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Invited review article

Regulation of basophil and mast cell development by transcription factors

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Abbreviations:
BaPs, basophil progenitors; BMCPs, basophil/mast cell progenitors; C/EBPα, CCAAT/enhancer binding protein-α; ChIP-seq, chromatin immunoprecipitation followed by high-throughput DNA sequencing; CLPs, common lymphoid progenitors; CMPs, common myeloid progenitors; GATA, GATA-binding protein; GMPs, granulocyte-monocyte progenitors; GPs, granulocyte progenitors; HSCs, hematopoietic stem cells; IRF8, interferon regulatory factor-8; MCPs, mast cell progenitors; MITF, microphthalmia-associated transcription factor; MPPs, multipotent progenitors; pre-BMPs, pre-basophil and mast cell progenitors; STAT5, signal transducer and activator of transcription-5

ABSTRACT

Basophils and mast cells play important roles in host defense against parasitic infections and allergic responses. Several progenitor populations, either shared or specific, for basophils and/or mast cells have been identified, thus elucidating the developmental pathways of these cells. Multiple transcription factors essential for their development and the relationships between them have been also revealed. For example, IRF8 induces GATA2 expression to promote the generation of both basophils and mast cells. The STAT5-GATA2 axis induces C/EBPα and MITF expression, facilitating the differentiation into basophils and mast cells, respectively. In addition, C/EBPα and MITF mutually suppress each other's expression. This review provides an overview of recent advances in our understanding of how transcription factors regulate the development of basophils and mast cells.

Introduction

Basophils and mast cells are important effector cells that contribute to host defense against parasitic infections and to allergic responses.[1–3] Basophils and mast cells express high-affinity immunoglobulin E (IgE) receptor (FcεRI) on their surfaces. Cross-linking of FcεRI by antigen stimulation causes the release of inflammatory cytokines and chemical mediators.[2,4]

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While basophils and mast cells have many similarities, they also have distinct characteristics.[3,5] Mast cells reside mainly in tissues and are barely detected in blood. However, basophils circulate in blood and migrate to tissues in response to stimuli. In addition, mast cells have a life span of several weeks to months, whereas basophils survive approximately 60 h. Nuclear morphology and expression of several surface receptors also differ between basophils and mast cells. Consequently, they possess non-redundant functions, although these distinctions are not described in detail here.

In recent years, basophil and mast cell development has been an active area of research, resulting in the identification of various progenitors and transcription factors that regulate their development. This review presents an outline of the mechanisms of developmental regulation of basophils and mast cells, based on currently available information.

Developmental pathways of basophils and mast cells

Basophils and mast cells develop from hematopoietic stem cells (HSCs) via common myeloid progenitors (CMPs) and granulocyte-monocyte progenitors (GMPs).[6,7] In addition, granulocyte progenitors (GPs),[8,9] capable of differentiating into granulocytes (neutrophils, eosinophils, and basophils) and mast cells; bone marrow pre-basophil and mast cell progenitors (pre-BMPs),[10] capable of differentiating into basophils and mast cells; spleen basophil-mast cell progenitors (BMCPs),[11] also capable of differentiating into basophils and mast cells; basophil progenitors (BaPs),[11] which differentiate only into basophils; and mast cell progenitors (MCPs),[11,12] which differentiate only into mast cells have been reported (Fig. 1).

GPs were originally identified as Sca-1$^-$ Lin$^-$ c-Kit$^+$ CD150$^-$ CD27$^+$ integrin β7$^-$ (SN) cells and classified into two types, Flt3$^+$ GPs and Flt3$^-$ GPs, based on the expression level of FMS-like tyrosine kinase 3 (FLT3).[8,9] Both GP populations differentiate primarily into granulocytes and possess some capability to give rise to mast cells, but Flt3$^-$ GPs have higher potential to develop into basophils and mast cells.[9]

Two types of bipotential progenitor populations, with the potential to differentiate into either basophils or mast cells, have been identified. One type is spleen BMCPs, discovered by Arinobu *et al.*,[11] and the other is bone marrow pre-BMPs, reported by Qi *et al.*[10] *In vitro* culture experiments have revealed that BMCPs produce basophils via BaPs and mast cells via MCPs (Fig. 1).[11] Pre-BMPs are a subpopulation of GMPs with high FcεRIα expression, and transplantation experiments have revealed that pre-BMPs have high potential to develop into basophils and mast cells.[10] Of note, pre-BMPs have been demonstrated to have higher potential to develop into basophils than BMCPs.[10]

BaPs are mainly present in the bone marrow, while MCPs are present not only in the bone marrow and spleen but also in the peripheral tissues, including the intestine.[11–13] After migrating to a tissue through the peripheral blood, MCPs eventually differentiate into mast cells.[3,14] BaPs and MCPs are believed to develop from the bipotential progenitors (BMCPs or pre-BMPs). However, there are reports showing that MCPs can also develop directly from upstream progenitors, such as CMPs or multipotent progenitors (MPPs).[8,12]

Because all of the abovementioned progenitor populations were identified using distinct sets of surface markers (Table 1),[8,10–13,15] the exact relationships among them are somewhat obscure. Furthermore, as in the case of GMPs that include pre-BMPs, whether or not individual progenitor populations are homogeneous awaits further investigation. Nevertheless, based on their differentiation potential, it is reasonable to assume that GMPs differentiate into GPs, which differentiate into bipotential progenitors (pre-BMPs or BMCPs) and then into unipotential progenitors (BaPs or MCPs) to give rise to basophils and mast cells (Fig. 1).

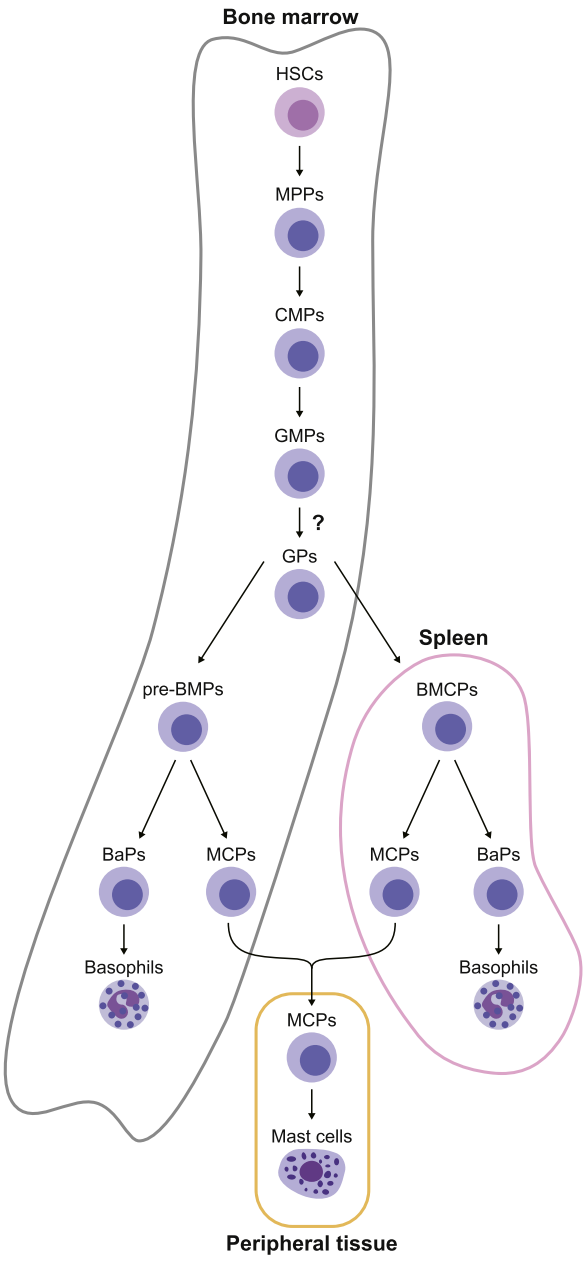


Fig. 1. A model for the developmental pathways of basophils and mast cells. Basophils and mast cells develop from HSCs via MPPs, CMPs, and GMPs. Bipotential progenitors capable of differentiating into either basophils or mast cells include bone marrow pre-BMPs and spleen BMCPs. These bipotential progenitors differentiate to unipotent progenitors, BaPs and MCPs. MCPs differentiate into mast cells after migration to tissue.

Expression of transcription factors important for basophil and mast cell development

Cellular processes, such as cell differentiation, that involve changes in gene expression patterns are regulated by various factors such as cytokines, micro-RNAs, epigenetic mechanisms, and transcription factors. Especially, transcription factors that bind to specific DNA sequences in the genome to directly regulate gene expression are key determinants of cell fate.[16] Indeed, multiple

Table 1
Surface markers on progenitors of the basophil and mast cell lineages.

Cell type	Surface expression	Reference
GMPs	Lineage markers (CD3, CD4, CD8, B220, Gr-1, TER119, CD19, IgM)$^-$, Sca-1$^-$, c-Kit$^+$, IL-7Rα$^-$, CD34$^+$, CD16/32^{hi}	15
Flt3$^+$ GPs	Lineage markers (CD3, CD4, CD5, CD8, CD11b, B220, Gr-1, TER119)$^-$, Sca-1$^-$, c-Kit$^+$, Ly6C$^-$, FcεRIα$^-$, CD71$^-$, CD41$^-$, CD27$^+$, integrin β7$^-$, Flt3 (CD135)$^+$, CD150$^-$	8
Flt3$^-$ GPs	Lineage markers (CD3, CD4, CD5, CD8, CD11b, B220, Gr-1, TER119)$^-$, Sca-1$^-$, c-Kit$^+$, Ly6C$^-$, FcεRIα$^-$, CD71$^-$, CD41$^-$, CD27$^+$, integrin β7$^-$, Flt3 (CD135)$^-$, CD150$^-$	8
pre-BMPs	Lineage markers (CD3, CD4, CD8, CD19, B220, Gr-1)$^-$, Sca-1$^-$, c-Kit$^+$, FcεRIα$^+$, CD34$^+$, CD16/32^{hi}	10
BMCPs	Lineage markers (CD3, CD4, CD8, CD19, B220, Gr-1)$^-$, c-Kit$^+$, integrin β7^{hi}, CD16/32^{hi}	11
BaPs	Lineage markers (CD3, CD4, CD8, CD19, B220, Gr-1)$^-$, c-Kit$^-$, FcεRIα$^+$, CD34$^+$	11
BM MCPs	Lineage markers (CD3, CD4, CD5, CD8, CD11b, B220, Gr-1, TER119)$^-$, Sca-1$^-$, c-Kit$^+$, Ly6C$^-$, FcεRIα$^-$, CD27$^-$, integrin β7$^+$, T1/ST2$^+$	12
SP MCPs	Lineage markers (CD3, CD4, CD5, CD8, CD11b, B220, Gr-1, TER119)$^-$, Sca-1$^-$, c-Kit$^+$, Ly6C$^-$, FcεRIα$^-$, CD27$^-$, integrin β7$^+$, T1/ST2$^+$	13
SI MCPs	Lineage markers (CD3, CD4, CD8, CD19, B220, Gr-1)$^-$, CD45$^+$, FcεRIα^{lo}, CD34$^+$, integrin β7$^+$	11

BM, bone marrow; SP, spleen; SI, small intestine.

transcription factors have been reported to be essential for basophil or mast cell development, including interferon regulatory factor-8 (IRF8), CCAAT/enhancer binding protein-α (C/EBPα), GATA-binding protein-1 (GATA1), GATA2, IKAROS family zinc finger-1 (IKAROS/IKZF1), microphthalmia-associated transcription factor (MITF), runt-related transcription factor 1 (RUNX1), and signal transducer and activator of transcription-5 (STAT5).[9–11,13,14,17–27]

The expression of these transcription factors changes during basophil and mast cell differentiation (summarized in Fig. 2A). GMPs and GPs express *Cebpa*, *Ikaros*, *Stat5*, and *Irf8*.[10,11,17,18] Upon differentiation to pre-BMPs, *Irf8* expression decreases, whereas the expression of *Cebpa*, *Stat5*, and *Gata2* increases.[10,13,17,24] On the other hand, with differentiation to BMCPs, not only *Irf8* but also *Cebpa* expression decreases, whereas the expression of *Gata1*, *Gata2*, and *Ikzf1* increases.[11,13,18] BaPs and basophils show high expression of *Cebpa*, but do not express *Mitf*.[10,11,18] In sharp contrast, mast cells show high expression of *Mitf*, but do not express *Cebpa*.[10] It is unclear at which differentiation stage *Runx1* is expressed. Below, we will describe the role of each of these transcription factors.

Developmental defects in basophil and mast cell lineages in mice deficient in STAT5, GATA2, C/EBPα, or MITF

We first describe the phenotype of mice deficient in STAT5, GATA2, C/EBPα, or MITF (Fig. 2B).[10,24–26,28–30] Basophil numbers reconstituted with fetal liver cells from *Stat5*$^{-/-}$ mice were markedly lower than those reconstituted with wild-type cells.[26] Conditional *Stat5* knockout mice in which the *Stat5a/b* locus is deleted in a polyinosinic–polycytidylic acid (poly I–C)-inducible manner,[10,31–33] also showed a decrease by half in the numbers of pre-BMPs, BaPs, and basophils, while the numbers of CMPs, GMPs, and BMCPs are unchanged (Fig. 2B).[10] In addition, tissue mast cells were significantly diminished in *Stat5*$^{-/-}$ mice or *Stat5*$^{-/-}$ bone marrow chimeric mice.[25] Therefore, STAT5, which is activated by cytokines such as IL-3 and stem cell factor (SCF) that promote basophil and mast cell development, is indispensable for the generation of both basophils and mast cells.[5,10,24–26]

Biallelic deletion of *Gata2* results in a marked reduction in basophils and mast cells *in vivo*, whereas monoallelic deletion of *Gata2* causes a decrease only in mast cells.[24] These results demonstrate that GATA2, known as a key regulator of HSCs and early progenitor cells, also contributes to the development of basophils and mast cells, although the need for GATA2 is greater for mast cells.[18,21,24]

C/EBPα, known to be essential for the development of various myeloid cells, is also required for basophil development.[10,11,13,18] Arinobu *et al.*, who performed a pioneering study on the mechanisms of basophil and mast cell development, revealed that conditional deletion of C/EBPα in BMCPs *in vitro*[28] caused the loss of potential for BMCPs to differentiate into basophils, and these cells differentiated exclusively into mast cells.[11] In addition, forced expression of C/EBPα in BMCPs facilitates differentiation into basophils. Recently, the percentage of pre-BMPs has been shown to be markedly lower in C/EBPα conditional knockout mice.[10] Conditional deletion of C/EBPα in pre-BMPs also abolished their potential to differentiate into basophils *in vitro*. Taken together, these results indicate that C/EBPα is required for basophil-mast cell bipotential progenitors to differentiate into basophils.[10]

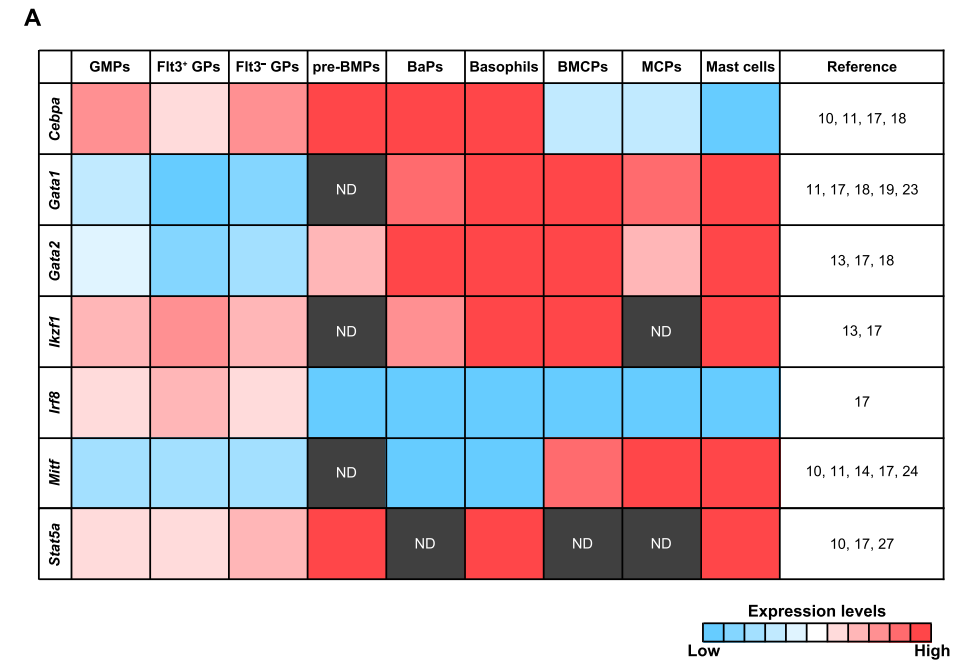
In MITF-deficient mice, the numbers of mast cell precursors and mast cells are markedly lower than in wild-type mice, demonstrating the essential role of MITF in mast cell development (Fig. 2B).[29,34,35]

Relationships between STAT5, GATA2, C/EBPα, and MITF in basophil and mast cell development

Qi *et al.* reported that STAT5 expressed in pre-BMPs facilitates basophil and mast cell development by inducing C/EBPα and MITF expression.[10] Thus, *Cebpa* expression is decreased in residual pre-BMPs in STAT5-deficient mice. In addition, wild-type GMPs cultured in the presence of IL-3 upregulate *Mitf* as their differentiation to the mast cell lineage proceeds, but this induction of *Mitf* expression does not occur in STAT5-deficient GMPs.[10] Interestingly, STAT5 directly binds to the promoter region of *Gata2*, and forced expression of GATA2 restores basophil and mast cell development in STAT5-deficient progenitor cells,[24] suggesting the importance of the STAT5-GATA2 axis. Because GATA2 is required for the expression of *Cebpa* and *Mitf* in basophils and mast cells, respectively,[24] the STAT5-GATA2 transcription factor cascade may operate in bipotential progenitors and downstream cell stages to support the development of both basophils and mast cells (Fig. 3).[10,24]

Introduction of GATA2 into common lymphocyte progenitors (CLPs), in which differentiation potential is naturally limited to lymphocytes, causes transdifferentiation to basophils and mast cells.[18] In addition, if CLPs are transduced by GATA2 and then by C/EBPα, differentiation is limited to basophils. Therefore, upregulation of GATA2 is important for the development of both basophils and mast cells, whereas upregulation of GATA2 followed by C/EBPα is important for basophil development, suggesting the importance of ordered expression of transcription factors.[18]

Moreover, Qi *et al.* clarified the mutual suppression mechanisms of C/EBPα and MITF.[10] The loss of C/EBPα upregulates *Mitf* expression in basophils, whereas the loss of MITF upregulates *Cebpa* expression in mast cells.[10] In addition, C/EBPα binds to the *Mitf* promoter region in basophils to suppress *Mitf* transcription, whereas MITF binds to the *Cebpa* promoter region in mast cells to suppress *Cebpa* transcription (Fig. 3).[10] Importantly, even



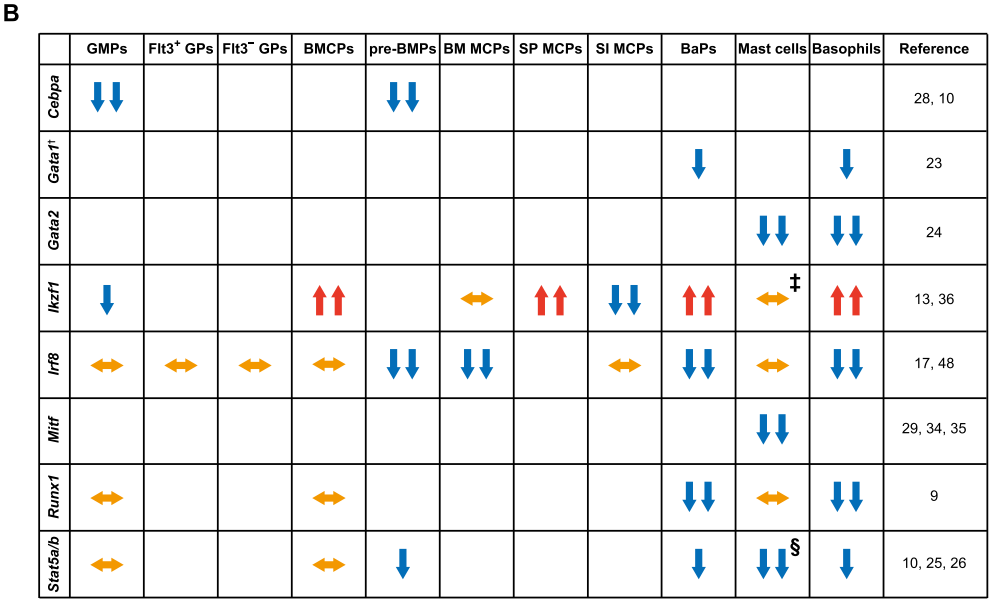


Fig. 2. Transcription factors regulating the development of basophils and mast cells. **(A)** A heat map representing relative changes in the expression levels of transcription factors in the basophil and mast cell lineages. **(B)** Changes in the numbers of basophil and mast cell lineage cells in mice deficient for the indicated transcription factor. BM, bone marrow; SP, spleen; SI, small intestine. † ΔdblGATA mice: deletion of GATA-binding site in the GATA-1 promoter. ‡ Intestinal mast cells were reduced in *Ikzf1*$^{-/-}$ mice. § *Stat5*$^{-/-}$ mice: deletion of *Stat5a* and *Stat5b* first coding exon (Reference 25).

mature basophils transdifferentiate into mast cell-like cells when C/EBPα is conditionally deleted.[10] Therefore, mutually exclusive expression of C/EBPα and MITF determines a basophil versus mast cell fate.

Regulation of C/EBPα expression and basophil development by IKAROS

IKAROS, encoded by *Ikzf1* and known to be particularly important for the development of lymphocytes, negatively regulates basophil development.[13,36] In IKAROS-deficient mice, the numbers of bone marrow BaPs and basophils are increased (Fig. 2B).[13] *In vitro* culture experiments using IKAROS-deficient bone marrow cells further demonstrated markedly enhanced differentiation into basophils in the absence of IKAROS. Chromatin immunoprecipitation (ChIP) and gene expression analyses revealed that IKAROS directly binds to *Cebpa* and *Hes1* loci, down- (*Cebpa*) or up- (*Hes1*) regulating histone H3 lysine 4 trimethylation (a chromatin signature of transcription initiation), and suppressing (*Cebpa*) or promoting (*Hes1*) expression of these genes.[13] *Hes1* encodes the transcription factor hairy and enhancer of split 1 (HES1) that has been shown to promote mast cell development and inhibit *Cebpa* expression *in vitro*,[37] although mast cells in HES1 knockout mice have not been analyzed. Thus, IKAROS acts in a cell-intrinsic way to inhibit

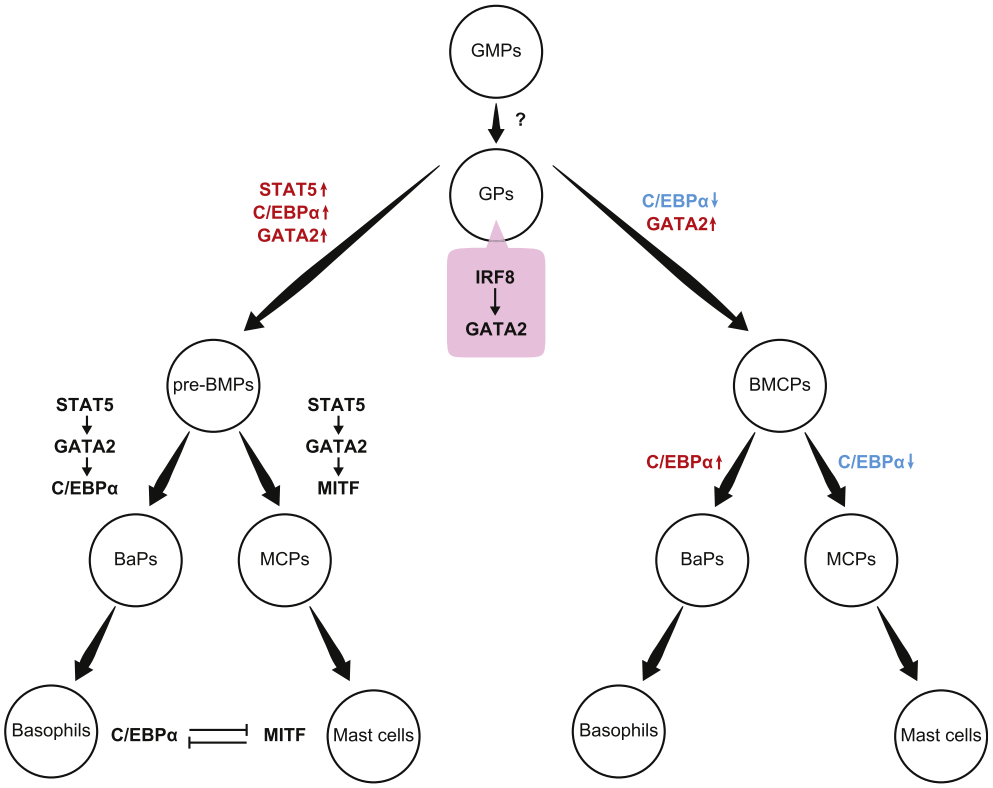


Fig. 3. A model for stage-specific regulation of basophil and mast cell development by transcription factors. IRF8, expressed in GMPs and GPs, induces GATA2 expression. When differentiation proceeds from GMPs (possibly through GPs) to BMCPs, GATA2 expression is upregulated, while C/EBPα expression is downregulated. When differentiation proceeds from GMPs (possibly through GPs) to pre-BMPs, the expression levels of STAT5, C/EBPα, and GATA2 are upregulated. BMCPs differentiate into BaPs upon upregulation of C/EBPα, whereas BMCPs differentiate into MCPs if C/EBPα remains suppressed. In pre-BMPs and downstream cells, the STAT5-GATA2 axis appears to induce the expression of C/EBPα or MITF. Induction of C/EBPα or MITF expression leads to the differentiation of these cells to BaPs or MCPs, respectively. C/EBPα and MITF mutually suppress each other's expression.

basophil development, probably by suppressing C/EBPα expression and promoting HES1 expression.[13]

Bone marrow MCPs and skin mast cells in IKAROS-deficient mice are as abundant as those in wild-type mice, and spleen BMCPs and MCPs are more abundant, suggesting that IKAROS is dispensable for the generation of these cells. Interestingly, however, the numbers of intestinal MCPs and mast cells are decreased (Fig. 2B).[13] This intestine-specific decrease is likely a result of the downregulation of α4 integrin expression in IKAROS-deficient mice, because α4 integrin is required for homing of mast cells to the intestine.[38]

Regulation of basophil and mast cell development by GATA1

GATA1 is known to be essential for the development of erythrocytes, megakaryocytes, and eosinophils.[39] Roles of GATA1 in basophil and mast cell development have been studied using three strains of mice[21–23,39]: mice in which both the enhancer and distal promoter but not coding region of *Gata1* are deleted (neoΔHS mice),[40,41] mice in which exons of GATA1 are conditionally deleted (*Gata1*$^{-/-}$),[42,43] and mice in which an enhancer with a high affinity double-GATA site but not coding region of *Gata1* is excised (ΔdblGATA).[44] The effects on mast cells vary among these strains. In neoΔHS mice, MCP and mast cell development is impaired,[22,45] whereas mast cells are present in the skin and stomach of *Gata1*$^{-/-}$ mice in numbers comparable to those in wild-type mice.[46] Although the number of mast cells in ΔdblGATA mice has not been investigated, mast cell development can be induced from bone marrow cells of these mice, similar to the wild type.[44,46] GATA1 was knocked out in *Gata1*$^{-/-}$ mice after birth, whereas neoΔHS mice had constitutively lower GATA1 expression from the embryonic stage, which may be the reason for the distinctive phenotypes of mast cell development. ΔdblGATA mice have been reported to possess a smaller number of BaPs and basophils (Fig. 2B).[23] The number of basophils in neoΔHS mice and *Gata1*$^{-/-}$ mice has not been investigated. Therefore, the induction of *Gata1* gene expression by auto-activation is necessary for BaP and basophil development *in vivo*, but probably is unnecessary for mast cell development.[23]

Regulation of basophil development by RUNX1

RUNX1, a key regulator of early hematopoiesis, also regulates basophil development.[9] *Runx1* is transcribed from both a distal promoter (P1) and a proximal promoter (P2) to produce transcripts with distinct 5′ UTRs.[47] In mice deficient in RUNX1 derived from the P1 promoter, the number of GPs, BMCPs, and mast cells do not change, but the numbers of BaPs and basophils are markedly reduced, suggesting a requirement for RUNX1 in the generation of basophils (Fig. 2B).[9] The molecular mechanism of RUNX1 regulation of basophil lineage development has not been clarified.

Functions of IRF8 in basophil and mast cell development

IRF8 is expressed in myeloid and B cell lineages and is required for the development of dendritic cells, monocytes, and eosinophils, while it suppresses neutrophil development.[48–52] Recently, we reported that IRF8 is important also for basophil and mast cell

development.[17] In *Irf8*$^{-/-}$ mice, the numbers of bone marrow pre-BMPs, BaPs, MCPs, and basophils are severely diminished, while bone marrow GPs, spleen BMCPs, tissue MCPs, and mast cells are as abundant as those in wild-type mice (Fig. 2B).[17,51] An analysis of IRF8 protein expression using IRF8-GFP chimeric knock-in mice[53] revealed that IRF8 is expressed in GPs but not in downstream progeny of the basophil and mast cell lineages.[17] Interestingly, transplantation and *in vitro* culture experiments revealed that IRF8 functions in GPs to promote the development of not only basophils but also mast cells.

A computational analysis of transcription factor binding motifs based on transcriptome data of wild-type GPs and IRF8-deficient GPs predicted that the GATA family transcription factors are likely to contribute to basophil and mast cell development downstream of IRF8. Although *Gata1* and *Gata2* mRNA expression levels are downregulated in *Irf8*$^{-/-}$ GPs, GATA1 protein is undetectable even in wild-type GPs, while GATA2 protein expression is readily detected in wild-type GPs and is markedly decreased in *Irf8*$^{-/-}$ GPs (Fig. 3). Moreover, it was revealed that when transduced into *Irf8*$^{-/-}$ progenitors, only GATA2 is able to restore differentiation into basophils and partially into mast cells. These results clarified that IRF8 induces GATA2 expression in GPs, facilitating basophil and mast cell development. Whether IRF8 directly induces *Gata2* remains unknown; however, we speculate that it may be indirect and mediated by other transcription factor(s), because the induction of GATA2 expression by forced expression of IRF8 requires substantial time.

Why tissue MCPs and mast cells are as abundant in *Irf8*$^{-/-}$ mice as in the wild type, despite the fact that *Irf8*$^{-/-}$ mice lack bone marrow MCPs and that IRF8 does promote mast cell development *in vitro*, has not been clarified. We speculate that both survival and proliferation of tissue MCPs and mast cells are enhanced, allowing them to reach normal levels, because of the extremely high blood IgE levels in *Irf8*$^{-/-}$ mice.[54] Such effects of IgE are known,[55] and tissue MCPs (but not bone marrow MCPs) and mast cells express FcεRI.

Analysis of genome-wide transcription factor binding regions in mast cells

For a comprehensive understanding of how transcription factors regulate cell differentiation, a genome-wide analysis using ChIP followed by high-throughput DNA sequencing (ChIP-seq) is a powerful approach. Göttgens *et al.* compared the SCF-dependent hematopoietic stem and progenitor cell (HSPC)-like cell line HPC7 with bone marrow-derived mast cells (BMMCs) and found that many transcription factors involved in development and function of HSPCs are expressed similarly in HPC7s and BMMCs.[56] These transcription factors include E2A/TCF3, ETS-related gene (ERG), Friend leukemia integration 1 (FLI1), GATA2, LIM domain only 2 (LMO2), Meis homeobox 1 (MEIS1), PU.1/SPI1, RUNX1, and stem cell leukemia (SCL): hereinafter referred to as "common transcription factors." Indeed, HSPCs and mast cells share characteristics such as the capacity for SCF-dependent self-renewal. However, these two cell types have substantially different morphologies and functions. Therefore, the authors performed ChIP-seq analyses of the common transcription factors in HPC7s and BMMCs.[56] The results showed that multiple transcription factors tend to bind contiguously to specific regions, but the genomic locations of these binding regions, as well as the gene expression patterns, differ substantially between HPC7s and BMMCs. An analysis of DNA motifs in the transcription factor binding sites revealed that the binding motifs of FBJ osteosarcoma oncogene (FOS) and MITF are significantly more overrepresented in BMMCs than HPC7s.[56] The authors then performed ChIP-seq analyses of FOS and MITF to demonstrate that these transcription factors, which are expressed more highly in mast cells than in HSPCs, indeed bind to the sites mentioned above in BMMCs. These results showed that the difference in binding sites of "common transcription factors" between HPC7s and mast cells may be caused by binding of cell-specific transcription factors. Interestingly, knockdown or knockout of the common transcription factor GATA2 in BMMCs resulted in downregulation of mast cell-specific genes such as *Kit* and *Mitf*.[56,57] Thus, common transcription factors and cell type-specific transcription factors are likely to cooperate to establish cell type-specific gene expression profiles.[56]

Recently, large active enhancer regions termed super-enhancers have been shown to be critical for the establishment of cell type-specific gene expression patterns.[58,59] In super-enhancers, there are region termed "hotspots" to which multiple transcription factors strongly bind.[58,60] We speculate that the regions discovered by Göttgens *et al.*, to which multiple transcription factors bind contiguously, include those in super-enhancers. Future analyses focusing on the role of super-enhancers in the development of basophils and mast cells may elucidate the mechanism by which the identities of similar but distinct cell types, basophils and mast cells, are established.

Discussion

As described above, the pathways and key transcription factors for the development of basophils and mast cells have been elucidated (Fig. 3). Specifically, GATA2 expression by IRF8 in GPs, and the induction of C/EBPα and MITF expression by the STAT5-GATA2 cascade in bipotential progenitors (pre-BMPs and possibly BMCPs) and downstream cells appear to be essential for promoting basophil and mast cell development. Because IRF8 is no longer expressed at the bipotential progenitor and downstream cell stages, it is conceivable that STAT5 takes over from IRF8 to induce GATA2 expression. Moreover, the mutually exclusive expression of C/EBPα and MITF underlies the basophil versus mast cell fate. In addition, IKAROS downregulates C/EBPα expression to suppress differentiation into basophils, presumably after the pre-BMP and BMCP stages.

Nevertheless, target genes of these transcription factors are not yet known on a genome scale. Therefore, ChIP-seq analyses of transcription factors and various histone modifications will be key for future studies. In addition, though various progenitors have been identified, their exact relationships and whether they are homogenous populations are still obscure. To resolve these issues, transcriptome analyses using single-cell RNA-seq and detailed cell tracking experiments are required. Indeed, in a recent study, single cell RNA-seq analysis of early myeloid progenitors (lineage markers^{-} Sca1^{-} c-Kit^{+} cells) indicated that these cells might be already transcriptionally primed toward a single cell lineage such as basophils.[61] Moreover, the analyses of several transcription factor-deficient mice would have to be re-conducted based on the current knowledge of the basophil and mast cell developmental pathways. Lastly, whether any changes in these developmental pathways occur with infections and allergies is still obscure. We expect that a deeper understanding of the differentiation of basophils and mast cells will contribute to the development of new therapies that control the production of basophils and mast cells.

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Conflict of interest

The authors have no conflict of interest to declare.

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