular phospho-STAT1 and phospho-STAT6 levels are significantly higher in peripheral eosinophils of untreated subjects with EoE vs subjects with EoE on therapy and HCs (A, B). Phospho-STAT1 and phospho-STAT6 levels are also significantly higher in peripheral CD3+ lymphocytes of untreated subjects with EoE vs HCs (C,D). Each point represents a single sample. EoE =eosinophilic esophagitis; HC = healthy control; NS =not significant.

TABLE 1

Clinical characteristics of study subjects undergoing phosphopeptide analysis

Group	No. subjects	Sex/age, y	GI symptoms	Prescribed therapy for EoE	Appearance of esophagus	No. eosinophils/hpf in esophagus	% eosinophils in blood	Food allergies	Other allergic disorders
Untreated EoE subjects	1*	F/3	Moderate epigastric pain	None	Furrowing	D = 16, P = 20	10.5	Eggs, milk, nuts	Allergic rhinitis
	2	M/4	Abdominal pain	None	Furrowing, white plaques	D = 25, P = 25	17.1	None	Allergic rhinitis
	3*	F/6	Vomiting, abdominal pain	None	Furrowing, narrowing	D = 35, P = 50	30.9	Eggs, nuts	Asthma
	4	M/8	Severe dysphagia, food impaction	None	Mild furrowing, white plaques	D = 12, P = 20	23.6	Eggs, legumes milk, nuts, soy	Asthma
	5	F/9	Vomiting	None	—	—	7.5	Nuts	Allergic rhinitis
	6	M/11	Food impaction	None	Slightly friable	D = 25, P = 25	20.8	Milk, nuts, potatoes, wheat	Allergic conjunctivitis, allergic rhinitis
	7	M/13	Vomiting	None	Mild furrowing	D = 16, P = 0	2.75	Beef, milk	None
	8	M/15	Moderate dysphagia	None	Furrowing, trachealization	D = 30, P = 30	11.3	Tomatoes	None
EoE subjects on therapy	9	M/6	Vomiting	None	Furrowing	—	18.2	Nuts	None
	1*	F/3	Mild abdominal pain	Elemental diet	Normal	D < 5, P < 5	1	Eggs, milk, nuts	Allergic rhinitis
	3*	F/6	None	Swallowed budesonide	Normal			Eggs, nuts	Asthma
	10	M/9	Asymptomatic	Swallowed budesonide	White plaques, friable tissue	D = 20, P = 20	19.1	Eggs, milk, pork, soy	Allergic rhinitis
	11	M/11	Asymptomatic	Anti-IL-5 therapy	White plaques	None	2.08	Eggs, nuts	Environmental allergies
	12	M/18	Asymptomatic	Swallowed budesonide	Furrowing, white plaques	D = 10, P = 50	15.5	Peanut, peas, potatoes	Environmental allergies
	13	M/19	Asymptomatic	Swallowed fluticasone	Furrowing, abscesses, trachealization	D > 20, P > 20	18.2	Eggs, milk	Allergic rhinitis
	14	M/3	Asymptomatic	Elemental diet		D > 20, P > 20		Milk	None

EoE = eosinophilic esophagitis; GI = gastrointestinal; IL = interleukin.

* Subjects 1 and 3 were included in the study at 2 time points, before any treatment and under ongoing therapy.

TABLE 2

Summary of cell-based phospho-STAT measurements

No. subjects	Eosinophil phospho-STAT1	Eosinophil phospho-STAT6
1	149	236
2	79	189
3	360	632
4	378	292
5	—	129
6	491	857
7	121	247
8	175	236
9	287	432
1 *	13	20
3 *	41	45
10	88	93
11	—	221
12	78	156
13	81	109
14	179	164

* Subjects 1 and 3 were included in the study at 2 time points, before any treatment and under ongoing therapy. Phospho-, phosphorylated; STAT, signal transducer and activator of transcription; —, data not available. All units are median fluorescence intensities (MFI).