

Mouse resident lung eosinophils are dependent on IL-5

To the Editor,

Eosinophils were traditionally considered terminally differentiated cells with little to no heterogeneity¹. Recent studies demonstrated that they can acquire unique phenotypes in response to diverse stimuli¹⁻³, and two subsets of eosinophils [i.e. resident (rEos) and inflammatory (iEos)] were described in the lungs of allergen-challenged mice⁴. rEos and iEos can be differentiated by the expression of Siglec-F, CD101 and CD62L, their functional activities and their dependency on IL-5⁴. In particular, survival of lung rEos, which were associated with immunoregulatory activities was described as IL-5-independent⁴. This distinction may have major therapeutic implications since neutralization of IL-5 via anti-IL-5 therapy may deplete iEos and keep rEos intact⁵. Conversely, depletion of eosinophils using antibody-dependent cell cytotoxicity via targeting anti-IL-5R α (e.g. benralizumab) may deplete both populations. Yet, experience in humans has not revealed adverse consequences nor distinct residual eosinophil populations following anti-IL-5 therapeutics¹. Herein, we demonstrate that rEos require IL-5 for their survival and that the expression of Siglec-F, is regulated by IL-5 drawing caution to previous conclusions⁴.

Wild type (WT) mice were challenged with house dust mite and the presence of lung eosinophils was determined (see methods online). At baseline, one population of lung rEos (Siglec-F^{int}/CD101⁻) were identified (Figure 1A-B). We could not identify the expression of CD62L, a suggested marker of resident lung eosinophils⁴ in any eosinophil population (not shown). On day 10, two eosinophil populations, which appeared to correspond with the previously identified rEos and iEos (e.g. Siglec-F^{int} and Siglec-F^{hi}) were observed (Figure 1A-B). However, by day 21 most lung

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eosinophils consisted only of Siglec-F^{hi} cells and expressed CD101 (Figure 1A-D). This suggested that an environmental factor, such as IL-5 regulates Siglec-F expression. Certainly, IL-5 increased the expression of Siglec-F in a concentration-dependent fashion on the surface of peritoneal eosinophils obtained from WT mice (Figure 1E). Similarly, eosinophils from the bone marrow, blood, spleen and lungs of *IL5^{Tg}* mice displayed elevated levels of Siglec-F expression (Figure 1F-I). Administration of anti-IL-5 neutralizing antibodies to naïve and day 10 allergen-challenged mice, which have Siglec-F^{int} and Siglec-F^{hi} eosinophil populations (Figure S1) decreased the expression of Siglec-F the peripheral blood and lung eosinophils from naïve mice (Figure 1J-M) as well as in blood, lungs and bronchoalveolar lavage fluid eosinophils from allergen-challenged mice (Figure 1N-S). Conversely, administration of IL-5 to the peritoneal cavity of WT mice, increased the expression of Siglec-F in peritoneal eosinophils (Figure 1T-U). These data suggest that Siglec-F^{hi} eosinophils in the lungs of allergen-challenged mice (Figure 1A) may comprise a mixture of rEos and iEos.

IL-5 neutralization in naïve mice markedly decreased the percentage and total numbers of resident lung and peripheral blood eosinophils (Figure 2A-B). Furthermore, IL-5 neutralization decreased the levels of blood, lung and BALF eosinophils in allergen-challenged mice (Figure 2C-D). Transcriptional profiling of lung rEos, allergen-challenged Siglec-F^{int}/CD101⁻ and Siglec-F^{hi}/CD101⁺ eosinophils revealed that lung rEos clustered with allergen-challenged Siglec-F^{int}/CD101⁻ cells whereas allergen-challenged Siglec-F^{hi}/CD101⁺ cells were distinct. Horizontal clustering demonstrated three main clusters that were unique for each population (Figure 2E, Tables S1 online). rEos were enriched with pathways that were associated with cell defense and innate immunity whereas allergen-challenged Siglec-F^{int}/CD101⁻

eosinophils were enriched with metabolic pathways suggesting an active cellular state⁶ (Figure 2F-H). Venn plot analysis revealed that allergen-challenged SiglecF^{hi}/CD101⁺ eosinophils uniquely upregulate 234 and 565 transcripts in comparison with rEos and allergen-challenged SiglecF^{int} cells, respectively (Figure 2I, Tables S2-3 online). Allergen-challenged SiglecF^{int}/CD101⁻ eosinophils uniquely upregulated 77 transcripts in comparison to resident eosinophils (Figure 2I, Table S4 online). The study bears the limitation that it was conducted in mice, which display additional IL-5R⁺ cells (e.g. neutrophils) and mouse eosinophils differ from human eosinophils. Nonetheless, these data show that anti-IL-5 treatment downregulates Siglec-F and depletes all lung populations of eosinophils. This is inconsistent with rEos being independent of IL-5 and further suggests that distinct eosinophil populations in the asthmatic lung represent a continuum of activation states rather than different cellular subsets.

Keywords

Eosinophils, Siglec-F, Asthma, Inflammation,

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Avishay Dolitzky¹

Sharon Grisaru-Tal¹

Shmuel Avlas¹

Hazut Inbal¹

Gordon Yaara¹

Michal Itan¹

Ariel Munitz¹

¹Department of Clinical Microbiology and Immunology, Faculty of Medicine, Tel Aviv University

Correspondence:

Ariel Munitz, PhD, Department of Clinical Microbiology and Immunology, The Sackler School of Medicine, Tel Aviv University, Ramat Aviv 69978, Israel. Tel. (Office): +972-3-640-7636, Fax: +972-3-640-9160, e-mail: arielm@tauex.tau.ac.il; ORCID: <https://orcid.org/0000-0003-1626-3019>;

Supplementary Table Legends

Table S1: Expression of transcripts comprising the three distinct clusters of lung eosinophils

Table S2: Fold-change of transcripts uniquely upregulated in Siglec-F^{hi}/CD101⁺ eosinophils in comparison with resident eosinophils

Table S3: Fold-change of transcripts uniquely upregulated in Siglec-F^{hi}/CD101⁺ eosinophils in comparison with Siglec-F^{hi}/CD101⁻ eosinophils

Table S4: Fold-change of transcripts uniquely upregulated in Siglec-F^{int} eosinophils in comparison with resident eosinophils

Figure legends

Figure 1. IL-5 regulates the expression of Siglec-F

Siglec-F expression in lung eosinophils ($CD45^+ CD11b^+/Siglec-F^{int}/CD125^{int}$, $CD45^+ CD11b^+/Siglec-F^{hi}/CD125^{int}$ cells) at baseline and following allergen challenge (A-B). Representative histograms and quantitation of CD101 expression is shown (C-D). Siglec-F expression in peritoneal eosinophils following IL-5 stimulation (E) and in bone marrow (F), blood (G), spleen (H) and lungs (I) of eosinophils from $Il5^{Tg}$ mice. Siglec-F expression in eosinophils from the blood (J-K, N-O), lungs (L-M, P-Q) and bronchoalveolar lavage fluid (BALF) (R-S) of naïve and allergen-challenged mice following IL-5 neutralization. Expression of Siglec-F on peritoneal eosinophils following intraperitoneal injections of IL-5 (T, U). Data are representative of $n=3$ experiments, each dot in the bar graphs represents one mouse, $**p<0.01$; $***p<0.001$.

Figure 2. Resident lung eosinophils are dependent on IL-5 and are distinct from allergen-challenge-induced Siglec-F^{int}/CD101⁻ and Siglec-F^{hi}/CD101⁺ cells

Percent (A, C) and total (B, D) blood, lung and bronchoalveolar lavage fluid (BALF) eosinophils under baseline conditions (A-B) and following allergen-challenge (C-D) is shown. Heat-map representation of differently expressed transcripts of sorted naïve lung eosinophils, and Siglec-F^{int}/CD101⁻ and Siglec-F^{hi}/CD101⁺ eosinophils following allergen challenge (E). Venn-plot analysis of upregulated transcripts in each eosinophil population (F). Gene ontology analysis of enriched pathways in the differentially expressed clusters (G-I). Each dot in the bar graphs represents one mouse, $**p<0.05$, $**p<0.01$; $***p<0.001$, RNAseq data was obtained from 2-3 samples/group consisting 3-4 pooled mice; Adjusted p value <0.05 , 2 $<$ fold change <-2 .

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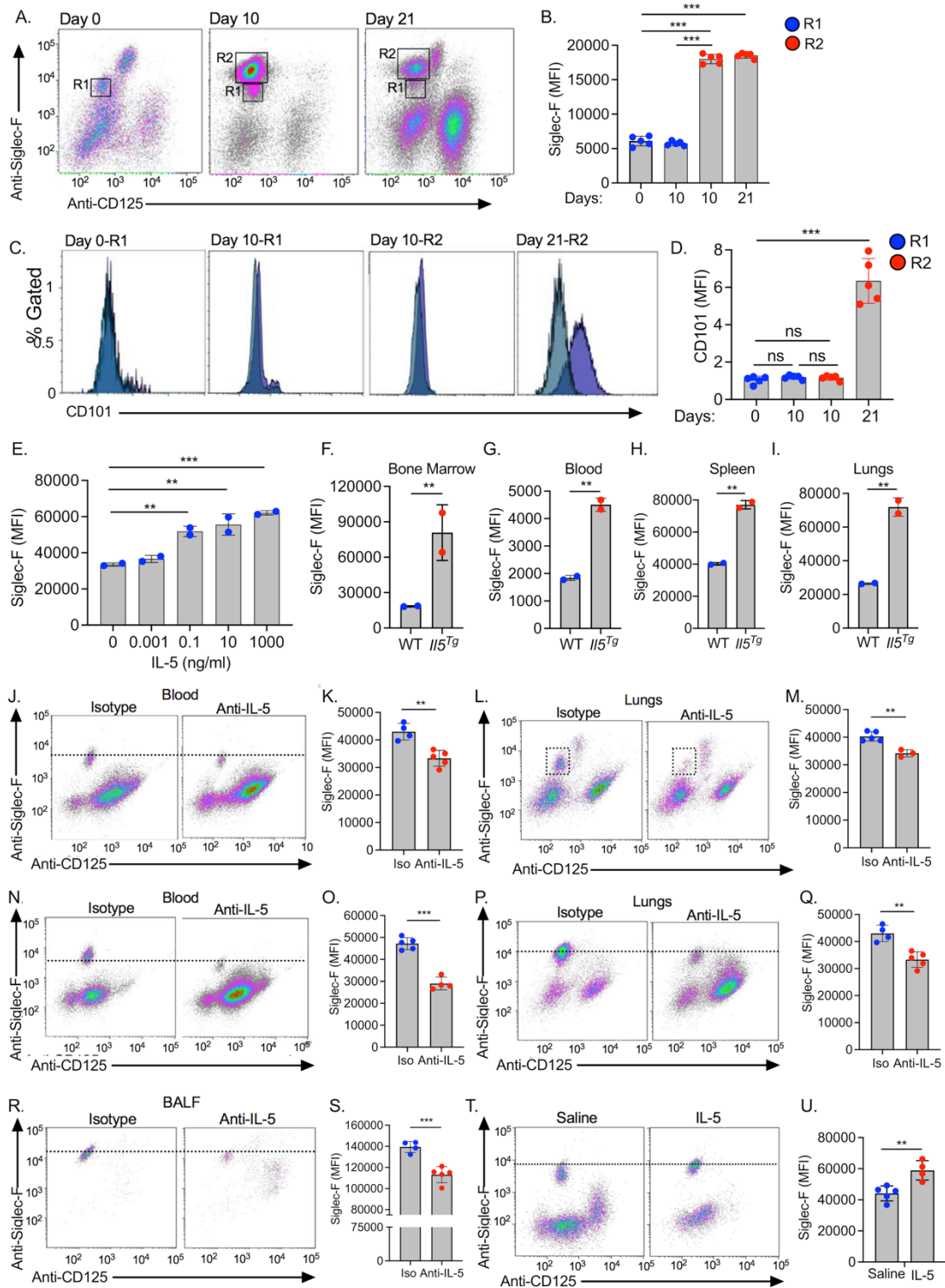


Figure 1_Dolitzky et al.

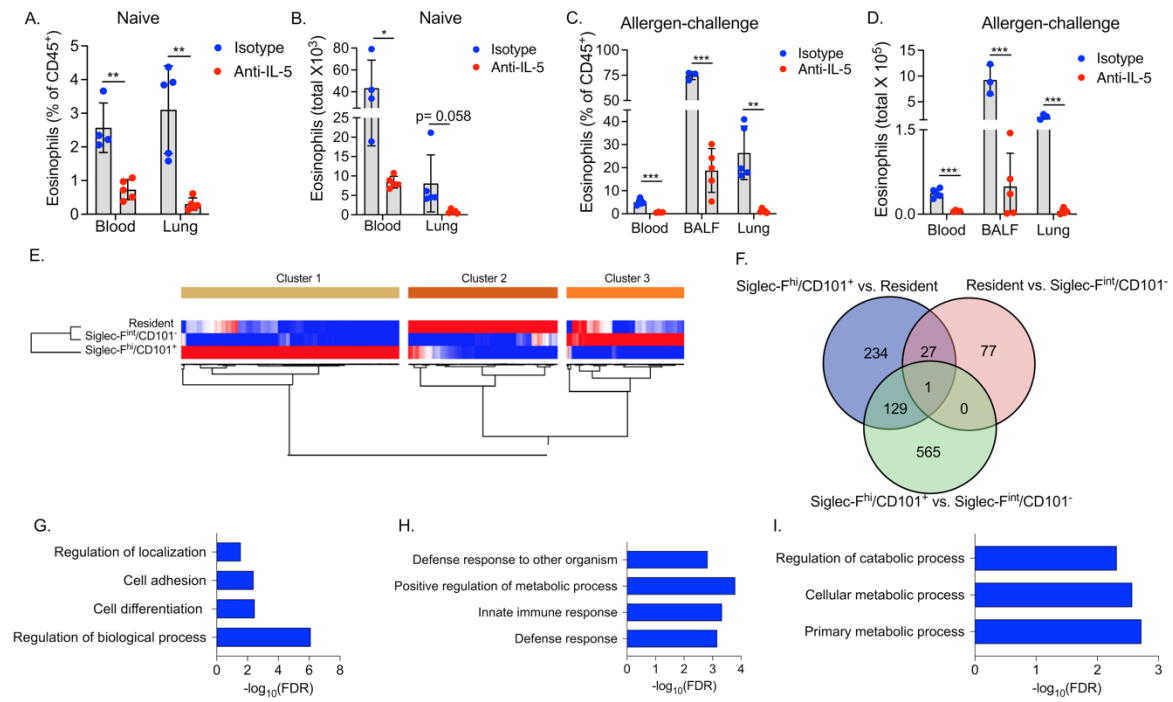


Figure 2_Dolitzky et al.