

SUPPLEMENTARY MATERIAL

Additional information regarding antibodies against surface and intracellular markers that are specific to this study and full methodology are given below.

SUPPLEMENTARY METHODS

Human subjects:

The study was performed according to Declaration of Helsinki guidelines. Proximal, middle (in some cases) and distal esophageal tissue and blood were obtained from subjects during a diagnostic endoscopy and subsequent biopsy. Biopsy samples were preserved as formalin-fixed and paraffin-embedded sections. Histological diagnosis of EoE was made by clinical pathology experts in gastroenterology at Stanford University School of Medicine by calculating the mean number of eosinophils per high powered field (HPF) in more than 3 fields per biopsy sample. Subjects who had no pathological basis for symptoms and who had a negative pH probe were considered healthy controls (HCs). Treatment regimens were customized to the patients' needs and compliance; they included elemental diet, elimination diet (to foods that were positive by skin and IgE Immunocap [Phadia, Sweden] testing), anti IL-5 monoclonal antibody therapy (Ception Therapeutics, Malvern, PA.) and/or local steroid therapy (swallowed fluticasone or swallowed budesonide) as per guidelines (1–3). No subject was taking systemic steroids at the time of sample collection. Allergic subjects were defined as having a total serum IgE of >25 IU/ml and positive skin prick testing as compared with positive histamine control.

Characteristics of the 6 subjects with allergic asthma and/or food allergy (atopic control subjects [AC]) are detailed in the supplementary table I.

Surface marker profiling:

Blood was first centrifuged (10 minutes, 3000G) to remove the plasma and resuspended to its original volume with phosphate buffered saline (PBS) containing EDTA (0.5%). For surface staining of eosinophils from whole blood, 50 μ l of blood were stained with the live/dead near infrared viability probe (Invitrogen, Carlsbad, CA) and several antibodies against surface determinants for 20 minutes at 4°C in darkness. These antibodies included anti-CD3 (Invitrogen, Carlsbad, CA; clone: UCHT1), anti-CD14 (Invitrogen; clone: Tük4), and anti-CD16 (BD Biosciences, San Jose, CA; clone: 3G8). After staining, cells were washed with excess PBS containing EDTA (0.5%) and fetal calf serum (FCS, 5%), centrifuged (5 minutes, 490G) and the supernatant was removed. Upon resuspension in 100 μ l of PBS containing EDTA, cells were fixed with 2 ml of 1X Lyse/Fix Phosflow (BD Biosciences) for 30 minutes at 4°C in the dark and then centrifuged (5 minutes, 490G).

Intracellular phosphoepitope profiling:

For each sample, 50 μ l of blood were stained with the live/dead near infrared viability probe and with antibodies that are resistant to methanol-based permeabilization (anti-CD3, -CD14, and -CD16) for surface staining to identify granulocyte and CD3+ lymphocyte subsets. Cells were then washed with an excess of PBS containing EDTA (0.5%) and FCS (5%), centrifuged (5 minutes, 490G), and resuspended. Subsequently, cells were fixed with 2 ml of 1X Lyse/Fix Phosflow Buffer (BD Biosciences), centrifuged (5 minutes, 490G), and permeabilized with 200 μ l of Perm Buffer III (BD Biosciences) for 30 minutes at 4°C in darkness. Cells were then washed twice with an excess of PBS containing EDTA and centrifuged after each wash (5 minutes, 490G). After permeabilization, cells were stained for the phosphoepitopes of interest for 20 minutes at 4°C in darkness. The antibodies used included: anti-phospho-STAT1 (BD Biosciences; pY701; clone: 4a), -phospho-STAT4 (BD Biosciences; pY693; clone: 38/p-Stat4), -phospho-STAT6 (BD Biosciences; pY641; clone: 18). After staining, cells were washed with an excess of PBS containing EDTA and FCS and centrifuged (5 min, 490G).

Six subjects with allergic asthma and/or food allergy without EoE were included in this study as controls. Intracellular phosphoepitope profiling in immune cells from these subjects was performed based on previously described methods (4).

Data acquisition:

Acquisition was controlled using the DiVa software (BD Biosciences). Compensation was done using single-stained beads or cells. Data was exported to Flowjo software (Treestar, Ashland, OR) and compensated by using single-stained beads or cells. Median fluorescence intensities were calculated and compared with the appropriate controls.

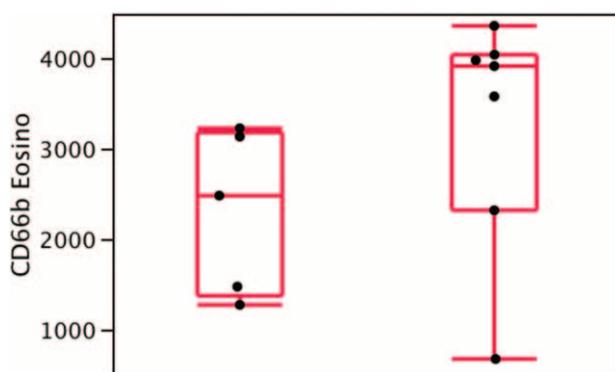
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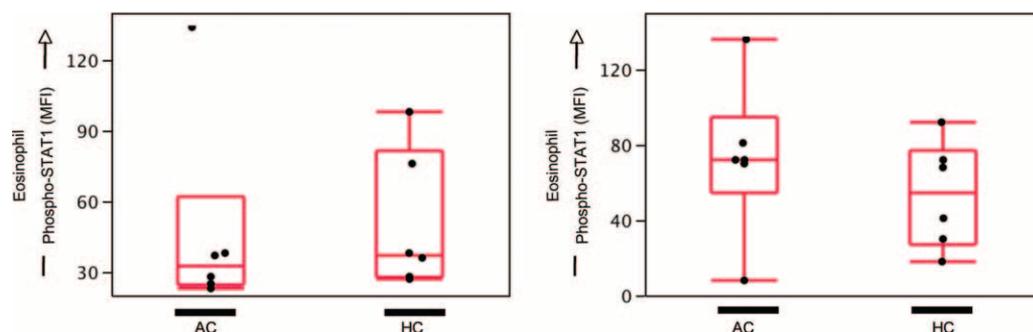
Supplementary table I: Characteristics of the 6 subjects with atopy (AC).

Subjects	Gender	Age (years)	Atopic Disorder
1	Female	56	Allergic asthma
2	Female	21	Allergic asthma
3	Male	32	Allergic asthma
4	Male	23	Allergic rhinitis
5	Female	31	Food allergy
6	Male	39	Allergic asthma Food allergy

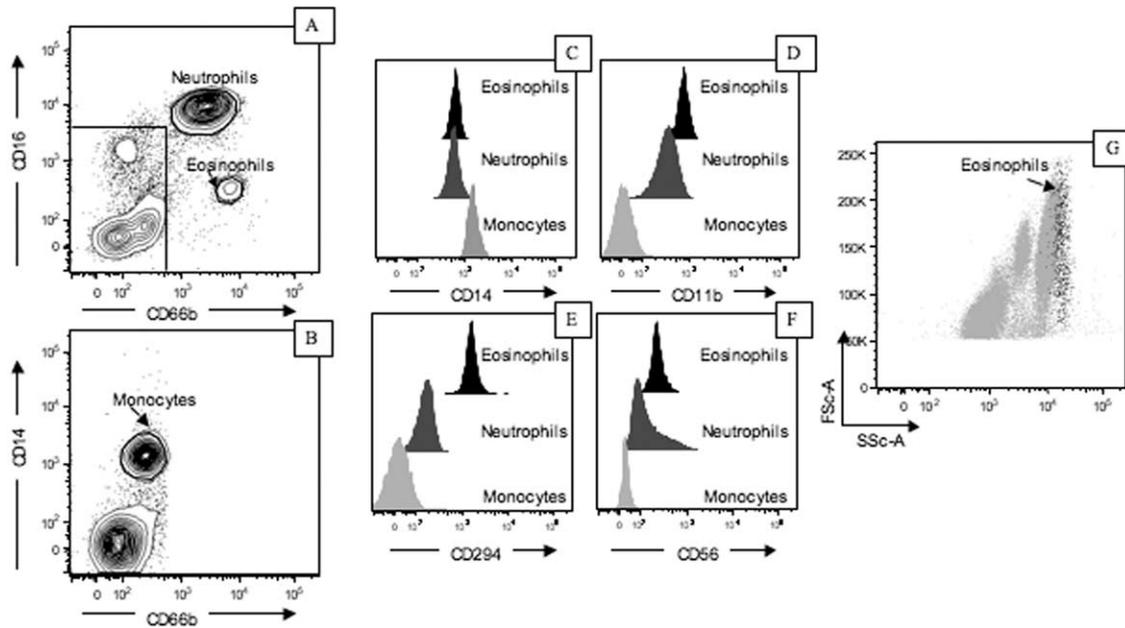
Supplementary Figure 1. Levels of surface activation marker CD66b in peripheral eosinophils from subjects with atopy and healthy controls. Surface CD66b expression on peripheral eosinophils from atopic control (AC) subjects (with allergic asthma and/or food allergy without EoE) was measured to test whether or not CD66b would also be elevated in subjects with atopic conditions other than EoE. Levels of CD66b on peripheral eosinophils were similar between AC vs. HC. Each point represents a single sample. NS: not significant.



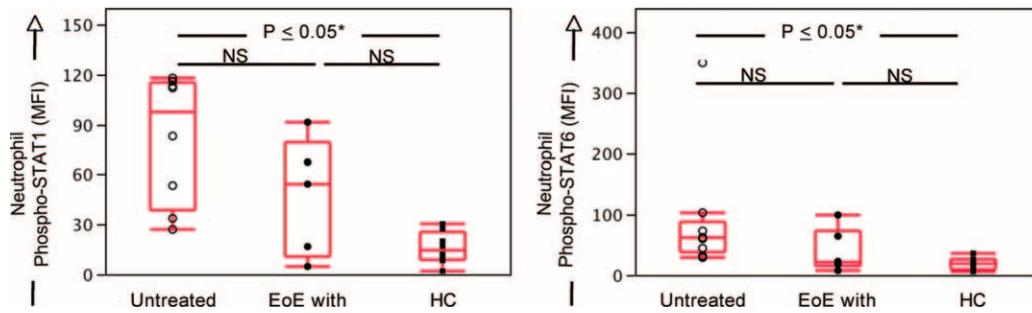
Supplementary Figure 2. Levels of intracellular phosphorylated STAT1 and STAT6 in peripheral eosinophils from subjects with atopy and healthy controls. Intracellular phospho-STAT1 and phospho-STAT6 expression in peripheral eosinophils from atopic control (AC) subjects (with allergic asthma and/or food allergy without EoE) was measured to test whether or not these phospho-STATs would also be elevated in subjects with atopic conditions other than EoE. Levels of phospho-STAT1 and phospho-STAT6 were similar between AC vs. HC. Each point represents a single sample. NS: not significant.



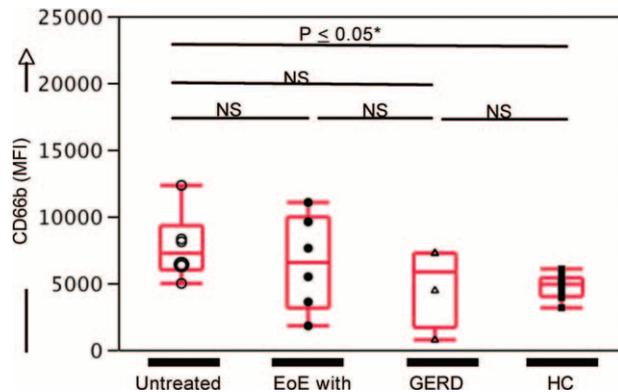
Supplementary Figure 3. Identification of eosinophils, neutrophils and CD14+ monocytes from whole blood. Eosinophils are identified as being CD16-negative, CD66b-positive (A). Monocytes are identified as CD14+ (B) Example histograms of median fluorescence intensities (MFIs) of CD14 (C), CD11b (D), CD294 (E) and CD56 (F) for neutrophils, eosinophils, and monocytes. Eosinophils are identified as negative for CD14, CD56 and positive for CD294 and CD11b. (G) Backgating of the eosinophils to the whole leukocytes population.



Supplementary Figure 4. Levels of intracellular phosphorylated STAT1 and STAT6 in peripheral neutrophils from untreated EoE, EoE with therapy and HC. Blood neutrophils from subjects with EoE were found to have a significant increase of phospho-STAT6 level in subjects with EoE compared to HC.



Supplementary Figure 5. Levels of surface activation marker CD66b in peripheral eosinophils from subjects with GERD compared to the same cohort of untreated EoE, EoE with therapy and healthy controls. Levels of CD66b on peripheral eosinophils were similar between GERD subjects vs. HC. Each point represents a single sample. NS: not significant.



Supplementary Figure 6. Levels of intracellular phosphorylated STAT1 and STAT6 in peripheral eosinophils from subjects with GERD compared to the same cohort of untreated EoE, EoE with therapy and HC. Intracellular phospho-STAT6 level in peripheral eosinophils from GERD subjects was significantly higher to HC. No difference was observed for intracellular phospho-STAT1 levels in peripheral eosinophils of GERD subjects compared to HC. Each point represents a single sample. NS: not significant.

