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Review

Review

The ontogenesis and heterogeneity of basophils

Jiyeon Park and Suk-Jo Kang*

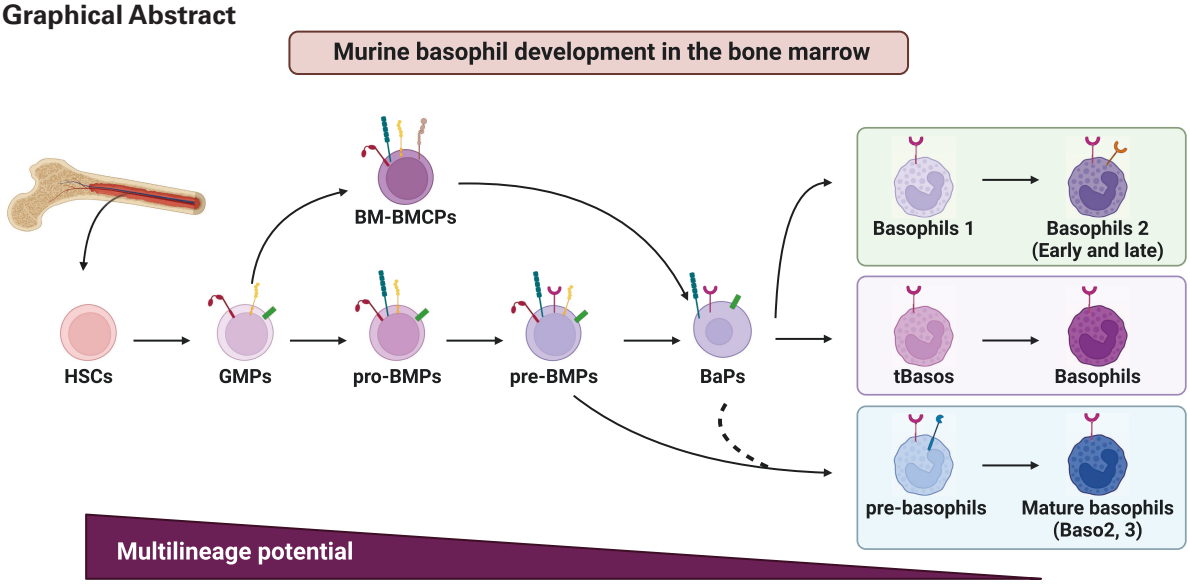
Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, Republic of Korea

*Correspondence: Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, Republic of Korea.
Email: suk-jo.kang@kaist.ac.kr

Summary

Basophils are the rarest leukocytes, but they have essential roles in protection against helminths, allergic disorders, autoimmune diseases, and some cancers. For years, the clinical significance of basophils has been neglected because of the lack of proper experimental tools to study them. The development of basophil-specific antibodies and animal models, along with genomic advances like single-cell transcriptomics, has greatly enhanced our understanding of basophil biology. Recent discoveries regarding basophils prompted us to write this review, emphasizing the basophil developmental pathway. In it, we chronologically examine the steps of basophil development in various species, which reveals the apparent advent of basophils predating IgE and basophil's IgE-independent regulatory role in primitive vertebrates. Then, we cover studies of basophil development in adult bone marrow, and compare those of murine and human basophils, introducing newly identified basophil progenitors and mature basophil subsets, as well as the transcription factors that regulate the transitions between them. Last, we discuss the heterogeneity of tissue-resident basophils, which may develop through extramedullary hematopoiesis. We expect that this review will contribute to a deeper understanding of basophil biology from the intricate aspects of basophil development and differentiation, offering valuable insights for both researchers and clinicians.

Graphical Abstract



Keywords: basophil, ontogenesis, hematopoiesis, heterogeneity

Abbreviations: AD: atopic dermatitis; AMs: alveolar macrophages; BaPs: basophil progenitors; BMCPs: basophil/mast cell progenitors; CLPs: common lymphoid progenitors; FcεRI: Fc epsilon receptor; GMPs: granulocyte/macrophage progenitors; HSCs: hematopoietic stem cells; IgE: immunoglobulin E; IL: interleukin; ILCs: innate lymphoid cells; MEPs: megakaryocyte/erythroid progenitors; pre-BMPs: pre-basophil and mast cell progenitors; pro-BMPs: pro-basophil and mast cell progenitors; tBasos: transitional basophils; TSLP: thymic stromal lymphopoietin.

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Introduction

Since the first description of human basophils by Paul Ehrlich in 1879 as leukocytes that are distinctly stained with basic dyes [1], it became evident that basophils play unique roles in host immunity against parasites and hypersensitivity [2–4]. The discovery of immunoglobulin E (IgE) and its high-affinity Fc epsilon receptor (FcεRI) provided crucial insights into the underlying mechanism of how basophils secrete the contents of intracellular granules to target parasitic helminths [5]. The interaction between IgE and its receptor on basophils triggers a cascade of signaling events that leads to degranulation which releases preformed mediators, including histamine and proteases, followed by release of newly generated lipid mediators, cytokines, and chemokines [6, 7]. These basophil effector molecules enable basophils to combat parasitic helminths and regulate various immune responses.

Basophils are rare circulating leukocytes with a relatively short lifespan of roughly 60 hours [8]. The contribution of basophils to allergic reactions was largely ignored because of their low abundance and similarity to mast cells, but basophils are now recognized as important effector cells in type 2 immune responses [9, 10]. Upon both IgE-dependent and -independent stimulations from cytokines, toll-like receptor ligands, complement proteins, or proteases, basophils release interleukin (IL)-4 and 13 to control type 2 immune responses under pathologic conditions [11]. Individuals who suffer from allergic conditions are found to have skin lesions associated with increased basophil infiltration [12, 13]. Similar basophil infiltration is also observed in the bronchial mucosa of asthma patients [14]. Basophils also regulate various aspects of allergic disorders, including the itchiness or pruritus of various skin diseases. This is mediated by IL-31-dependent neuroimmune communication [15, 16] involving basophils [17].

Basophils and mast cells are considered valuable targets for drug interventions. In fact, the primary objective in treating patients with allergies is blocking the effector function of basophils and mast cells. Glucocorticoids can be effective for allergy patients as they affect both basophils and mast cells [18, 19]. Omalizumab (Xolair®) is a humanized monoclonal antibody that blocks the binding of IgE to its receptor. Omalizumab treatment is effective and approved for patients with chronic idiopathic urticaria (CIU) and allergic asthma [20] and is under consideration for other allergic diseases [21]. Omalizumab improves clinical outcomes at least in part by reducing basophil numbers [22]. Typical prescriptions for alleviating itch and reducing allergic responses overall include drugs that target histamine, which is a mediator released during allergic reactions. In addition, an IL-31 antagonist is being developed for the treatment of itch associated with atopic dermatitis (AD) [23]. A deeper understanding of basophil biology could significantly accelerate the development of therapeutic agents for allergic diseases.

Basophils originate primarily from hematopoietic stem cells (HSCs) and complete development in the bone marrow [24]. Their differentiation involves a series of sequential steps, starting from HSCs and progressing through various progenitor stages before ultimately producing mature basophils, but the whole process is not yet fully delineated. Furthermore, the discovery of lung-resident basophils (rBasos) in perinatal mouse lungs and their role in regulating alveolar macrophages (AM) recently expanded the scope of basophil research [25]. Thus, in this review, we will first review the roles of basophils in different species to help clarify the most ancient functions of basophils as well as the functions that have been both conserved and newly acquired in mammals. Then, we will revisit basophil development in the bone marrow of mice and humans, including the terminal maturation process and regulatory transcription factors. Last, we will cover basophil heterogeneity and lung-resident basophils, which suggest extramedullary ontogeny and functions in tissue homeostasis.

Species differences concerning basophils

Basophils are conserved among vertebrates, appearing in ray-finned bony fish and extending to mammals [26–28]. Basophils in all these species are distinguished by their large cytoplasmic granules, but the lobulation of the nucleus appears to have developed over evolutionary time (Fig. 1). There are also species variances in the hematological characteristics of granules. Mouse basophils have sparser and less concentrated granules than other species including humans [29, 30]. Although few basophils exist in fish [31, 32], they appear frequently in amphibians and reptiles [33, 34]. Basophil percentages vary among frog species, appearing abundantly in African clawed frogs (*Xenopus laevis*) and American bullfrogs (*Lithobates catesbeiana*) [35] but more sparsely in DuBois's tree frogs (*Polypedates teraiensis)* [36]. In salamanders, basophils comprise 2–10% of leukocytes in the peripheral blood where basophil maturation takes place [37, 38]. Basophils are the most dominant granulocytes in snapping turtles (*Chelydra serpentine*), representing over 50% of circulating blood cells, but they are scarce in marine turtles [39, 40]. In mammals such as guinea pigs and rabbits, basophils are more abundant than mast cells, whereas mice, rats, and humans show the opposite [27]. These species-specific differences in the presence and abundance of basophils likely reflect distinct roles in defense or tissue homeostasis.

Basophils and mast cells in mammals mediate IgE-dependent type I hypersensitivity. Mammals have five immunoglobulin classes: IgM, IgD, IgA, IgG, and IgE. In contrast, vertebrates such as amphibians, reptiles, and birds have IgX and IgY instead of IgA, IgG, and IgE [41]. IgX is considered equivalent to IgA, and a duplication of the IgY gene over 200 million years ago is thought to have resulted in the advent of IgG and IgE in mammals (Fig. 1). Similarly, Fc receptors (FcR) for immunoglobulins first appeared during early mammalian evolution. Phylogenetic analysis suggested that FcR-like (FcRL) molecules in bony fishes are the ancient forms of the FcεRI and FcγRI-IV IgG receptors in mammals [42]. Thus, basophils first appeared before IgE or FcεRI (Fig. 1) [41, 43]. For this reason, basophils in early vertebrates can be detected not by FcεRI or surface IgE expression, but rather only by metachromatic staining-based cytochemistry.

The basophils of non-mammalian vertebrates release granules containing proteases and inflammatory mediators such as histamine in response to non-IgE immunoglobulins or allergy-associated molecules such as proteases, chitin (a polysaccharide of arthropods, insects and fungi), and compound 48/80 (a condensate of *N*-methyl-*p*-methoxyphenethylamine crosslinked with formaldehyde) [28, 44, 45]. Fugu, a teleost fish, lacks IgE or IgG but exhibits basophil degranulation upon IgM engagement or treatment with papain or chitin [44]. Turtle basophils release histamine in response to anti-turtle Ig serum stimulation [45]. Avian basophils release histamine in

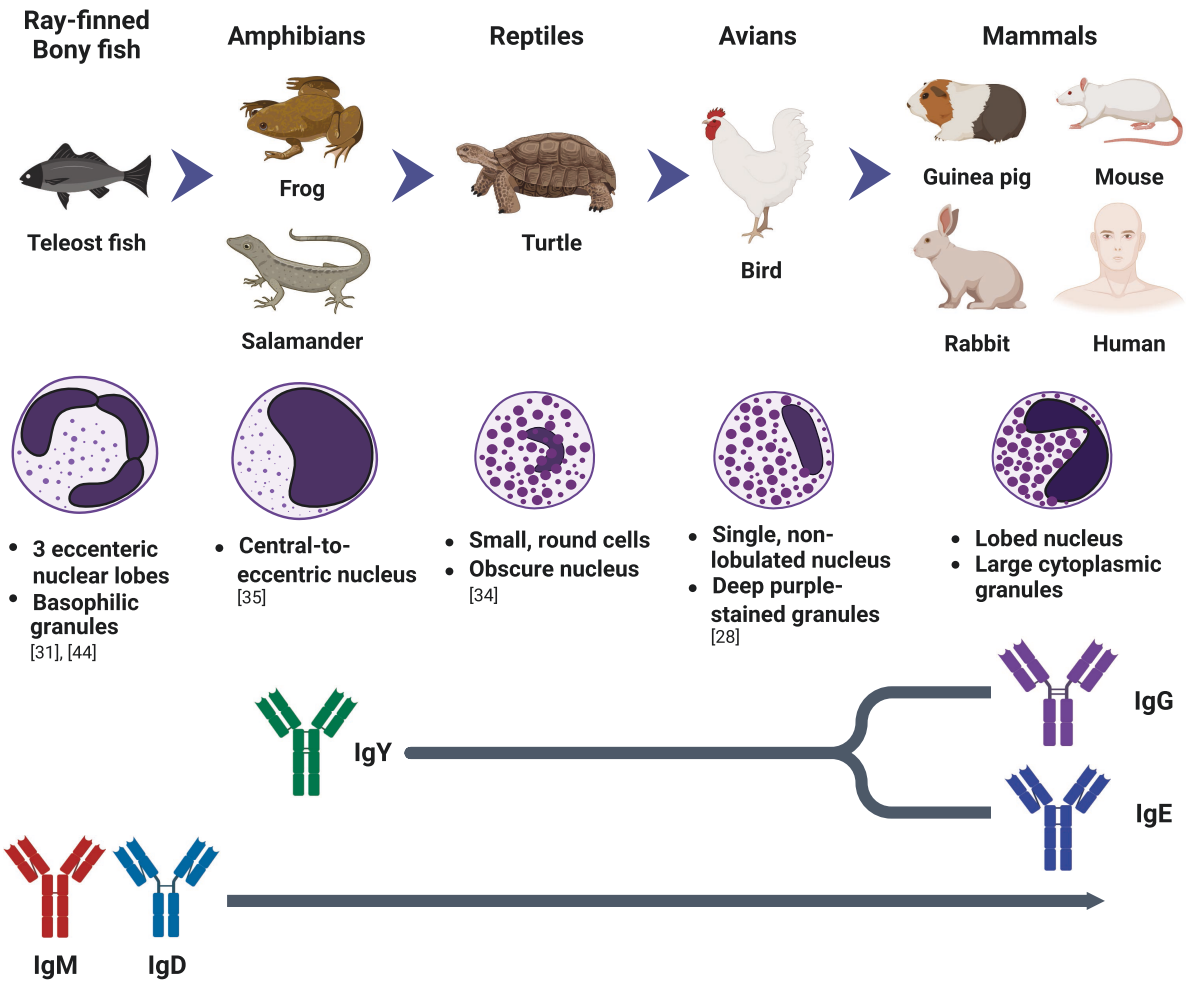

Figure 1: the evolutionary conservation of basophils. Basophils are well-conserved through the various vertebrates. Over evolutionary time, cell size shrank and cytosolic granules grew denser. IgY, the immunoglobulin ancestral to IgG and IgE, appeared after the advent of basophils. IgM and IgD are conserved in all vertebrates

response to compound 48/80, which is a basic secretagogue that can induce mast cell degranulation [28]. Intriguingly, some non-IgE-mediated basophil activation seems to be preserved in mammals, implying that it predates IgE-mediated activation [11]. Notably, the ancient immunoglobulin IgD binds its receptor CD44 and galectin 9 on basophils, leading to the production of IL-4 and IL-13 [46, 47]. Antibody isotype switching to IgE requires the type 2 cytokines IL-4 and IL-13. These cytokines and their receptors are detected as early as the jawed vertebrates [48]. Together, these results argue that mammalian basophils coopted type 2 cytokines to exert their effects via IgE. Still, it will be interesting to investigate the biological meaning of the fact that basophils and the contents of their prominent granules predate the appearance of IgE and its receptor, as well as the reason IgE is used now for allergen sensing and basophil activation in mammals. The answers to these questions may help uncover the most ancient roles of basophils and the origin of allergens.

The medullary development of basophils

Basophil-committed progenitors in mice

The classical hematopoiesis model places HSCs at the apex of a hierarchy of discrete stepwise differentiation toward the various blood lineages following a concrete, but stratified transition in cellular states in which stemness is lost and lineage commitment occurs [49]. Based on this model, cells at certain stages of differentiation are homogeneous and undergo bifurcations to produce more lineage-committed cells lower in the hematopoiesis hierarchy. Under the classical model, HSCs give rise to multipotent progenitors (MPP), which then produce lymphoid lineage cells via lymphoid-primed multipotent progenitors (LMPP) or common lymphoid progenitors (CLP) and myeloid lineage cells via the common myeloid progenitors (CMP) known as granulocyte/macrophage progenitors (GMP) and megakaryocyte/erythroid progenitors (MEP) [50–52]. Progenitors committed to basophil lineages diverge at the GMP stage and complete their maturation process in the bone marrow by passing through several progenitor stages, some of which are shared by mast cells [53]. These stages include pre-basophil and mast cell progenitors (pre-BMPs, Lin^- c-Kit^+ $Sca\text{-}1^-$ $CD34^+$ $CD16/32^{high}$ $Fc\varepsilon RI\alpha^+$), which have been described as $Fc\varepsilon RI\alpha$-expressing GMPs [54], and basophil progenitors (BaPs, $CD34^+$ c-Kit^- $Fc\varepsilon RI\alpha^+$) in the bone marrow [55]. Pre-BMPs differentiate into basophils or mast cells through the BaP or mast cell progenitor (MCP) stages, respectively. Another basophil/mast cell-committed population referred to as basophil/

mast cell progenitors (BMCPs) was identified as Lin$^-$ c-Kit$^+$ integrin β7^{high} CD16/32^{high} FcεRIα$^-$ cells falling outside GMP gates in the spleen (SP-BMCPs [55]) and in bone marrow (BM-BMCPs [56]). Advanced single-cell analysis techniques revealed that E-cadherin (E-cad) expression marks basophil or mast cell-committed progenitors and mature cells [57]. An E-cad-expressing, FcεRIα-negative sub-population of GMPs was designated pro-basophil and mast cell progenitors (pro-BMPs, Lin$^-$ c-Kit$^+$ integrin β7^{low} CD16/32$^+$ FcεRIα$^-$ E-cad$^+$). Pro-BMPs undergo differentiation to become pre-BMPs and subsequently give rise to either BaPs or MCPs [57]. To summarize, basophils in mice develop as follows: GMPs differentiate into BM-BMCPs or pro-BMPs and then to pre-BMPs followed by BaPs, which become fully mature basophils (Fig. 2 and Table 1).

Recent advancements in the tracking of single-cell fates have challenged the classical hematopoiesis model and revealed that HSCs and other progenitors are more heterogeneous than previously thought, with the presence of bi- or oligo-potent progenitors indicating that cell lineage commitment occurs in earlier progenitor populations [68]. This suggested a new continuum hematopoiesis model in which hematopoiesis occurs continuously among cells in developmental stages that are neither homogeneous nor discrete. Using PU.1 or GATA-1 reporter mice, MPPs were found to be heterogeneous, with PU.1$^+$ MPPs showing granulocyte/monocyte/lymphoid-lineage potential and GATA-1$^+$ MPPs showing megakaryocyte/erythrocyte-lineage potential [69]. Single-cell RNA sequencing of pre-granulocyte macrophage progenitors (pre-GMs, Lin$^-$ Sca-1$^-$ c-Kit$^+$ CD41$^-$ CD16/32$^-$ CD150$^-$ CD105$^-$) that act upstream of GMPs [70] further revealed two separate pathways for granulocyte development in which basophil/mast cell/eosinophil lineage potential co-segregates with megakaryocyte/erythrocyte lineage potential (EMkMPP branch), while neutrophil lineage potential co-segregates with monocyte and lymphoid potential (LMPP branch) [71].

The relationship among pro-BMPs, SP-BMCPs, BM-BMCPs, and pre-BMPs remains unclear. Pro-BMPs and pre-BMPs were identified as a subpopulation of GMPs, whereas BMCPs lie outside of GMP gates. Flow cytometric gating for BM-BMCPs and pro-BMPs based on surface marker expression showed that 85–90% of BM-BMCPs and pro-BMPs fall outside the gates for the other cell type [57]. Furthermore, it remains unclear whether BM-BMCPs transit through the pre-BMP stage during their maturation. In single-cell culture conditions, pro- or pre-BMPs make more basophil colonies than mast cells [54, 57], whereas, in contrast with SP-BMCPs [54, 55, 65], BM-BMCPs differentiate equally into basophils and mast cells [56]. It is worth noting that unlike BMCPs [55, 56], pro- and pre-BMPs can differentiate into erythroid cells and megakaryocytes when exposed to megakaryocyte/erythroid-supportive conditions [57]. Pro-BMPs, but not pre-BMPs, can also generate neutrophils, eosinophils, and undefined Ly6C^{high} CD11b$^+$ Ly6G$^-$ cells [57]. Thus, it seems likely that pro-BMPs represent a mixed population of progenitors for all myeloid cells, while pre-BMPs lie in the EMkMPP branch [71]. Consistent with a previous study that showed co-segregation of progenitor potentials based on GATA-1 expression [71], pro-BMPs may be divided

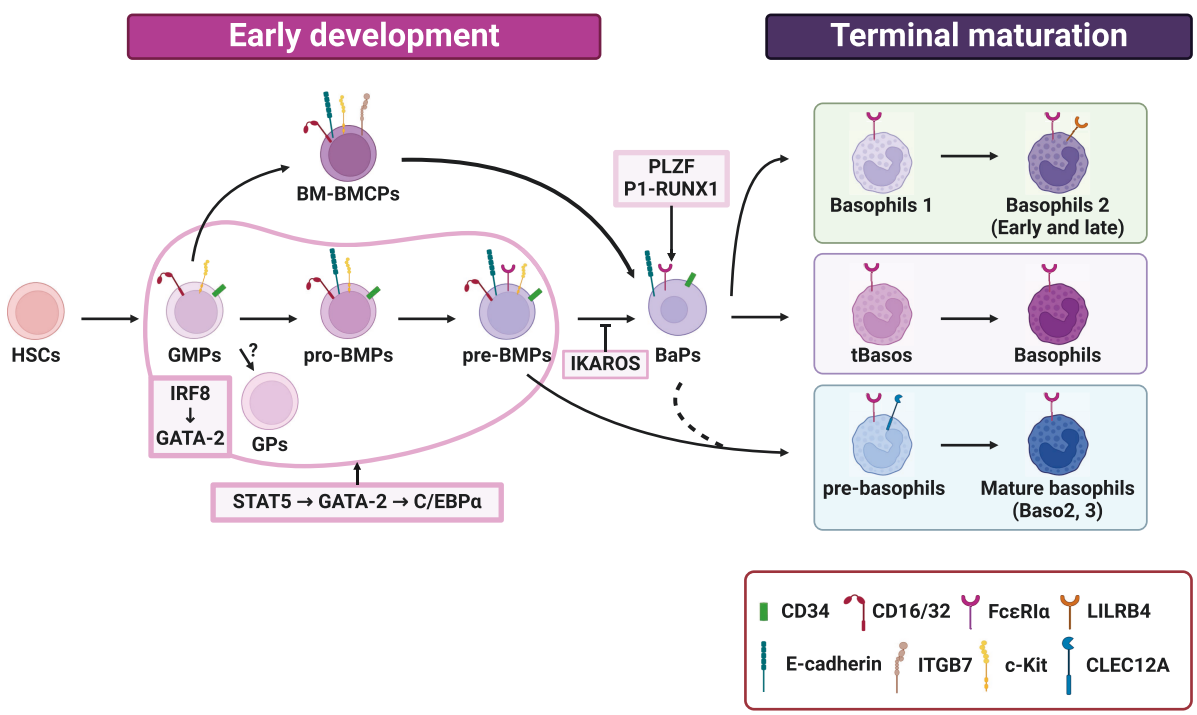

Figure 2: the development of basophils in mouse bone marrow. Basophil development is completed in the bone marrow. The transition from GMPs, pro-GMPs, pre-BMPs, or BM-BMCPs to BaPs is regulated by serial upregulation of STAT5, GATA-2, and C/EBPα. The terminal basophil maturation process was recently dissected by three studies [58–60]. BaPs differentiate into mature basophils (late Basophils 2) via newly identified precursor cells, i.e. Basophils 1 and early Basophils 2 [58], pre-basophils [59], or tBasos [60]. Some surface proteins are displayed only in certain stages of basophils for simplicity: for example, E-cadherin is shown only in early basophil progenitors despite being demonstrated to be expressed in mature basophils. More information is provided in the text and Table 1.

Table 1: cell types in basophil development

Cell type	Surface marker	Transcription factor	Note	Ref.
GMP	Lin$^-$ Sca-1$^-$ c-Kit$^+$ CD34$^+$ CD41$^-$ CD150$^-$ CD16/32^{high} CD105$^-$	Sequential expression of GATA-2 and C/EBPα	• GMPs can differentiate into granulocytes with the appropriate expression of transcription factors and treatment with growth factors	[61]
GP	Lin$^-$ Sca-1$^-$ c-Kit$^+$ integrin β7$^-$ CD150$^-$ CD27$^+$	IRF8, GATA-2	• GPs strongly express IRF8 • IRF8 deficiency reduces the number of pre-BMPs and BaPs but not GPs.	[62, 63]
Pro-BMP	Lin$^-$ Sca-1$^-$ c-Kit$^+$ CD34$^+$ CD16/32$^+$ integrin β7^{low} FcεRIα$^-$ E-cad$^+$	GATA-2	• Single-cell analysis revealed that E-cad-expressing HSPCs increased their expression of genes responsible for basophil and mast cell lineage development. • GATA-2 (but not GATA-1) upregulates E-cad expression in GMPs • Their flow cytometry gates do not overlap with those of BM-BMCPs	[57]
Pre-BMP	Lin$^-$ Sca-1$^-$ c-Kit$^+$ FcεRIα$^+$ CD34$^+$ CD16/32^{high}	STAT5, GATA-2 then C/EBPα in series	• FcεRI-expressing GMP subset	[54,64]
BM-BMCP	Lin$^-$ c-Kit$^+$ integrin β7^{high} CD16/32^{high}	Unknown	• Very rare, detected via single-cell analyses	[55,56]
BaP	Lin$^-$ c-Kit$^-$ FcεRIα$^+$ CD34$^+$	Promoting factors: C/EBPα, PLZF (ZBTB16), P1-RUNX1 Suppressing factors: MITF, IKAROS (IKZF1)	• Unipotent basophil progenitors that suppress mast cell differentiation. • Constitutive C/EBPα expression maintains basophil identity by preventing MITF from becoming mast cells.	[55,65–67]
Pre-basophils	Lin$^-$ c-Kit$^-$ CD34$^-$ CLEC12A^{hi} CD9^{lo}		• Basophil precursor population upstream of mature basophils (CLEC12A^{lo}CD9^{hi}). • Encompass some BaP population.	[59]
Transitional basophils (tBasos)	Lin$^-$ c-Kit$^-$ CD34$^-$ CD200R3$^+$ FcεRIα^{hi} CD49b^{lo}		• Basophil precursor population. • Direct descendants of BaPs and further develop into mature basophils with low FcεRIα expression. • Notable cytokine production.	[60]
Basophils 1	Lin$^-$ c-Kit$^-$ CD34^{low} LILRB4$^-$ FcεRIα^{high}		• A subset of basophil precursor population upstream of Basophils 2	[58]
Basophils 2 (Early and late)	Lin$^-$ c-Kit$^-$ CD34$^{low/-}$ LILRB4$^+$ FcεRIα^{mid}		• Early Basophils 2 express genes regulating oxidative phosphorylation and unfolded protein responses. • Late Basophils 2 express genes regulating inflammatory responses.	[58]

by their expression of GATA-1, with the GATA-1-expressing pro-BMPs being basophil/mast cell-committed [57].

Granulocyte progenitors at the unipotent post-GMP stage continue to mature into terminally differentiated mature cells like neutrophils. The steps of their maturation were delineated through a microscopic analysis of cellular morphology focused on nuclear lobulation and cytosolic granule number, size, and density [72]. The recent development of more sensitive separation protocols for neutrophil precursors based on flow cytometric parameters and technological advancements in transcriptomic analysis significantly transformed our understanding of neutrophil granulopoiesis and heterogeneity [73]. Terminal granulocyte differentiation is accompanied by a loss of mitotic potential and a concomitant acquisition of lineage-specific function [74]. The differentiation process can be divided into mitotic precursors, including early neutrophil progenitors (NePs [75] or proNeu1 and 2 [76]) and neutrophil precursors (NeuPs [77] or preNeus [78]), and post-mitotic cells, including immature band neutrophils and mature neutrophils [78]. We suspect it will be valuable to explore the cell-cycle-coupled cellular transformations that occur during basophil terminal differentiation after the precursors enter the unipotent BaP stage.

Recently, three research groups independently uncovered one or more precursor stages prior to the mature basophil stage in the terminal basophil maturation process (Fig. 2) [58–60]. Using a pseudotime analysis of basophil differentiation trajectory, Matsumura *et al*. found differential expression of the leukocyte immunoglobulin-like receptor B4, LILRB4 (*Lilrb4a* and *Lilr4b* in mice are orthologs for human LILRB4) [58]. They categorized basophil-lineage cells in the maturation process from BaPs to mature basophils into three distinct groups: Basophils 1 (CD34^{low} LILRB4$^-$ FcεRIα^{high}), early Basophils 2 (CD34^{low} LILRB4$^+$ FcεRIα^{high}), and late Basophils

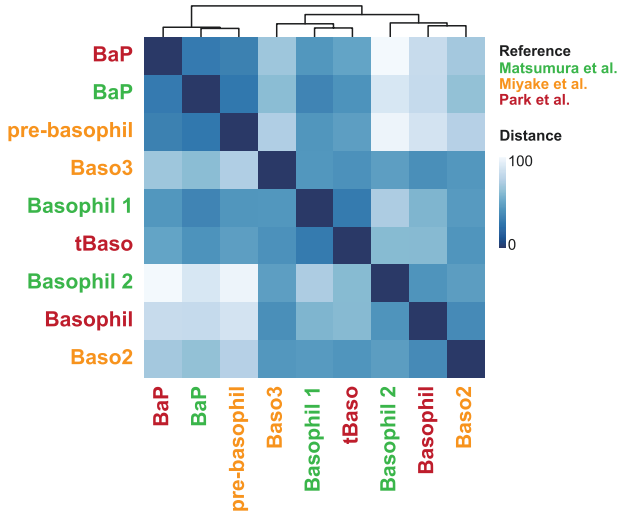

Figure 3: comparison of basophil precursors using a similarity distance matrix. A heatmap displaying the distance matrix of single cell (Matsumura *et al.* [58] and Miyake *et al.* [59]) and bulk RNAseq (Park *et al.* [60]) data provides a comparison of basophil populations for the similarities and differences. The original data were obtained from GEO depositories (GSE206589, GSE207688, and GSE148857) and subsequently re-analyzed using R (v4.2.3). In the clustering analysis, each single-cell data set was transformed into pseudo-bulk countmatrix data, and their similarity was calculated. Cell populations from the same study share the same color.

2 ($CD34^-$ $LILRB4^+$ $Fc\varepsilon RI\alpha^{mid}$). As basophils acquire LILRB4 expression, they undergo notable morphological changes, including a reduction in the cytoplasm and cell size, as well as a ring-like condensation of the nucleus. LILR family members play regulatory roles in a variety of immune cells [79], but the significance of LILRB4 expression in basophils remains unknown.

Two very similar basophil precursor populations, 'pre-basophils' [59] and 'transitional basophils (tBasos)' [60] were identified by Miyake *et al.* and Park *et al.*, respectively. Pre-basophils ($CLEC12A^{hi}$ $CD9^{lo}$) are separable from mature basophils ($CLEC12A^{lo}$ $CD9^{hi}$) [59], while tBasos (Lin^- $CD34^-$ $c\text{-}Kit^-$ $CD200R3^+$ $Fc\varepsilon RI\alpha^{high}$ $CD49b^{int}$) are distinguished from mature basophils (Lin^- $CD34^-$ $c\text{-}Kit^-$ $CD200R3^+$ $Fc\varepsilon RI\alpha^{int}$ $CD49b^{high}$) [60]. These groups employed different gating strategies, but pre-basophils and tBasos exhibit overlaps in flow cytometry analysis [60]. Although both precursor cells possess the ability to proliferate and differentiate into mature basophils, pre-basophils encompass some BaP ($CD34^+$ $CD200R3^+$ $CLEC12A^{hi}$) or BaP-like populations, suggesting a possible direct transition from pre-BMPs without going through the BaP stage to mature basophils. On the other hand, tBasos are direct descendant cells that lie downstream of BaP populations and develop into mature basophils [60].

Pre-basophils and tBasos have shared and distinct functional features. Pre-basophils and tBasos produced more type 2 cytokines than mature basophils when stimulated with cytokines [59, 60]. Intriguingly, Park *et al.* reported that tBasos exhibit a dichotomous fashion in cytokine-induced cytokine production: tBasos produce IL-4 in response to IL-3 stimulation, but IL-13 with IL-33 stimulation [60]. The biological context for this differential cytokine production and its underlying mechanisms merit further investigation. During their terminal maturation, maturing basophils from pre-basophils or tBasos acquire IgE-dependent degranulation capacity [59, 60]. Miyake *et al.* showed that in the context of helminth infection, pre-basophils leave the bone marrow, migrate to helminth-infected skin, and protect the tissue with their mitotic capacity retaining [59], which resembles emergency granulopoiesis reported in neutrophils [80].

To gain a better understanding of the terminal basophil maturation process and the relationship among the newly identified basophil precursors, we re-analyzed the gene expression profiles of basophil precursors (Fig. 3). This analysis reveals three clustering populations: (1) BaPs and pre-basophils, (2) Basophils 1, Baso 3, and tBasos, and (3) Basophils 2, Baso2, and (mature) basophils. The reason that pre-basophils resemble BaPs more than other precursor cells could be that pre-basophils include BaPs and even pre-BMPs [59]. Altogether, these provide evidence for a multi-step progression in basophil terminal maturation. Further studies and more sophisticated analyses are required to delineate their relationships further and transitions from mitotic to post-mitotic basophils, which will enhance our understanding of the programs underlying the acquisition of basophil effector functions.

Basophil development in human

In mice, pre-BMPs represent a shared step in the development of both basophils and mast cells immediately upstream of BaPs, which are basophil-restricted unipotent progenitors. It remains unclear, however, whether this is also the case in humans. Combining single-cell RNA-seq with single-cell cultures of human hematopoietic stem and progenitor cells (HSPCs, Lin^- $CD34^+$ $CD38^-$), another group found cell lineages that are already primed at the HSPC stage and HSPCs that are continuously acquiring new lineage priming into the eosinophil/basophil/mast cell lineage, the megakaryocyte/erythroid cell lineage, or the neutrophil and monocyte/dendritic cell lineages [81]. Similar results were also reported from analyses of HSPCs isolated from umbilical cord blood [82]. Consistent with observations of early murine hematopoiesis [71], single-cell analysis of CMPs (Lin^- $CD34^+$ $CD38^+$ $CD123^+$ $CD45RA^-$) downstream of HSPCs showed a separation of myeloid lineage potentials into either an EMkMPP branch that includes $CD131^+$ CMPs or an LMPP branch that includes $CD114^+$ CMPs [83]. Of note, human GMPs (Lin^- $CD34^+$ $CD38^+$ $CD123^+$ $CD45RA^+$) express CD114 but not CD131 and generate neutrophils and monocytes under pan-myeloid culture conditions. Some early studies reported instead a population of progenitor cells shared between the basophil and eosinophil lineages [84, 85]. More recent studies showed that basophil-like cells cultured from human umbilical cord blood with IL-3 contained eosinophil-associated transcripts [86]. Furthermore, a common progenitor of both eosinophils and basophils, referred to as eosinophil/basophil progenitors (EoBPs, $CD34^+$ $CD133^{low/-}$), was discovered in an analysis of $CD34^+$ human cord blood cells [87]. That study did not, however, explore whether EoBPs can generate mast cells. Notably, EoBPs were not reported in mice through single-cell trajectory studies, calling for additional investigation into whether the lineage relationship between eosinophils and basophils differs between humans and mice [56, 58]. In summary, our understanding of human basophil development is far from complete and the identification of more uni-, bi-, or oligo-potent basophil progenitors should continue.

Transcription factors for basophil development

The regulation of the intricate transitions between basophil progenitors relies on the coordination of multiple transcription factors (Fig. 2). Interferon regulatory factor 8 (IRF8) is important for the development of various myeloid cells [88]. IRF8 is highly expressed in granulocyte progenitors (GPs) defined as Lin$^-$ Sca-1$^-$ c-Kit$^+$ CD150$^-$ integrin β7$^-$ CD27$^+$ cells [62] but not in pre-BMPs, BaPs, or BMCPs [63]. IRF8 deficiency leads to a loss of pre-BMPs and BaPs but not GPs or BMCPs. It also reduces the expression of GATA-2, a transcription factor that is important for basophil and mast cell development [61]. This indicates that IRF8 acts at the GP stage by promoting GATA-2 expression. The differential requirement of IRF8 for the generation of pre-BMPs and BMCPs confirms the distinction of their lineages.

Signal transducer and activator of transcription 5 (STAT5) and GATA-2 are required for the progression from pre-BMPs to basophils and mast cells [54, 64]. Expression of both STAT5 and GATA-2 increases at the pre-BMP stage, with STAT5 increasing GATA-2 expression by binding to its promoter and intronic regions. GATA-2 regulates the expression of basophil- and mast cell-related genes, including *Fcer1a*, *Hdc* (histidine decarboxylase, an enzyme synthesizing histamine), *Il4*, *Il13,* and *Cdh1* (E-cadherin) [57, 64]. In pre-BMPs, STAT5 also induces the expression of CCATT-enhancer binding protein alpha (C/EBPα) [54], which promotes basophil differentiation [61]. The sequential order and balance of the expression of GATA-2 and C/EBPα in GMPs is crucial in determining cell fate [61]. When GATA-2 expression precedes C/EBPα expression, GMP development is biased toward basophils and mast cells, whereas the opposite expression pattern or forced expression of GATA-2 alone promotes the development of eosinophils. Differential GATA-2 expression in GPs and pre-BMPs may contribute to the ordered or balanced expression of GATA-2 and C/EBPα at each stage.

Sustained expression of C/EBPα is essential in limiting the trajectory of differentiation toward basophils rather than mast cells. C/EBPα is also subject to reciprocal regulation by multiple factors that promote mast cell differentiation. HES-1, which is a Notch signaling target gene, also functions as a mast cell-specifying factor by suppressing C/EBPα expression [89]. From the pre-BMP stage onward, C/EBPα suppresses the expression of the mast cell-driving factor microphthalmia transcription factor (MITF) by directly binding its promoter [54]. MITF then exerts reciprocal inhibition of *Cebpa* gene expression. IKAROS (IKZF1) deletion results in the expansion of BaPs, SP-BMCPs, and mature basophils, indicating that baseline levels of IKAROS suppress basophil differentiation [66]. IKAROS suppresses C/EBPα expression by binding the *Cebpa* promoter and reducing its H3K4me3 histone modification. Intriguingly, IKAROS also binds the *Hes1* promoter, but this increases its H3K4me3 modification and HES-1 expression.

Runt-related transcription factor, the expression of which is controlled by distal promoter P1 (P1-RUNX1), is critical for the differentiation of basophils but not mast cells or other granulocytes [65]. Deletion of *P1-Runx1* decreases BaPs but not GPs or SP-BMCPs. An analysis of pre-BMPs in *P1-Runx1*-deficient mice would clarify the stage at which P1-RUNX1 acts. A study tracking the expression of promyelocytic leukemia zinc finger (PLZF; ZBTB16) revealed prominent expression in BaPs, mature basophils, and mast cells but low expression in CMPs and GMPs [67]. Consistent with this expression pattern, PLZF-deficient mice show reduced BaPs and mature basophils and reduced basophil effector function. It remains unclear, though, whether PLZF deficiency also affects mast cells. MYB also plays a crucial role in regulating the early hematopoiesis of various cell types [90]. A recent study found *Myb* -68 enhancer activity in pre-GMs and GMPs that was maintained in basophils and mast cells, as well as *Myb* -74 enhancer activity in immature T and B cells [58]. Functionally, the *Myb* -68 enhancer regulates the expression of MYB and basophil and mast cell differentiation. GATA-1 expression is used to separate progenitors in the EMkMPP and LMPP branches at the pre-GM and GMP stages [71]. It should be investigated whether *Myb* -68 enhancer activity is correlated with or regulates GATA-1 expression (and vice versa). The role of GATA-1 in basophil development is rather contradictory. GATA-1 is highly expressed in basophils, and ΔdblGATA mice, which have a deletion of a high-affinity double GATA site in the *Gata1* promoter region, show impaired basophil development with reduced BaPs and basophils [91]. Conversely, downregulating GATA-1 by deleting the upstream enhancer and promoter of the *Gata1* gene increases basophils compared to controls [57]. Considering that distinct *Myb* enhancers are used in different cell lineages, these apparent discrepancies in the functions of GATA-1 could be resolved with further investigations into GATA-1 regulatory regions that either directly or indirectly affect basophil development.

The transcription factors mentioned above regulate the early stages of basophil development. Although several studies have documented impaired basophil function and even defective basophil development when these transcription factors are compromised, the details of how and when they regulate the distinct functions of basophils remain unknown. Future studies of their temporal regulation using omics technology will be required to clarify their interplay and the stages in which they act.

Discovery of tBasos revealed a temporal acquisition of unique basophil functions during development [60]. The transcriptome analysis of BaPs, tBasos, and basophils revealed that the expression of the transcription factor nuclear factor interleukin-3 (NFIL3, also known as E4 binding protein 4 (E4BP4)) increases during basophil maturation [60]. NFIL3 is a key regulator of immune cell development and function [92] that is reportedly a basophil signature gene [93], but the functional role of NFIL3 in basophils is unknown. NFIL3 is required in basophils for the expression of genes related to inflammatory responses, IgE-dependent degranulation, and cytokine production and basophil-specific NFIL3 deficiency suppressed skin inflammation and IL-4 secretion in a mouse AD model [60]. Still, the detailed mechanisms by which NFIL3 regulates IgE-dependent signaling in basophils should be further explored. Future research to identify and elucidate the roles of late-stage transcription factors for regulating basophil terminal differentiation that is coupled to mitotic exit and the acquisition of effector functions will deepen our understanding of basophil biology.

Basophil heterogeneity and extramedullary development

IL-3 or TSLP-derived basophils

IL-3 is a hematopoietic cytokine that drives a wide spectrum of myelopoiesis [94]. IL-3 mediates diverse inflammatory

responses by binding to the IL-3 receptor α (CD123) and β common chains (β_C, CSF2RB and β-IL3, CSF2RB2), which are expressed on a variety of cells [95]. IL-3 is produced mainly by T cells but also by many other immune and non-immune cells, including basophils and mast cells. In basophils, IL-3 is a versatile cytokine, functioning as a growth factor, an anti-apoptotic factor, and even as a stimulating factor for the release of mediators that induce inflammatory responses.

Regarding IL-3's role as a growth and survival factor, murine bone marrow cells cultured with IL-3 alone generated basophils [96]. Recently, IL-3 was found to increase the number of pre-BMPs and their descendants (including basophils and mast cells) by elevating the expression of *Il3ra* and *Gata2* [97] *in vitro*. Of note, *in vivo* delivery of IL-3 expanded pre-BMPs but not pro-BMPs or GMPs. IL-3 was also found to enhance basophil viability via NF-κB [98] or PIM1 [99]. But neither basophil number nor differentiation changed in naïve IL-3 [100, 101] or IL-3R β chain (*Csf2rb2*$^{-/-}$*Csf2rb*$^{-/-}$) deficient [102] conditions. Therefore, there must be an unknown niche in basophil development for factors other than IL-3. Further investigations will be necessary to identify the factor driving basophil differentiation in IL-3-depleted conditions.

IL-3 alone without IgE engagement can stimulate basophils to produce IL-4 [103] and IL-6 [104, 105]. IL-3 pre-treatment primes basophils and augments their production of type 2 cytokines and histamines in response to both IgE-dependent and -independent stimulations, such as stimulations by C5a and *N*-formylmethionyl-leucyl-phenylalanine (fMLP) [95]. This priming effect is achieved in an autocrine way, with IL-3 produced by basophils immediately binding the IL-3 receptor on the basophil's membranes. This process facilitates basophil viability and cytokine production. IL-3 deficiency was found to suppress anti-parasite immune responses in helminth-infected conditions, as well as delayed-type hypersensitivity reactions [101, 106]. IL-3 also mediates basophil recruitment to draining lymph nodes in helminth infections [107] and in an MC903-induced AD model [108].

Thymic stromal lymphopoietin (TSLP) is produced by immune cells such as basophils and mast cells and by non-immune cells such as epithelial cells. TSLP acts on a variety of cell types to regulate their development and functions [109]. TSLP, IL-25, and IL-33 comprise a set of epithelial cell-derived cytokines that promote type 2 immune response and play critical roles in allergic disorders. More than a decade ago, basophils were found to differentiate into two distinct subtypes when stimulated by either TSLP or IL-3 [110]. IL-3-elicited and TSLP-elicited basophils show differential expression of cytokine and metabolism-related genes. Functionally, only IL-3-elicited basophils degranulate upon IgE-dependent crosslinking. In contrast, TSLP-elicited basophils produce more cytokines (IL-4, IL-6, TNFα) and chemokines in response to IL-3, IL-18, or IL-33 than IL-3-elicited basophils. This association of IgE responsiveness with IL-3 but not TSLP is interesting and maybe clinically relevant.

TSLP and IgE appear to mediate different types of allergic disorders. While Omalizumab treatment is effective for CIU and allergic asthma patients [20], it is less effective in treating eosinophilic esophagitis (EoE) [111], AD [112], non-allergic asthma [113], and subsets of CIU [114]. Increased TSLP and TSLP gain-of-function mutations have been found in patients with AD, non-allergic asthma, allergic rhinitis, and EoE [115]. An experimental EoE mouse model confirmed that TSLP and its receptor signaling are necessary and sufficient for the development of EoE-like pathogenesis, which is basophil-dependent but IgE-independent [116].

Although a previous study showed that treatment of cultured bone marrow cells or cultured BaPs with TSLP expands basophils and prolongs their survival [110], TSLP is not required for basophil development at baseline because TSLP receptor deficiency did not alter basophil numbers [102, 117]. Instead, IL-3 and TSLP may have a role in the expansion of basophils during infection or disease conditions. Notably, Lin$^-$ CD34$^+$ c-Kit$^+$ FcεRIα$^-$ GMP-like cells were observed in the spleens of mice with systemic overexpression of TSLP [118]. These TSLP-elicited GMP-like cells were found to be multipotent, being capable of generating basophils, mast cells, and other myeloid cells including macrophages, dendritic cells, neutrophils, and eosinophils. It remains unclear whether this extramedullary hematopoiesis generates basophils and mast cells via SP-BMCPs.

A recent mass cytometric analysis of human basophils (CD45$^+$ HLA-DR$^-$ CD123$^+$) revealed significant heterogeneity, dividing them into four subpopulations based on their differential expression of CD16, FcεRI, and CD244 [119]. Two of the subpopulations behaved like classical basophils, expressing CD244 and FcεRI at high levels and upregulating CD203c (a basophil activation marker) upon IgE crosslinking or IL-3 stimulation. Interestingly, these two groups expressed eosinophil markers while the other two groups showed similar morphology and gene expression profiles to neutrophils. Further studies are required to determine the functional roles of each of these subtypes and the lineage relationships among them and with neutrophils and eosinophils. It remains unclear whether the co-expression observed in these different lineages is simply vestigial or whether it indicates that the lineages share common progenitors such as EoBPs.

Tissue-resident basophils

Most immune cell types take up residency in peripheral and lymphoid tissues. These include not only myeloid cells, such as macrophages, dendritic cells, and granulocytes but also lymphoid cells, such as T, B, NK, and innate lymphoid cells (ILCs) [120]. Resident immune cells regulate homeostasis, inflammation, infections, and tissue repair. Studies of macrophage ontogenesis established the notion of tissue residency and its regulation. Tissue-resident macrophages are heterogeneous in terms of ontogeny, residency niche and duration, and inflammatory condition [121]. Microglia and brain macrophages reside in the brain, a tissue that closes early in embryogenesis. They originate from early erythro-myeloid precursors (EMPs) derived from yolk sac (YS) blood islands via primitive hematopoiesis and are maintained during adulthood through self-renewal. Next, in waves of fetal hematopoiesis, the hemogenic endothelium of the YS generates late EMPs, and these begin definitive hematopoiesis. Late EMP-derived fetal liver monocytes replace YS macrophages in many organs during the perinatal period, including the liver, lung, and epidermis [122]. Some tissue-resident macrophages (especially those in the heart, pancreas, gut, and dermis) are further replenished in adults by HSC-derived blood circulating monocytes. These also replace embryonic macrophage precursors. One study identified macrophage precursors of different origins (including the YS, fetal liver, and bone marrow) that can generate AMs that self-maintain, emphasizing the importance of tissue

Table 2: features of lung-resident granulocytes

Features	Cell types		
	Basophil	**Neutrophil**	**Eosinophil**
Anatomical location	Parenchyma, proximal to alveoli	Intravascular region	Parenchyma, not in the peri-bronchial area
Inter- and intra-cellular signaling	Interact with alveolar epithelial cells and AMs through GM-CSF(CSF2)-CSF2RB and IL33-IL1RL1(ST2) pathways	Immune-suppressive through PGE_2/PKA/TGM2-mediated signals in neutrophils with reduced TNFα secretion	Inhibit nearby DC maturation and its proallergic functions, reducing Th2 response
Role in lung homeostasis	Promoting M2 phenotype (or maturation) in AMs.	Undetermined	Undetermined
Distinct phenotype compared to circulating counterparts	High expression: CSF2RB, IL1RL1, IL6, IL13, CSF1, HGF, OSM	High expression: CXCR4, CD14, IL6, CD101, SIGLECF Low expression: CXCR2, CD62L	High expression: CD62L, RUNX3 Low expression: CD101, SIGLECF, CXCR2, IL6
Ref.	[25]	[130]	[131]

factors for regulating residency [123]. Precursor cells show better plasticity or adaptability to local cues compared to terminally differentiated tissue-resident cells.

ILCs reside in various organs and are maintained by self-renewal [124]. As with macrophages, various organs are populated with ILCs derived from waves of progenitors from different developmental stages (i.e. fetal, postnatal, and adult) exhibiting a layered ontogeny [125]. Perinatal ILC2 precursors seed fetal tissues and acquire tissue-specific signatures, contributing to adult ILC2 pools. These adult pools are then replaced by *de novo*-generated ILC2s to varying degrees in different tissues [125]. Mast cells are long-lived cells that take up residence in connective or mucosal tissues. Similar to what was observed for macrophages and ILCs, two recent fate-mapping studies showed that mast cells are derived sequentially from YS-originated early EMPs, which are replaced by late EMPs and fetal HSCs during fetal development, and HSC-derived early EMPs in adults [126, 127].

At a steady state, granulocytes enter various naïve tissues and play homeostatic roles [73, 128, 129]. Normal lungs have resident neutrophils (rNeus) and resident eosinophils (rEos) (Table 2). rNeus exhibit distinct gene expression profiles that set them apart from bone marrow or blood neutrophils [130]. Within the steady-state lung environment, prostaglandin E2 (PGE_2) is an environmental factor promoting the generation and maintenance of rNeus. In the context of experimental acute respiratory distress syndrome (ARDS), PGE_2 plays a regulatory role in controlling the secretion of pro-inflammatory cytokines, such as TNFα and IL-1β, through the PGE_2/protein kinase A (PKA)/transglutaminase 2 (TGM2) pathway [130]. Notably, TGM2 represses the production of pro-inflammatory cytokines, including TNFα, in response to bacterial infection. Although the specific locations of rNeu residence have not yet been specified, this regulatory mechanism highlights a protective function for rNeus in preserving lung integrity. Neutrophils in the fetal liver are derived from late EMPs [132] but their contribution to tissue-resident neutrophils remains unknown. rEos are distinguished as Siglec-F^{int} $CD62L^{+}$ $CD101^{low}$ cells localized within the lung parenchyma and maintained independently of IL-5. IL-5-dependent circulating eosinophils (Siglec-F^{high} $CD62L^{-}$ $CD101^{high}$) are inflammatory and recruited to the lung upon allergen challenge. Although the roles rEos play in normal lungs are not clearly defined, rEos suppress responses to inhaled allergens, inhibiting the type 2 immune response [131].

Although one previous study reported a lack of expression of eosinophil or basophil markers at the embryonic stage [132], a more recent single-cell analysis of mouse lung during development revealed basophils in the fetal lung [25]. These emerge in the lung as early as embryonic day 12.5 (E12.5) when fetal liver hematopoiesis has begun and are found close to the alveoli at 30 hours after birth. rBasos persist throughout lung development until its completion and are also found in adult mice. It is unclear whether adult lung basophils originate from EMPs, HSCs, or both via layered ontogeneses like macrophages and ILCs. The details regarding the embryonic progenitors and ontogenic transition process for rBasos still need experimental verification. Lung-resident basophils have a distinct gene expression pattern compared to bone marrow-derived circulating basophils, with the unique expression of cytokines (*Il6*, *Il13*, *Tnf*), receptors for GM-CSF (*Csf2rb)* and IL-33, *(Ilr1rl1*), and transcription factors (*Pou2f2, Nr4a1*). It appears that basophils establish residency by acquiring tissue niche cues via interactions with other immune cells and non-immune cells in the lung. A ligand-receptor pairing analysis revealed intricate interactions in basophils between GM-CSF(CSF2)-CSF2RB and IL33-IL1RL1(ST2).

The identification of rBasos enhanced our understanding of the AM differentiation process (Fig. 4). YS-derived macrophages first appear in the fetal lung at E12.5 and are then replenished by fetal liver monocytes starting around E16.5 [123, 133, 134]. The fetal monocytes then differentiate into AMs around E18.5, coinciding with the initiation of alveolarization, which requires GM-CSF [133, 135]. At birth, mouse lungs pass through a saccular stage (E18.5 to postnatal day 5) in which alveolar precursors called sacs develop [136]. During postnatal days 5–30, which is the final stage of alveolarization, the sacs divide and secondary septa are formed to produce alveoli, the final functional units for gas exchange. During the postnatal period, IL-33 expands and activates ILC2s to produce IL-13. IL-13 then polarizes AMs, causing them to become M2-type cells [137, 138]. In a more recent analysis of lung development, GM-CSF and IL-33 were found to prime rBasos such that they contribute

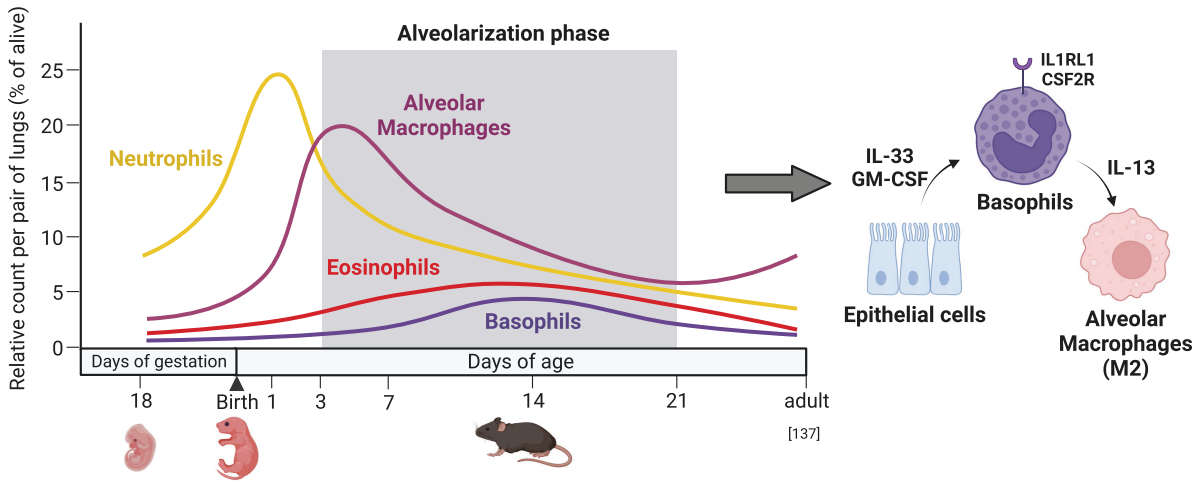

Figure 4: lung-resident basophils. Lung-resident basophils in the alveolarization phase (gray box) of lung development receive growth factors from lung type 2 epithelial cells and transmit signals to AMs to polarize them and guide their differentiation into M2-type cells.

further production of GM-CSF [25]. When lung basophils are depleted via an antibody-mediated strategy (MAR-1 antibody), both AM-specific signatures (*Il1m, Ear1, Lpl, Clec7a, and Siglec5*) and M2-associated genes (*Clec7a, Ccl17*) are reduced in the bronchoalveolar lavage fluid of newborn mice (postnatal 30 hours). Constitutive basophil ablation using basophil-specific Cre expression ($Mcpt8^{YFP\text{-}Cre/+}$; $R26^{DTA/+}$), however, marginally or negligibly reduced AM number [25, 139]. Thus, further studies will be required to clarify the roles rBasos play in the development and maintenance of AMs. Alternatively, lung basophils may cooperate with lung-resident ILCs in regulating the differentiation of AMs to M2-type cells and suppressing inflammatory responses during bacterial lung infections [138].

IL-33 is a member of the IL-1 family of cytokines that regulates type 2 barrier immunity. It is considered an alarmin produced by tissue damage and is thus an important player in sensing and maintaining tissue homeostasis [140]. Early life allergen exposure and the post-natal first breath trigger IL-33 production in the perinatal lung [137], presumably because they cause tissue injury or stress [138]. The first postnatal breath, in particular, suggests the intriguing possibility that tissue damage during lung development that is not so inflammatory is associated more with type 2 immune responses. This suggests the M2 polarization of AMs early in lung development is critical for proper alveolarization. This scenario parallels mammary gland development, where deficiencies of the type 2 cytokines IL-4 and IL-13 or of the transcription factor STAT6 can delay mammary gland development, leading to reduced branching morphogenesis and alveolar budding of the luminal epithelial cells at gestation [141]. T and B cells were further excluded as the source of the type 2 cytokines. Consistent with the results of this study, Th1 cells suppress luminal cell branching via IFNγ, while Th2 cells accelerate it. This emphasizes the importance of maintaining a type 2 immune environment during the alveolarization of mammary organs [142]. Thus, it would be interesting to investigate experimentally whether a type 2 immune setting with M2 polarization of AMs regulates alveolarization. This may finally reveal the true steady-state role of rBasos.

Basophil-mediated modulation of macrophages leading them to acquire the M2 phenotype has also been observed in the skin and liver. Under AD conditions, basophils exhibit skin-homing characteristics in response to alarmins like IL-18 and IL-33 [143]. They infiltrate the skin in the early phases of AD and stably persist there [13, 144, 145]. Basophils play a significant role in resolving skin barrier impairments [146]. Their presence in the skin leads to the expansion of M2-like macrophages through the actions of basophil-derived IL-4 and M-CSF. It is these M2-like macrophages that then help restore the skin barrier. In the liver, infection with *Listeria monocytogenes* induces the death of Kupffer cells (liver resident macrophages) and triggers hepatocytes to release IL-33. This then triggers basophils to secrete IL-4. Basophil-derived IL-4 induces M2 phenotypic changes in infiltrating monocytes so that they can fulfill the role of the deceased Kupffer cells, restoring liver homeostasis [147]. These results demonstrate that basophils contribute to the healing and restoration of damaged tissues by participating in processes related to tissue repair, such as promoting pro-resolution, orchestrating immune responses, and influencing the behavior of other immune cells. Their regulatory functions highlight the importance of basophils in tissue remodeling and maintaining tissue homeostasis.

Conclusion

In this review, we discussed the evolution of basophils and some recently revealed complexities of basophil development. Basophils are well-conserved from the most primitive vertebrates to mammals with their initial appearance predating the advent of IgE and its high-affinity receptors (FcεRI). This implies a role for basophils in protecting ancient non-mammals from threats such as parasites. The recent discovery of lung-resident basophils and their potential roles in lung development may indicate another original role of basophils in regulating tissue development and homeostasis. With the interesting correlation that basophils appear in all lung-breathing species, future studies will be required to resolve the mystery of the primordial basophil function.

Efforts to overcome the difficulties of studying basophils led to the identification of unrecognized basophil/mast cell-committed populations, and these discoveries helped clarify the process of basophil development in the bone marrow. The

pseudotime tracking of basophil lineages and the discovery of precursor populations (tBasos, pre-basophils, and Basophils 1 and early Basophils 2) appearing in the terminal stage revealed the existence of previously unappreciated steps of basophil development. These steps represent a critical transition in which mitotic potential is lost and *bona fide* basophil functions are acquired. We have reviewed medullary basophil development in adults, and some recently discovered population heterogeneity. Considering that basophils are essential in allergic disorders and other related diseases, studying basophil biology in more depth is clinically beneficial. In addition to expanding our understanding, unraveling the complexity of basophil developmental paths and functions may open new opportunities for developing innovative therapeutic approaches. This holds promise for more effective interventions and better outcomes for people suffering from basophil-related disorders, allergic illnesses, and associated diseases.

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Conflict of interests

The authors declare no competing interests.

Author contributions

J.P. and S-J. K designed and wrote the manuscript.

Ethical approval

Not applicable.

Data availability

The RNA-seq data of GSE206589 (Matsumura *et al.* [58]), GSE207688 (Miyake *et al.* [59]) and GSE148857 (Park *et al.* [60]) are used for generating Figure 3.

Clinical trial registration

Not applicable.

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