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Modulation of surface CD11c expression tracks plasticity in murine intestinal tissue eosinophils

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Abstract

Intestinal eosinophils are implicated in the inflammatory pathology of eosinophilic gastrointestinal diseases and inflammatory bowel diseases. Eosinophils also contribute to intestinal immunologic and tissue homeostasis and host defense. Recent studies in allergic airway disease suggest functional subphenotypes of eosinophils may underly their pathogenic versus protective roles. However, subphenotypes of intestinal eosinophils have not been defined and are complicated by their constitutive expression of the putative eosinophil inflammatory marker CD11c. Here, we propose a framework for subphenotype characterization of intestinal eosinophils based on relative intensity of surface CD11c expression. Using this flow cytometry framework in parallel with histology and BrdU tracing, we characterize intestinal eosinophil subphenotypes and monitor their plasticity at baseline and within the context of acute allergic and chronic systemic inflammation. Data reveal a conserved continuum of CD11c expression amongst intestinal eosinophils in health and acute disease states that overall tracked with other markers of activation. Oral allergen challenge induced recruitment of eosinophils into small intestinal lamina propria surrounding crypts, followed by in situ induction of CD11c expression in parallel with eosinophil redistribution into intestinal villi. Allergen challenge also elicited eosinophil transepithelial migration and the appearance of $CD11c^{lo}CD11b^{hi}$ eosinophils in the intestinal lumen. Chronic inflammation driven by overexpression of TNFα led to a qualitative shift in the relative abundance of CD11c-defined

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AUTHORSHIP

L. A. S. conceptualized the study; L. D. L., K. D., C. L. O., and I. M. C. performed the experimental investigations; L. D. L., K. D., C. L. O., and L. A. S. analyzed the data; L. D. L. and L. A. S. wrote the original manuscript draft; all authors contributed to review, editing, and revision of the manuscript.

DISCLOSURE

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eosinophil subphenotypes favoring CD11c^{hi}-expressing eosinophils. These findings provide new insights into heterogeneity of intestinal tissue eosinophils and offer a framework for measuring and tracking eosinophil subphenotype versatility in situ in health and disease.

Keywords

allergic inflammation; eosinophil; eosinophil subphenotypes; intestinal innate immune cell; phenotypic plasticity

1 | INTRODUCTION

Eosinophils are innate immune leukocytes that develop in the bone marrow before being released into the blood circulation as terminally differentiated, mature cells. Primarily tissue-dwelling cells, eosinophils, are short lived in blood, representing an overall minor percentage of WBCs (<5%) in healthy individuals, and home from the blood into several tissues in the steady state, including the gastrointestinal (GI) tract, lung, adipose tissue, and thymus, where they are longer lived.[1] The largest reservoir of eosinophils in health is found within the GI tract, where intestinal tissue eosinophils contribute to innate and adaptive immune homeostasis, tissue repair and remodeling, systemic metabolic homeostasis, and host defense. In contrast to their homeostatic functions, in eosinophilic GI diseases, including eosinophilic esophagitis and gastroenteritis, excessive infiltrations of eosinophils are hallmarks of disease and are believed to contribute to tissue pathology and fibrosis.[2–6] Activated intestinal eosinophils are also implicated in the pathogenesis of inflammatory bowel diseases.[7–9] Growing evidence suggests functional subphenotypes of tissue eosinophils, shaped by systemic and local microenvironmental pressures, may underly their diverse contributions to homeostasis and/or disease[10–12]; however, subphenotypes of intestinal eosinophils are poorly defined. With the recent availability of eosinophil-targeting biologics that ablate bone marrow-derived eosinophils,[9,13–16] there is an urgent need to better identify and characterize functional subphenotypes of tissue-dwelling eosinophils within the context of health and disease to understand the full consequences of global eosinophil ablation. Moreover, next-generation therapeutic approaches to manage eosinophil-associated GI diseases may benefit from targeting disease-exacerbating eosinophils while sustaining or enriching subphenotypes equipped for homeostatic functions.

Recent studies in mice have demonstrated at least 2 eosinophil subphenotypes in the lung. A regulatory, steady-state resident population localized to lung parenchyma actively dampens Th2 immunity and is characterized by the surface phenotype SiglecF^{int}CD62L^{+}CD11c^{-}. A separate allergen-driven population is characterized by the surface phenotype SiglecF^{hi}CD62L^{-}CD11c^{+} and exhibits Th2-driving proinflammatory functions.[17,18] The latter inflammatory eosinophil population expresses CD11c (αM integrin), localizes to peribronchial regions, undergoes transepithelial migration, and is recovered within bronchoalveolar lavage (BAL) fluid from allergic mice and asthmatic patients.[18,19] Likewise, regulatory (SiglecF^{int}) eosinophils are implicated in disease resolution in a mouse model of rheumatoid arthritis (RA), and eosinophil depletion through anti-IL-5 treatment

induced a relapse of arthritis in RA patients with concomitant asthma.[20] These collective data contribute to an emerging paradigm in mice of tissue resident homeostatic eosinophils at functional odds with a recruited, inflammatory subpopulation, the latter marked by enhanced expression of Siglec F and induction of CD11c. However, utilization of surface CD11c expression as a binary indicator of "inflammatory" eosinophils is complicated by the finding of constitutive CD11c expression on intestinal, thymic, and uterine eosinophils.[21,22]

The purpose of this study was to develop a strategy to characterize and monitor intestinal tissue eosinophil heterogeneity in health and disease. Using relative expression of CD11c as a framework, we employed parallel flow cytometry, BrdU tracing, and histology approaches to track eosinophil plasticity and tissue localization in the steady state and in models of acute allergen challenge and chronic intestinal inflammation. Data demonstrate a dynamic continuum of CD11c expression on intestinal eosinophils from lamina propria (LP) and intraepithelial (IE) compartments that correlated with other markers of eosinophil activation. Acute allergen challenge revealed in situ plasticity in CD11c expression and elicited transepithelial migration of eosinophils into the intestinal lumen. Chronic inflammation led to a qualitative shift within the intestinal eosinophil compartment favoring $CD11c^{hi}$-expressing phenotypes. These findings provide insights into the localization and heterogeneity of intestinal eosinophils within the context of local allergen-driven, and chronic systemic, inflammation, and propose a framework for monitoring plasticity of intestinal eosinophil subphenotypes.

2 | MATERIALS AND METHODS

2.1 | Mice and experimental models

Studies received prior approval from the University of Colorado School of Medicine (CUSOM) Institutional Animal Care and Use Committee. Eight to 12-week-old BALB/c and C57Bl/6 wild-type (WT) mice were originally obtained from the Jackson Laboratory (Bar Harbor, ME) and subsequently bred and maintained in-house under specific pathogen-free conditions. Mice were subjected to an acute food allergen challenge model as previously described.[23] Briefly, mice were sensitized to OVA through i.p. injections of 44 μg of grade VI OVA (Sigma–Aldrich) in sterile PBS admixed with 9 μl of Imject Alum (ThermoScientific) on days 0, 7, and 14. Sensitized mice were challenged daily on days 21–23 by oral gavage with 50 mg of grade V OVA (Sigma–Aldrich) dissolved in sterile PBS. In some experiments involving C57Bl/6 mice, a second round of oral gavage challenges was performed on days 28–30, as indicated. Control cohorts were sensitized with PBS + alum and challenged with PBS. $TNF^{\Delta ARE/+}$ mice[24,25] were bred and maintained as heterozy-gotes in-house.

2.2 | In vivo BrdU tracing

In some experiments, newly differentiated, bone marrow-derived eosinophils were labeled by i.p. injections of 2 mg of BrdU resuspended in a total volume of 200 μl sterile PBS, given approximately 6 h after each oral gavage (days 21–23).

2.3 | Staining and imaging of intestinal tissues

For immunohistochemistry (IHC) analyses, formalin-fixed, paraffin-embedded tissues were sectioned and eosinophils identified by staining with antibodies against major basic protein (rat anti-mouse MBP, clone MT-14.7.3, provided by Dr. Elizabeth Jacobsen, Mayo Clinic Arizona) following antigen retrieval as described.[23] Nuclei were labeled by counterstaining with methyl green. Eosinophils were manually counted from blinded slides that included 5–6 jejunum rings per mouse. For immunofluorescence (IF) analyses, tissue was fixed overnight in 4% paraformaldehyde followed by overnight cryoprotection in 30% sucrose prior to embedding in OCT. Frozen sections were mounted in fluorescence mounting medium supplemented with Hoechst dye to visualize nuclei. Images for IHC and IF stained slides were acquired using an Olympus IX83 and CellSense software (Olympus).

2.4 | Recovery of intestinal leukocytes from LP and IE compartments

Single-cell LP and IE suspensions were obtained from intestinal tissues as previously described.[26] Briefly, whole small intestine was collected, Peyer's patches excised, and tissue pieces incubated in mucolytic DTT-containing buffer (1 mM DL-dithiothreitol, 10% FBS, 1 mM HEPES, 2.5 mM NaHCO3 in $HBSS^{-/-}$) to release IE cells. Following recovery of IE cell-containing supernatant, remaining tissue pieces were incubated with EDTA containing buffer (1.3 mM EDTA, 1× Pen-Strep in $HBSS^{-/-}$) to remove epithelial cells. LP cells were recovered by digesting tissue pieces in a collagenase containing buffer (175 U/ml collagenase type 1, 10% FBS, 0.5 mM $CaCl_2$, 0.5 mM $MgCl_2$, 1× Pen-Strep in RPMI 1640 with L-glutamine and 25 mM HEPES) prior to mechanical disruption and passage through a 70 μM cell strainer. Both IE and LP cell preparations were further purified by discontinuous Percoll gradient centrifugation as described.[26] Briefly, cell pellets were resuspended in Percoll (Cytiva, Upsala, Sweden) diluted to 44% in RPMI containing phenol red and underlaid with Percoll diluted to 67% in RPMI without phenol red prior to centrifugation at 1000 × *g*. Leukocytes were collected from the interface between 44% and 67% layers.

2.5 | Intestinal lavage

For recovery of leukocytes from the intestinal lumen, whole small intestines were flushed with 10 ml of ice-cold RPMI supplemented with 3% FBS. Intestinal lavage fluid (ILF) was collected, centrifuged, and leukocytes were further enriched through discontinuous Percoll gradient centrifugation as described above for LP and IE cell preparations.

2.6 | Flow cytometric assessment of tissue eosinophils

Single-cell suspensions of cells recovered from the LP, IE, and ILF were incubated with the following antibodies or their relevant isotype controls. Aqua fixable live dead stain was from Invitrogen; CD45 (30-F11), Siglec F (E50–2440), and CD125 (T21) were from BD Bio-sciences; CD11c (N418), CD11b (M1/70), and CD62L (MEL-14) were from Biolegend. Gating was determined using appropriate isotype control antibodies. Data were acquired using a BD FACs Canto II and FACsDiva software and analyzed using FloJo. Eosinophils were identified as live, $CD45^{+}SSC^{hi}SiglecF^{+}CD11b^{+}FSC^{lo}$ cells as previously described.[27] ΔMFI was calculated as [(GeoMFI relevant Ab – GeoMFI isotype control)/GeoMFI isotype] × 100.

2.7 | Statistical analysis

Statistical analyses of data outcomes were calculated by Student's *t*-test or one-way ANOVA with post-hoc analyses as appropriate and as indicated in respective figure legends. Data are expressed as means ± SD. A p value of ≤0.05 was considered statistically significant.

3 | RESULTS AND DISCUSSION

3.1 | CD11c expression tracks continuum of intestinal eosinophil activation phenotypes in the steady state

Although up-regulation of Siglec F is a marker of inflammatory eosinophils in mice, high baseline expression of its human ortholog Siglec 8 does not appear to be further up-regulated upon activation of human eosinophils.[28] Therefore, to design a gating strategy likely applicable to both mouse and human eosinophils, we used CD11c as a generalized marker of eosinophil activation status. Unlike blood and resident lung eosinophils, CD11c is expressed, in the steady state, on uterine, thymic, and intestinal eosinophils.[21,29] Intestinal eosinophils are predominantly localized to the LP in health; however, we previously demonstrated that a small subset of eosinophils is also constitutively recovered with the IE leukocyte compartment in both mice and humans[29] and expresses higher levels of Siglec F, CD11b, and CD11c than LP eosinophils.[29] Of note, we confirmed in that same study that exposure to collagenase had no impact on surface expression levels of CD11c on intestinal eosinophils.[29] The broad, multipeaked histograms of LP and IE eosinophils suggest an overlapping continuum of CD11c expression (Figure 1(A)). Using contour plots to roughly identify cellular foci, we assigned gates to divide this continuum into $CD11c^{-/lo}$, $CD11c^{int}$, and $CD11c^{hi}$ subsets (Figure 1(B)). Applying this framework to map intestinal eosinophils in BALB/c WT mice, $CD11c^{int}$ eosinophils were found within both compartments, with LP also highly enriched for $CD11c^{-/lo}$ eosinophils, while the highest frequency of $CD11c^{hi}$ eosinophils localized to the IE compartment (Figure 1(C)). Small intestinal eosinophils from C57Bl/6 mice revealed a similar distribution of CD11c-defined subphenotypes in the steady state (Figure S1). These data provide further context to the overlapping and distinct phenotypes between eosinophils localized to the LP and IE compartments.

We hypothesized that markers of eosinophil activation would increase in parallel along the continuum of CD11c expression on intestinal eosinophils. We first compared overall levels of Siglec F and found surface expression progressively increased on LP and IE $CD11c^{-/lo}$ < $CD11c^{int}$ < $CD11c^{hi}$ eosinophil subpopulations (Figure 1(D)). CD11b, an adhesion molecule up-regulated on eosinophils from asthmatics,[30] exhibited the same stepwise pattern of overall increasing expression (Figure 1(E)). Forward (FSC) and side scatter (SSC) characteristics were assessed as an overall measure of cell size and complexity, respectively. Although no differences were apparent in FSC between CD11c-defined subpopulations (not shown), a statistically significant decrease in SSC was observed between $CD11c^{-/lo}$ compared with $CD11c^{int}$ and $CD11c^{hi}$ eosinophils (Figure 1(F)). Decreased granularity as measured by the SSC parameter has been used to distinguish normodense and hypodense eosinophils in mice[11] and humans.[31] Hypodense eosinophils were originally described in the early 1980s as a characteristic of activated blood eosinophils from patients with idiopathic hypereosinophilic syndrome, helminth infection, or asthma.[32–34] Their higher SSC suggests

that the CD11c$^{-/lo}$ subpopulation represents normodense (putatively nonactivated) cells, whereas CD11c$^{int\text{-}hi}$ eosinophils more closely resemble hypodense (activated) cells.

Interpreted together, these data demonstrate that intestinal eosinophils coexist in a spectrum of activation states at baseline and that relative levels of surface CD11c expression provide a viable strategy to characterize and evaluate intestinal eosinophil subphenotypes. These findings further suggest that CD11c$^{-/lo}$ eosinophils represent a less activated (i.e., higher SSC, lower Siglec F and CD11b) subpopulation compared with CD11c^{int} and CD11c^{hi} eosinophils.

3.2 | IL-5Rα expression remains stable across CD11c-defined eosinophil subpopulations

Eosinophils are reported to down-regulate IL-5Rα expression upon entry into tissue and/or upon engagement of IL-5.[35–37] Since newly available biologics targeting the IL-5 pathway aim to eliminate eosinophils,[9,13,16] we queried whether levels of surface IL-5Rα expression might be differentially expressed by CD11c-defined eosinophil subphenotypes, thereby impacting their susceptibility to targeting with anti-IL-5 biologics. As seen in Figure 1(G), IL-5Rα was uniformly detected across all CD11c-defined subgroups, suggesting these populations would be equally vulnerable to anti-IL-5 therapies, at least at baseline.

3.3 | In contrast to resident lung eosinophils, intestinal eosinophils are uniformly CD62L^{-}

We further queried whether the CD11c$^{-/lo}$ eosinophil subpopulation might represent an intestinal counterpart to homeostatic lung eosinophils. Lung-resident eosinophils are characterized by maintained expression of CD62L (L-selectin),[17] a homing adhesion molecule recognizing sialylated carbohydrate groups that is constitutively expressed on blood eosinophils. As anticipated, blood eosinophils expressed CD62L (Figure 1(H), left panel). However, in contrast to resident lung eosinophils, intestinal eosinophils from all CD11c-defined subpopulations were uniformly negative for CD62L (Figure 1(H)).

3.4 | Oral allergen challenge elicits recruitment, villus redistribution, and in situ induction of CD11c expression on LP eosinophils

To investigate plasticity between CD11c-defined eosinophil subpopulations within the context of allergic inflammation, we utilized an acute food allergen challenge model. As depicted in Figure 2(A), BALB/c WT mice were sensitized over 3 weeks with OVA admixed with alum adjuvant before receiving OVA allergen challenges by oral gavage on 3 consecutive days. Sham control mice were sensitized with saline admixed with alum and challenged with oral saline. Mice were sacrificed 1 (d24) or 4 (d27) days after allergen challenge, and eosinophils quantified from jejunum tissue sections. Oral allergen challenge of sensitized mice elicited a significant increase in total jejunal eosinophils on d24 and numbers of eosinophils remained elevated but were not further increased 3 days later (d27) (Figure 2(B)). Although the absolute number of eosinophils did not increase between days 24 and 27, their tissue distribution was altered, as demonstrated by a shift in the ratio of eosinophils localized to LP surrounding crypts relative to those localized to villus-associated LP. These data suggest that eosinophils immigrate into LP surrounding crypts on day 24 followed by redistribution into intestinal villi by day 27 (Figure 2(Bi)).

Whole small intestine tissue digests demonstrated an enrichment specifically in CD11c$^{-/lo}$ eosinophils (which most closely resemble blood eosinophils) 1 day after final allergen challenge, corresponding to the increase in eosinophils within LP surrounding crypts in BALB/c (Figure 2(C), left panel) and C57Bl/6 (Figure S1) mice. BrdU pulse labeling on days 21–23 (see Figure 2(A)) in saline- or allergen-challenged mice revealed newly bone marrow-derived (BrdU^{+}) eosinophils within LP surrounding crypts (Figure 2(D)) and confirmed BrdU^{+} cells contributed to the CD11c$^{-/lo}$ LP eosinophils on day 24 (Figure 2(E)). Therefore, intragastric allergen exposure elicits immigration of bone marrow-derived, CD11c$^{-/lo}$ eosinophils from the blood circulation into intestinal LP surrounding crypts.

To track tissue localization and CD11c expression of recruited eosinophils over time, BrdU^{+} eosinophils were assessed from intestinal tissue digests on days 24 and 27. BrdU^{+} eosinophils recovered from the LP on day 27 exhibited higher CD11c expression compared with BrdU^{+} eosinophils isolated on day 24 (Figure 2(Ei)) and were detected within LP both surrounding crypts and within villi at this time point (Figure 2(D)). Together, these data suggest eosinophils recruited into small intestinal LP surrounding crypts in response to ingested allergens up-regulate CD11c in situ in parallel with their redistribution into intestinal villi.

Notably, CD11c^{lo} eosinophils were also transiently detected within the IE compartment on day 24 in OVA-challenged, but not saline-challenged mice (d24, Figure 2(C) right panel, and Figure S1). This finding is particularly notable since CD11c$^{-/lo}$ eosinophils were rarely observed within the IE compartment at baseline (see Figure 1(C)). BrdU studies confirmed the origin of this allergen-driven subpopulation to be newly recruited eosinophils (Figure 2(F)). The fate of these newly recruited BrdU^{+} CD11c^{lo} IE eosinophils has yet to be determined. It is plausible that these cells populate the resident CD11c$^{int\text{-}hi}$ IE eosinophil compartment. Similar to LP eosinophils, increased CD11c expression was evident on BrdU^{+} IE eosinophils recovered on day 27 compared with day 24 (Figure 2(Fi)). However, whether CD11c^{lo} BrdU^{+} eosinophils up-regulate CD11c locally within the IE compartment or whether the BrdU^{+} CD11c$^{int\text{-}hi}$ eosinophils detected within the IE compartment on day 27 represent movement of CD11c^{int} LP eosinophils into the IE space remains to be determined. It is also plausible that allergen-elicited BrdU^{+} CD11c^{lo} IE eosinophils detected on day 24 exit the tissue through transepithelial migration, as discussed in Section 3.5 below. Of note, BrdU^{+} eosinophils were still detected within both the LP and IE compartments on day 38 (11 days post allergen challenge and BrdU pulse) and expressed intermediate to high levels of CD11c, respectively, at this time point (Figure S2). Biologic significance of the resident subset of CD11c^{hi} eosinophils constitutively isolated with IE leukocytes (Figure 1 and Ref.[29]) remains to be determined.

3.5 | Oral allergen challenge drives transepithelial migration of CD11c^{lo}CD11b^{hi} eosinophils

In human asthma and mouse models of allergic airway inflammation, activated eosinophils transmigrate across respiratory epithelium, moving from lung parenchyma into the airways, wherein they can be quantified in experimental or clinical settings by BAL. BAL eosinophils in both allergic mice and human asthmatics are CD11c^{+}, indicative of an activated

phenotype.[18] We hypothesized that allergen challenge would similarly elicit epithelial transmigration of intestinal eosinophils, and that eosinophils recovered from the intestinal lumen would phenocopy the CD11c^{hi} expression of IE eosinophils. To readily identify luminal eosinophils by flow cytometry and microscopy in parallel, we utilized eosinophil reporter mice on the C57Bl/6 background wherein GFP expression is driven in eosinophils downstream of a membrane-specific promotor, while all non-eosinophils express membrane-targeted tdTomato red (tdTomRed), as previously described.[29] Jejunum rings encompassing intestinal lumen were isolated 4 days after oral OVA challenge. Fluorescence microscopy revealed sparse cells within the intestinal lumen, the majority of which expressed tdTomRed (Figure 3(A)). Although infrequent, GFP^{+} eosinophils were observed transmigrating across villus epithelium (Figure 3(Ai)), and within the intestinal lumen (Figure 3(Aii)). To quantify eosinophils from intestinal lumen, whole small intestines were lavaged and ILF was collected. Eosinophils were rarely detected (<1%) among total CD45^{+} leukocytes in naïve mice and remained modest (up to 3%) following oral allergen challenge (Figure 3(B)). Unexpectedly, unlike IE eosinophils, the majority of eosinophils recovered from the intestinal lumen following allergen challenge were CD11c^{lo} (Figure 3(C)). To determine whether the low frequency of eosinophils detected within ILF and the predominance of the CD11c^{lo} phenotype reflected the lower allergic potential of the C57Bl/6 background strain, experiments were repeated in a cohort of BALB/c WT mice. ILF collected from BALB/c mice on day 24 revealed similar frequencies of luminal eosinophils (Figure 3(D)), which were also enriched for the CD11c^{lo} subpopulation (Figure 3(E)). Taken together, luminal eosinophils post allergen challenge from both C57Bl/6 and BALB/c background strains more closely resembled the CD11c-defined phenotype of eosinophils from respective LP rather than IE compartments (compare Figure 3(E) with Figure 1(C)).

Transepithelial migration of intestinal eosinophils had been previously described and found to be dependent upon eosinophil expression of CD11b.[38] Therefore, we investigated levels of CD11b expression on lumen-derived, transmigrated eosinophils. ILF eosinophils were uniformly CD11b^{hi} (Figure 3(F)).

Although we cannot rule out the possibility that CD11c is selectively shed or cleaved from eosinophils undergoing transepithelial migration, taken together with our detection of a transient, BrdU^{+}, CD11c$^{-/lo}$ eosinophil population within the IE compartment 1 day after allergen exposure (Figures 2(C) and 2(F)), we favor the interpretation that oral allergen challenge may drive a fraction of newly recruited eosinophils to transmigrate across intestinal epithelium, resulting in a dominant CD11c^{lo}CD11b^{hi} phenotype of ILF eosinophils. According to this interpretation, allergen-driven transmigrating eosinophils would be distinct from the CD11c^{hi}CD11b^{hi} eosinophils resident within the IE compartment (Figure 1 and Ref.[29]). Additional studies are needed to confirm the fate of allergen-elicited CD11c$^{-/lo}$ IE eosinophils and to determine the origin and function of resident CD11c^{hi} IE eosinophils.

3.6 | Chronic inflammation shifts CD11c-defined intestinal eosinophil heterogeneity to favor CD11c^{hi} subphenotype

Although oral OVA challenge drove recruitment, villus redistribution, and CD11c induction on small intestinal eosinophils, the relative abundance of CD11c-defined eosinophil subphenotypes 4 days after acute allergen challenge was not significantly different from baseline (see Figure 2(C)). To determine whether chronic inflammation might shift the relative balance of CD11c-defined eosinophil subphenotypes, we utilized TNF$^{\Delta ARE/+}$ mice. TNF$^{\Delta ARE}$ mice carry a genetic deletion in the AU-rich elements (ARE) contained within the 3′ untranslated region of TNFα mRNA transcripts, which leads to systemic overproduction of TNFα protein downstream of enhanced TNFα mRNA stability. Heterozygous TNF$^{\Delta ARE/+}$ mice exhibit chronic intestinal inflammation and spontaneously develop ileitis resembling Crohn's disease by 8 weeks of age.[24,39,40] We first analyzed ileal tissues recovered from 12 week old WT and TNF$^{\Delta ARE}$ mice and found eosinophils to be a substantial component of the inflammatory cell infiltrates appearing in patches along the ilea (Figure 4(A)). Whole small intestinal tissue digests were performed on pairs of age and gender-matched littermate controls aged 12–21 weeks old. At each age interval, intestinal LP eosinophil subpopulations were skewed in favor of CD11c^{int} and CD11c^{hi} eosinophil subpopulations (Figure 4(B)), suggesting a local inflammatory microenvironment reshapes the composition of the intestinal eosinophil compartment.

Intestinal eosinophils are long lived (relative to most other organs) and constitutively exposed to microenvironmental pressures that may uniquely shape their functional and phenotypic repertoire.[41] Subphenotypes of intestinal eosinophils had not been defined; their characterization complicated by a high level of heterogeneity even at baseline and constitutive expression of putative inflammatory markers (e.g., CD11c). Here, we propose a strategy to characterize the heterogeneous intestinal eosinophil compartment based on relative levels of CD11c expression. Applying this framework, we demonstrate a dynamic coexpression of phenotypically "inflammatory" subpopulations within the intestinal LP and IE eosinophil compartments. These data provide new and deeper insights into the heterogeneity and plasticity of intestinal eosinophils and provide a framework to quantify and monitor tissue eosinophil subphenotypes in health and across disease states. Further work is needed to more fully characterize CD11c-defined subphenotypes and, most importantly, to delineate their functional attributes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

EGID	eosinophilic gastrointestinal disease

GI	gastrointestinal
IF	immunofluorescence
IHC	immunohistochemistry
ILF	intestinal lavage fluid
LP	lamina propria
RA	rheumatoid arthritis
SSC	side scatter
WT	wild-type

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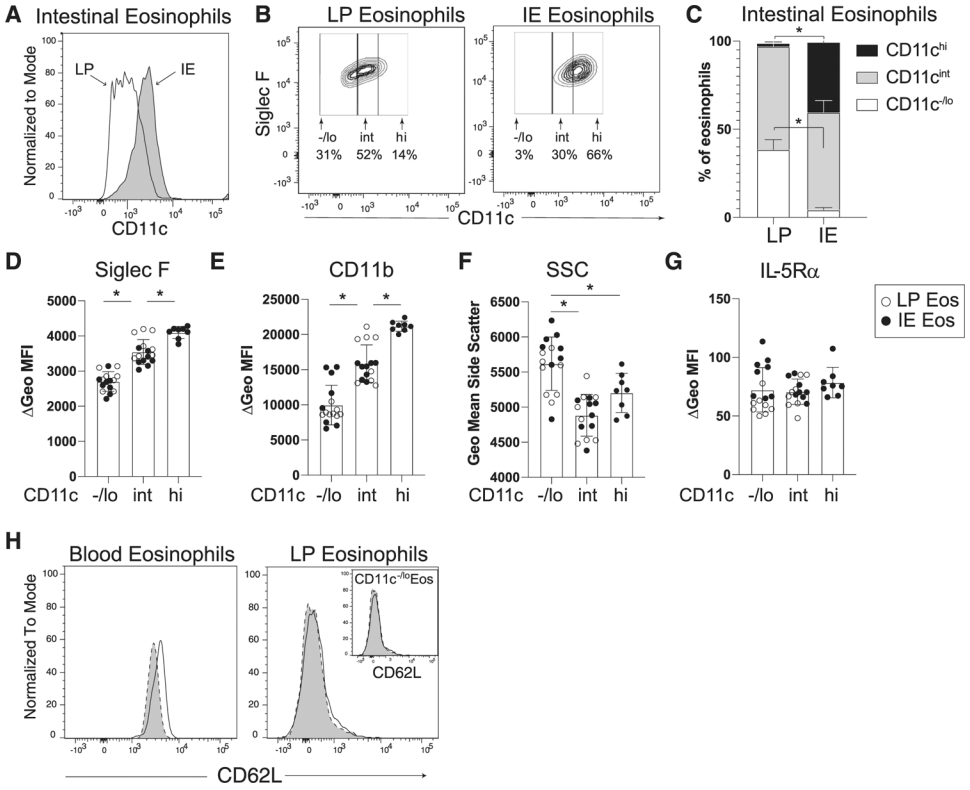

FIGURE 1.

CD11c expression tracks plasticity along a continuum of eosinophil activation phenotypes. (A) Single-cell suspensions from both LP and IE compartments were assessed by flow cytometry for expression of CD11c. (B) Gating strategy to assign intestinal eosinophils into $CD11c^{-/lo}$, $CD11c^{int}$, or $CD11c^{hi}$ subpopulations. (C) Breakdown of LP and IE associated eosinophils based on CD11c-defined subpopulations ($N = 5$). Expressions of Siglec F (D), CD11b (E), SSC parameter (F), and IL-5Rα (G) were quantified on CD11c-defined subpopulations from LP (open circles) and IE (filled circles) compartments. Each circle represents data from a single mouse ($N = 8$). (H) CD62L expression assessed on blood (left panel) and total LP (right panel) eosinophils. Shaded histograms, isotype control; open histograms, CD62L. Inset gated on $CD11c^{-/lo}$ LP eosinophils. Error bars, SD. Statistical analysis, Student's *t*-tests. *, $p < 0.05$

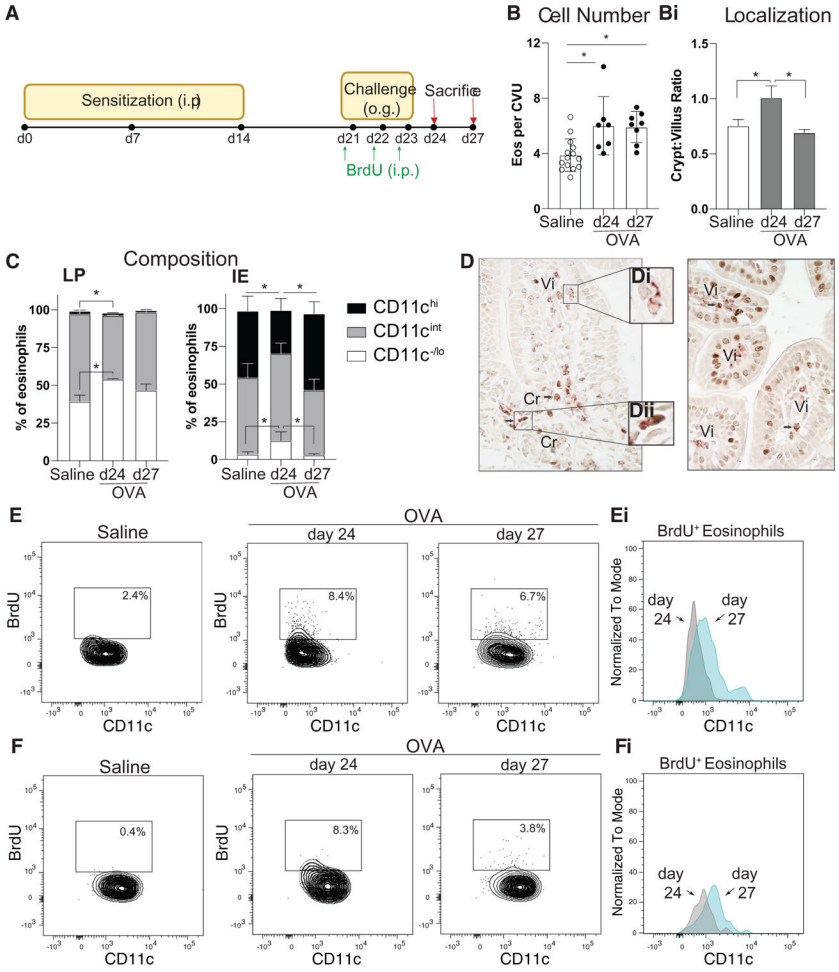

FIGURE 2.

Redistribution and CD11c plasticity of intestinal eosinophils following acute food allergen challenge. (A) Experimental timeline for acute food allergy challenge. (B) Eosinophils quantified from jejunum sections stained with anti-MBP are expressed as total eosinophils (eos) per crypt villus unit (CVU) (B) or as a ratio of eosinophils detected within LP surrounding crypts to eosinophils within LP of villi (Bi). Open circles, no OVA; closed circles, +OVA. (C) Breakdown of LP and IE eosinophils based on CD11c subphenotypes in saline- versus OVA-challenged mice at the indicated time points, as determined by flow cytometry of whole small intestine tissue digests ($N \geq 3$ mice per time point). (D–F) $BrdU^+$ eosinophils detected from jejunum sections (D) or whole small intestine tissue digests (E and F) in saline- or OVA-challenged mice. In (D), jejunum sections from an OVA-challenged mouse on day 27, showing a crypt:villus unit (left panel) or cross sectional villi (right panel) are stained with MBP (eosinophils, red) and BrdU (nuclear, brown). Insets in (D) show $BrdU^-$ (Di) and $BrdU^+$ (Dii) eosinophils. (Ei and Fi) CD11c expression on $BrdU^+$ eosinophils from LP (Di) or IE (Fi) eosinophils of OVA-challenged mice recovered on day 24 (gray histograms) or day 27 (blue histograms). Arrows identify $BrdU^+$ eosinophils. Cr, crypt; Vi, villus. Data in (D)–(F) are representative of $N = 4$ (saline), $N = 4$ (OVA, day 24) or $N = 3$ (OVA, day 27) mice per group, from 2 separate experiments. Error bars, SD. Statistical analyses, Student's *t*-tests. *, $p < 0.05$

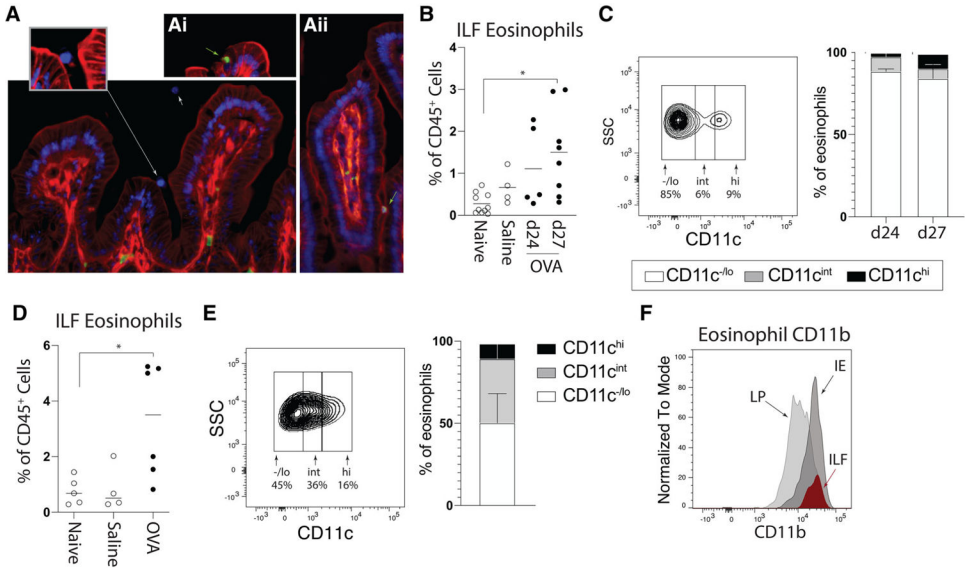

FIGURE 3.

Lumen transmigrated eosinophils are predominantly CD11c^{lo} CD11b^{hi}. (A) Frozen sections of jejunum tissue rings from eosinophil reporter mice 4 days after oral allergen challenge. Magnification, 200×. White arrows, noneosinophil (tdTomRed^{+}); green arrows, eosinophils (GFP^{+}). (B) Frequency of eosinophils (CD45^{+}GFP^{+}tdTomRed^{-}) amongst total CD45^{+} leukocytes recovered from ILF of the small intestine from naïve, saline-, or allergen-challenged C57Bl/6 eosinophil reporter mice. (C) Representative plot from a single mouse (left panel) and compiled data from $N = 6$ mice (right panel) showing the CD11c subphenotype composition of lumen-migrated eosinophils (d27). (D) Frequency of eosinophils (CD45^{+}SSC^{hi}SiglecF^{+}FSC^{lo}) amongst total CD45^{+} leukocytes recovered from ILF of small intestine from naïve, saline-, or OVA-challenged BALB/c WT mice. (E) Representative plot from a single mouse (left panel) and compiled data from $N = 3$ mice (right panel) showing the CD11c subphenotype composition of lumen-migrated eosinophils (d24) from BALB/c mice. (F) Comparison of CD11b surface expression on eosinophils from LP, IE, and intestinal lumen (ILF). In (B) and (D) open circles, no OVA; closed circles, +OVA. Statistics in (B) and (D) reflect one-way ANOVA comparing all means back to naïve mice using Dunnett's multiple comparisons post-hoc test. *, $p < 0.05$

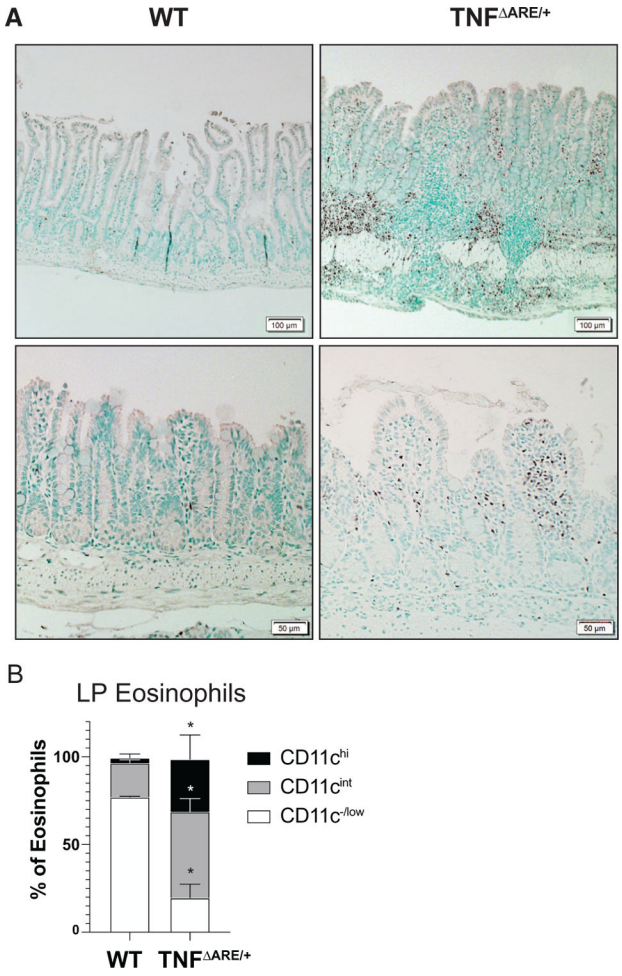

FIGURE 4.

Chronic inflammation shifts the CD11c subphenotype composition of intestinal eosinophils to favor CD11c$^{int/hi}$ subpopulations. (A) Ileal sections from 12 w/o TNF$^{\Delta ARE/+}$ (right panels) or WT littermate controls (left panels) were stained with anti-MBP to identify eosinophils (DAB-Ni, dark gray cells). (B) CD11c-defined subphenotype composition of LP eosinophils recovered from WT versus age- and gender-matched TNF$^{\Delta ARE/+}$ mice, aged 12–21 w/o. Data combined from $N = 4$ matched pairs. *, $p < 0.05$, Student's *t*-tests