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Mouse resident lung eosinophils are dependent on IL-5

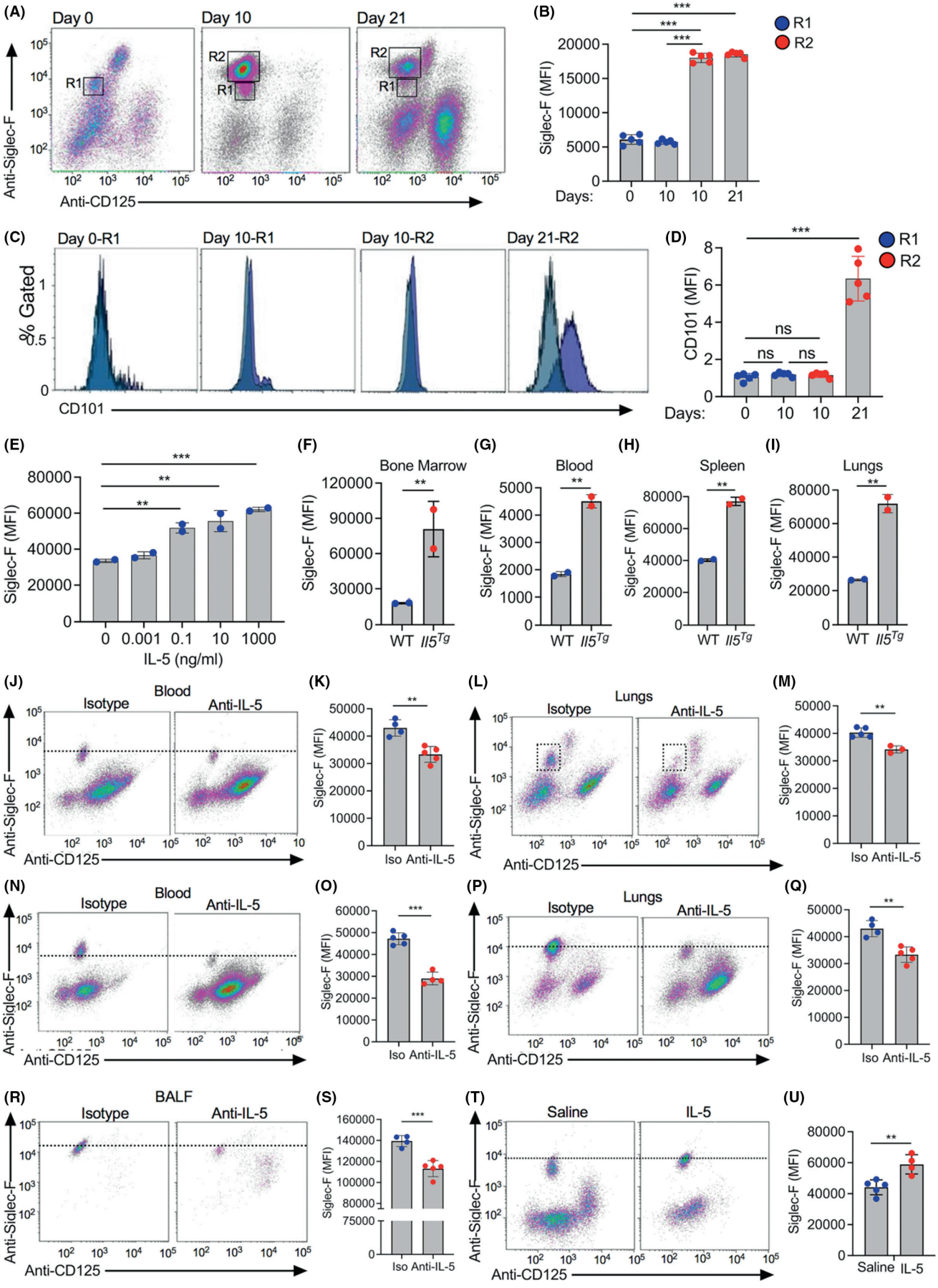
To the Editor,

Eosinophils were traditionally considered terminally differentiated cells with little to no heterogeneity.[1] Recent studies demonstrated that they can acquire unique phenotypes in response to diverse stimuli,[1–3] and two subsets of eosinophils [i.e. resident (rEos) and inflammatory (iEos)] were described in the lungs of allergen-challenged mice.[4] rEos and iEos can be differentiated by the expression of Siglec-F, CD101 and CD62L, their functional activities and their dependency on IL-5.[4] In particular, survival of lung rEos, which were associated with immunoregulatory activities was described as IL-5-independent.[4] This distinction may have major therapeutic implications since neutralization of IL-5 via anti-IL-5 therapy may deplete iEos and keep rEos intact.[5] Conversely, depletion of eosinophils using antibody-dependent cell cytotoxicity via targeting anti-IL-5Rα (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 therapeutics.[1] 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 conclusions.[4]

Wild type (WT) mice were challenged with house dust mite, and the presence of lung eosinophils was determined (see Methods S1). 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 eosinophils[4] 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 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). 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 state[6] (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-S3). Allergen-challenged SiglecF^{int}/CD101^{-} eosinophils uniquely upregulated 77 transcripts in comparison with resident eosinophils (Figure 2I, Table S4). 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

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, $^{**}p < 0.01$; $^{***}p < 0.001$


(A)
Day 0
Day 10
Day 21
R1
R2
Anti-Siglec-F
Anti-CD125
(B)
Siglec-F (MFI)
Days: 0 10 10 21
R1
R2
(C)
Day 0-R1
Day 10-R1
Day 10-R2
Day 21-R2
% Gated
CD101
(D)
CD101 (MFI)
ns
Days: 0 10 10 21
(E)
Siglec-F (MFI)
IL-5 (ng/ml)
0 0.001 0.1 10 1000
(F)
Bone Marrow
(G)
Blood
(H)
Spleen
(I)
Lungs
WT Il5Tg
(J)
Blood
Isotype
Anti-IL-5
(K)
Iso Anti-IL-5
(L)
Lungs
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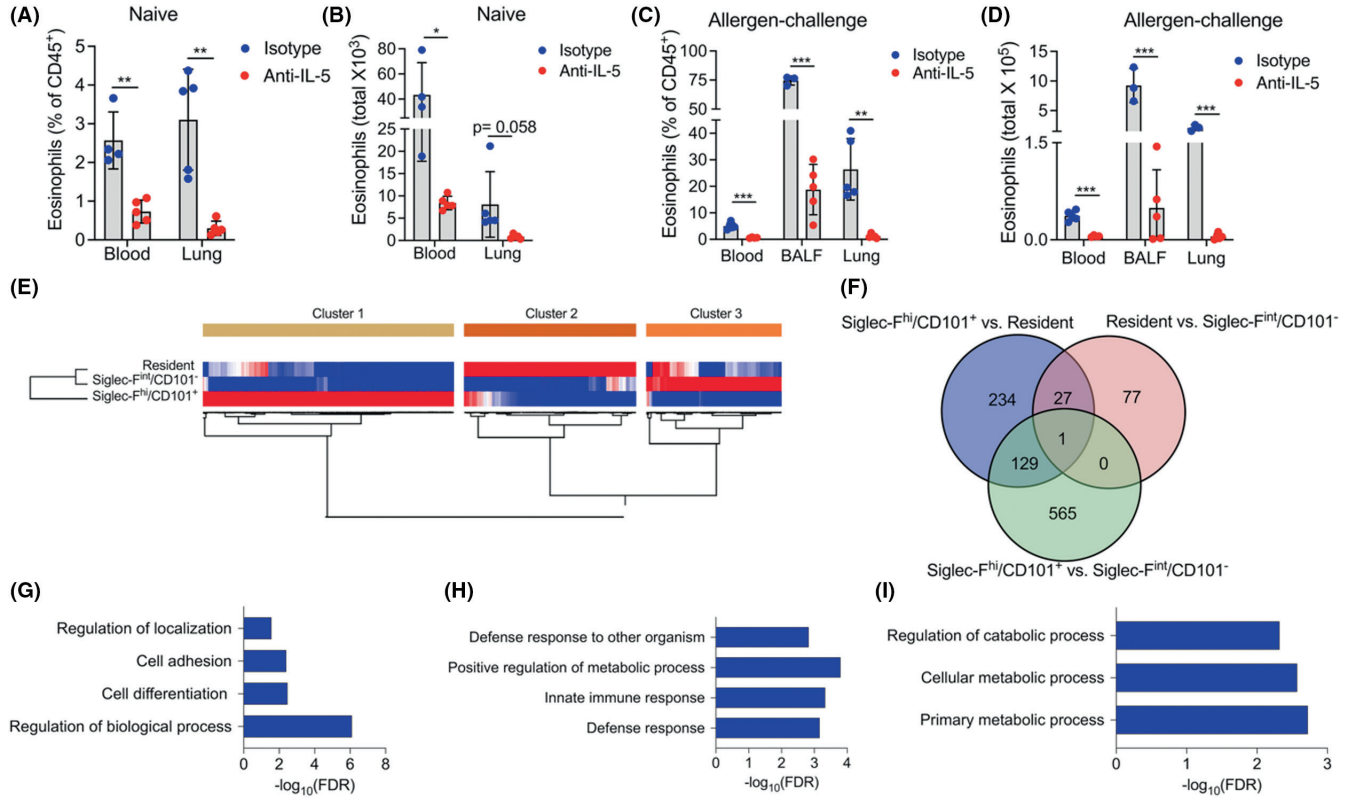

FIGURE 2 Resident lung eosinophils are dependent on IL-5 and are distinct from allergen-challenge-induced Siglec-F^{int}/CD101^{-} and Siglec-F^{hi}/CD101^{+} cells. Percent (A, C) and total (B, D) blood, lung and bronchoalveolar lavage fluid (BALF) eosinophils under baseline conditions (A-B) and following 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 were obtained from 2–3 samples/group consisting 3–4 pooled mice; Adjusted p value <0.05, 2 < fold change<−2

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.

AUTHOR CONTRIBUTIONS

AD and AM involved in conception and/or design of the work. AD, SGT, SA, IH, YG and MI assisted with data collection. AD, SGT and AM performed data analysis and interpretation. AD and AM involved in drafting the article. SGT, SA, IH, YG and MI involved in critical revision of the article. AM involved in final approval of the version to be published.

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KEYWORDS

asthma, eosinophils, inflammation, Siglec-F

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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Similar IgE binding patterns in Gulf of Mexico and Southeast Asian shrimp species in US shrimp allergic patients

To the Editor,

Shellfish allergy (SA) is a leading cause of food-induced anaphylaxis[1] and one of the most common causes of adult-onset food allergy worldwide, with 1%–3% of the United States (US) population affected.[2–4] Nearly half (45%) of US adults with SA report utilizing emergency services for SA symptoms over their lifetime,[2] remaining at-risk for lethal allergic reactions. Several allergenic proteins have been identified across shellfish species, including tropomyosin (TM), arginine kinase (AK), myosin light chain (MLC), sarcoplasmic calcium-binding protein (SCP), hemocyanin, troponin C, and triosephosphate isomerase.[5] (Table 1.) However, there are a large number of shrimp allergens that have been detected, but not yet characterized.[6] The allergens of major importance in SA are the muscle proteins TM and AK. TM, the major allergen with specific-IgE antibodies in ≤90% of SA patients, is associated with severe clinical reactivity. AK is a pan-allergen with cross-reactivity with crustaceans and cephalopods.[5]

Cross-reactivity has been observed clinically when SA patients ingest various invertebrate species with subsequent allergic reactions, but further study of shrimp sIgE binding between different shrimp species is needed.[7,8] This study examined the sIgE binding patterns to 2 shrimp species from the Gulf of Mexico and Southeast Asia in US SA patients.

Shellfish allergy patients with a history of shrimp-induced allergic reactions, allergic reaction with clinical oral food challenge and positive immediate skin prick testing (IHST) and/or shrimp sIgE ImmunoCAP™ levels were recruited from the Baylor College of Medicine (BCM) Allergy and Immunology Clinics. The study was approved by the BCM IRB and all participants provided written, informed consent. The patients underwent IHST to shrimp extract (mixture of *Penaeus borealis*, *Penaeus monodon*, *Metapenaeus barbata*, *and Metapenaeopsis joyner)*, *Dermatophagoides pteronyssinus (Der p1*, *Der p 10)*, *Dermatophagoides farinae (Der f1)*, cockroach, codfish, crab, lobster, and oyster using extracts from Greer™. The patients underwent prick and prick IHST to raw fresh shrimp (*Penaeus aztecus*), and cooked fresh shrimp (*Penaeus aztecus*). ImmunoCAP™ and ISAC™ customized testing by ThermoFisher™ assessed total IgE as well as sIgE levels for shrimp, recombinant *Penaeus aztecus* (TM), *Der p10*, *Der p1*, *Der p2*, recombinant *Penaeus monodon* AK, MLC, SCP, troponin C, crab, lobster, cockroach, clam, and oyster. Western blot (WB) analysis of sIgE binding profile to

Abbreviations: Der f1, *Dermatophagoides farinae*; Der p1, *Dermatophagoides pteronyssinus*; rPen a, recombinant *Penaeus aztecus*; rPen m, recombinant *Penaeus monodon*; SA, shrimp allergy; sIgE, specific IgE.