

Origin of Basophils and Mast Cells

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ABSTRACT

Basophils and mast cells are major players in the progression of allergic disorders. Although both cell types originate from hematopoietic stem cells, their lineage commitment pathways and mechanisms have been unsolved issues in hematology. Recent advances in the multicolor FACS system enable the prospective isolation of progenitor populations whose readouts are restricted to basophil and/or mast cell lineages. These newly-isolated progenitor subsets are helpful to understand the developmental machinery of basophil and mast cell lineages, leading to the possible exploitation of a novel therapeutic strategy for allergic and autoimmune disorders. In this review, we summarize the recent progress in our understanding of the basophil/mast cell ontogeny on a cellular basis.

KEY WORDS

basophils, C/EBP α , GATA-2, mast cells, progenitor

INTRODUCTION

It has been well established that mast cells play a pivotal role in allergic disorders.¹ Mast cells possess the $\alpha\beta\gamma 2$ form high-affinity receptor for IgE (Fc ϵ RI) and, upon crosslinking of the Fc ϵ RI-bound IgE with bivalent or multivalent antigens, they release diverse pre-formed and lipid mediators as well as cytokines, leading to the immediate hypersensitivity with local symptoms and the sequential inflammatory process. Recent studies demonstrated that mast cells also play a critical role in development of autoimmune disorders^{2,3} such as rheumatoid arthritis,⁴ multiple sclerosis,⁵ and systemic sclerosis.⁶ Thus, mast cells are now considered to be “a linker” between innate and acquired immunity.⁷

Basophils, another cell type which expresses Fc ϵ RI, have not been a subject of intensive research presumably because of their sparse distribution in hematopoietic organs, which represents less than 1% of blood leukocytes, and of their short life span. Furthermore, lack of basophil-deficient mouse models made it difficult to explore the *in vivo* function of basophils. In recent years, however, we are getting a new insight into the function of basophils in allergic disorders. Mukai and colleagues elegantly showed the crucial role of basophils in the development of

IgE-mediated chronic allergic inflammation by utilizing the Fc ϵ RI deficient mouse model.^{8,9} Basophils are involved in a variety of immune reactions including initiation of Th2 differentiation,¹⁰ progression of IgG-mediated systemic anaphylaxis,¹¹ and enhancement of humoral immune memory responses.¹² These observations collectively suggest that basophils besides mast cells are critical immunoregulators in diverse immune responses, and could be cellular targets to control allergic and autoimmune disorders. Thus, understanding the developmental machinery of basophils and mast cells is critical.

Both basophils and mast cells develop from hematopoietic stem cells. Basophils typically become mature in the bone marrow, and then enter the circulation. On the other hand, mast cell precursors leave the bone marrow before their terminal maturation, and home vascularized peripheral tissues such as a digestive tract where they become mature.^{13,14} The origin and developmental relationships of basophils and mast cells have long been the major unsolved issue in hematology. Focusing on adult murine hematopoiesis, this review summarizes the recent progress in our understanding of the cellular origin and developmental mechanisms of basophil/mast cell lineages.

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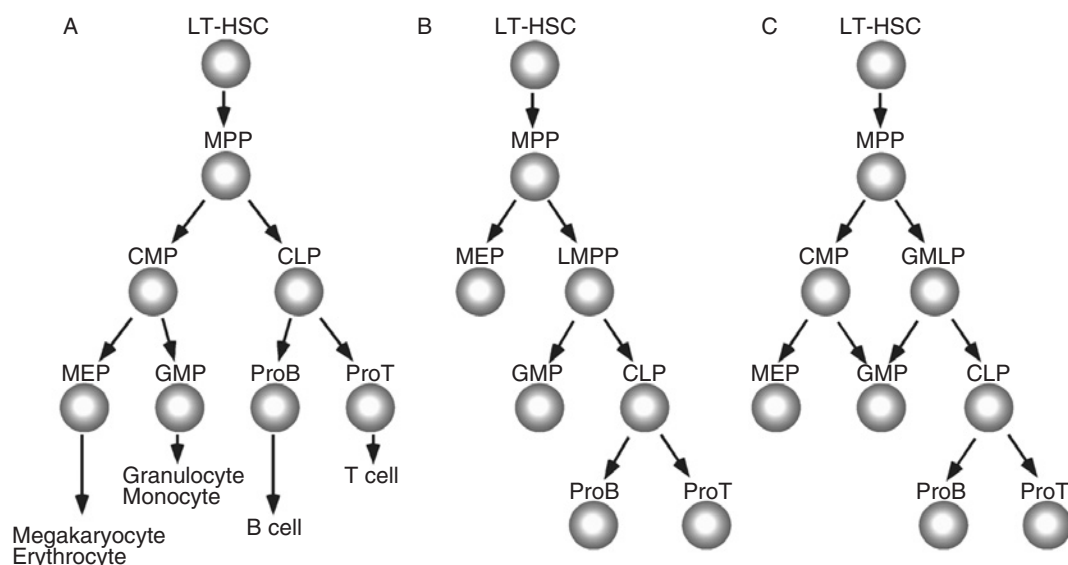


Fig. 1 Differentiation pathways in adult murine hematopoiesis. Hierarchical hematopoietic developmental pathways have been proposed based on the result of prospective purification of lineage-restricted progenitors. **(A)** Conventional model. First commitment step is the strict segregation of myeloid and lymphoid lineages. **(B and C)** Alternative models that are proposed by Adolfsson *et al.* **(B)** and our group **(C)**. LT-HSC, long-term hematopoietic stem cells; MPP, multipotential progenitors; CLP, common lymphoid progenitors; CMP, common myeloid progenitors; GMP, granulocyte/monocyte progenitors; MEP, megakaryocyte/erythrocyte progenitors; LMPP, lymphoid-primed multipotent progenitors; GMLP, granulocyte/monocyte/lymphoid progenitors.

HIERARCHICAL LINEAGE COMMITMENT IN ADULT MURINE HEMATOPOIESIS

The most primitive HSCs with a long-term (LT) self-renewal potential can be purified as Thy1^{lo} or CD34⁺ cells within the Lin-Sca-1⁺c-Kit⁺ (LSK) fraction.^{15,16} Thy1⁺ or CD34⁺ LSK cells are short-term (ST)-HSCs or multipotential progenitors (MPPs), and are capable of reconstituting multi-lineage hematopoiesis only for ~3 months. The myeloid vs. lymphoid lineage commitment occurs after the MPP stage. In the myeloid pathway, common myeloid progenitors (CMPs), granulocyte/monocyte progenitors (GMPs), and megakaryocyte/erythrocyte progenitors (MEPs) were purified within the Lin-Sca-1⁺c-Kit⁺ fraction.¹⁷ In the lymphoid pathway, common lymphoid progenitors (CLPs) were isolatable as the IL-7R α ⁺Lin-Sca-1^{lo}c-Kit^{lo} population.¹⁸ Based on the existence of these prospectively isolatable stem and progenitor cells, the hierarchical hematopoietic map has been established (Fig. 1A).

However, this conventional model has been challenged by several recent studies. Adolfsson *et al.* reported that a fraction of MPPs expressing Flt3 at a high level mostly lacked a megakaryocyte/erythroid (MegE) potential and was largely primed for the lymphoid lineage.¹⁹ They claimed that MPPs might sequentially lose lineage potentials starting with the

MegE then the granulocyte/monocyte (GM) readout during the lymphoid lineage commitment (Fig. 1B). More recently, our group identified precursors of CMPs or CLPs in the MPP fraction by tracing the transcriptional activation of GATA-1, a MegE lineage-related transcription factor,²⁰ and PU.1, a GM/lymphoid transcription factor,²¹ respectively.²² The GATA-1⁺ LSK cells were precursors of conventional CMPs and the PU.1⁺ LSK cells represented the granulocyte/monocyte/lymphoid-restricted progenitor (GMLP) activity. This new finding reconciled inconsistency of previous models, and we have proposed the revised high-resolution map of hematopoietic development (Fig. 1C). In this new model, GMPs develop from both CMPs and GMLPs. Taken together, the recent identification of these new progenitor subsets led to better understanding of the early commitment process.

ONTOGENY OF BASOPHILS AND MAST CELLS

The developmental pathway of basophils has been controversial.²³ By utilizing colony formation assays, several groups claimed that basophils developed from the common progenitors for basophil and eosinophil lineages.^{24,25} It was also reported that granulocytes with the hybrid characteristics of basophils and eosinophils developed from normal cord

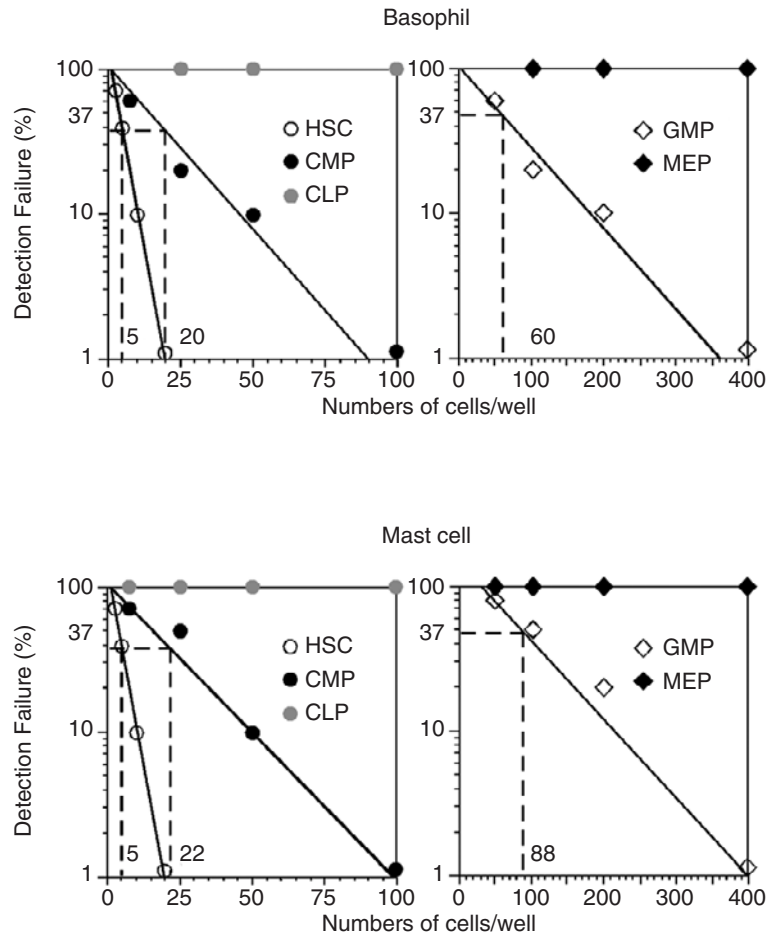


Fig. 2 Limiting dilution analyses of basophil and mast cell development from purified stem and progenitor cells. Basophils and mast cells are efficiently developed in the presence of Slf, IL-3, IL-6 and IL-9 when enumerated on day 7 and 21, respectively. One in 60 and 88 single GMPs give rise to basophils and mast cells, respectively.

blood progenitors *in vitro*.²⁶ In contrast, cells possessing the ultra-structural features typical of both basophils and mast cells were identified in patients with myeloproliferative disorders.²⁷ The monoclonal antibody 97A6, which recognizes ectonucleotide pyrophosphatase/phosphodiesterase-3 (E-NPP3; CD203c), was shown to react only with basophil/mast cell lineage cells.²⁸ Furthermore, the existence of metachromatic cells with features of both basophils and mast cells was reported in patients with asthma.²⁹ This controversy regarding the basophil/mast cell/eosinophil development should be resolved by tracing their developmental pathway based on prospective isolation of progenitors committed for each lineage.

Mast cells reside predominantly in peripheral tissues.³⁰ Mast cell precursors leave the bone marrow prior to completion of their maturation, migrate into various tissues, and then differentiate into mature functional mast cells. The limit dilution analyses or

colony formation assays demonstrated that mast cell colony-forming cells reside within the bone marrow, spleen, peripheral blood, mesenteric lymph node, and gut mucosa.^{31,32} Rodewald *et al.* first identified the committed progenitors for the mast cell lineage in mouse fetal blood.³³ This mast cell progenitor was defined by the phenotype of Thy-1^{lo}c-Kit^{hi} and lacked the expression of FcεRIα transcript. In adult murine hematopoiesis, however, neither mast cell progenitors nor basophil progenitors had been identified.

IDENTIFICATION OF BASOPHIL/MAST CELL LINEAGE COMMITTED PROGENITORS IN ADULT MURINE HEMATOPOIESIS

To track the basophil and mast cell potentials, the frequency of basophil and mast cell readouts was estimated in the culture of purified stem and progenitor subsets (Fig. 2). HSCs and CMPs but not CLPs were capable of generating basophils and mast cells.

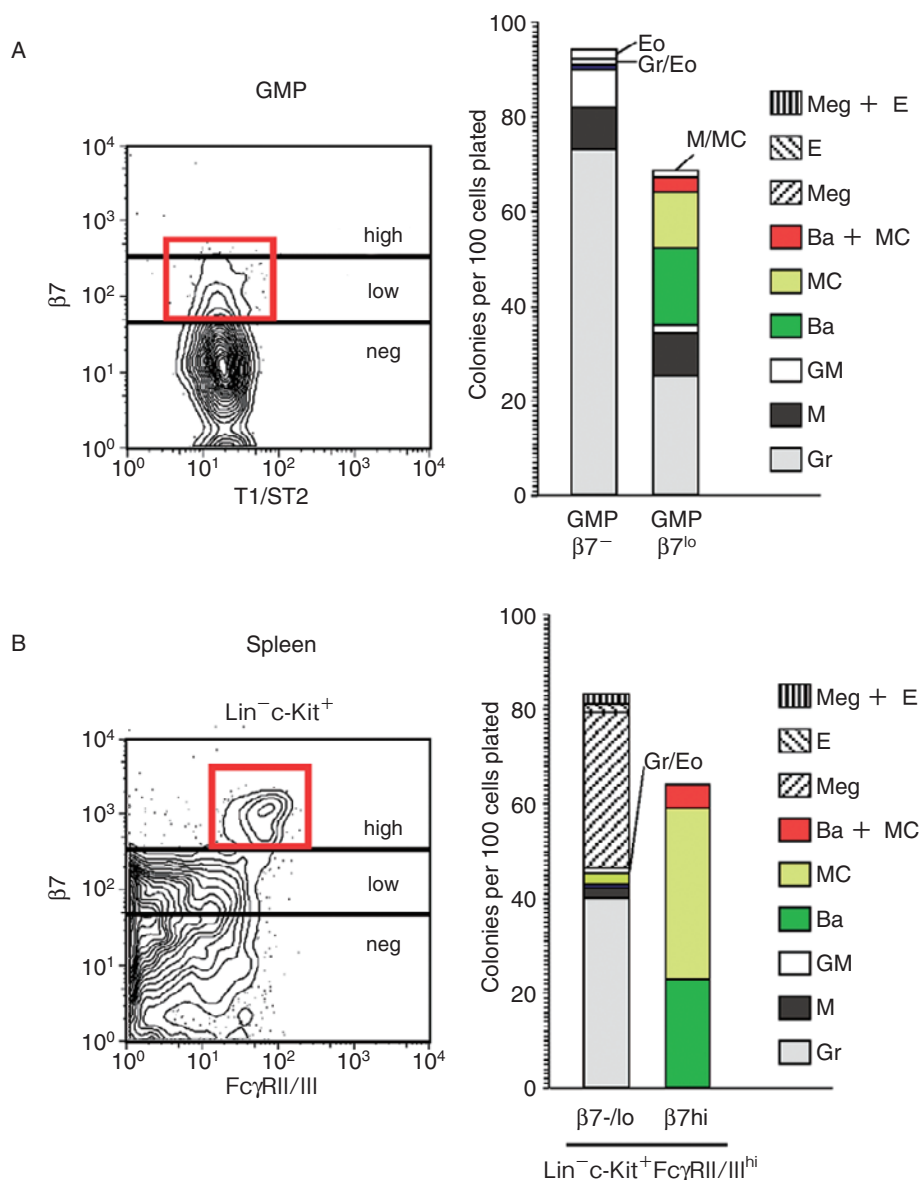


Fig. 3 Identification of basophil/mast cell committed progenitors in adult murine hematopoiesis. **(A)** The expression of $\beta 7$ and T1/ST2 of GMPs (left) and clonogenic analysis of $\beta 7^{lo}$ GMPs (right). **(B)** The expression of $\beta 7$ and Fc γ RII/III of Lin $^-$ c-Kit $^+$ spleen cells (left) and clonogenic analysis of Lin $^-$ c-Kit $^+$ $\beta 7^{hi}$ spleen cells (right).

Downstream of CMPs, GMPs but not MEPs differentiated into both lineages. These data collectively suggest that the basophil and mast cell development occurs along with the granulocyte/monocyte but not lymphoid or erythroid differentiation pathway.

$\beta 7$ -integrin is a type I transmembrane glycoprotein expressed mainly on lymphocytes. It plays a pivotal role in the trafficking of T cells into mucosal organs.³⁴ Mucosal mast cells also possess $\beta 7$ -integrin,³⁵ mediating the tissue specific homing of intestinal mast cell precursors.³⁶ Accordingly, this molecule might be useful marker to identify the committed mast cell pro-

genitors. Galli's group first reported the identification of the mast cell committed progenitors (MCPs) in adult murine bone marrow.³⁷ They fractionated the Lin $^-$ Sca-1 $^+$ c-Kit $^+$ bone marrow progenitors according to the expression profile of $\beta 7$ -integrin and CD27 and found that the mast cell colony forming activity was concentrated in the $\beta 7^+$ fraction. This $\beta 7^+$ fraction was further subdivided into the T1/ST2 $^+$ and the T1/ST2 $^-$ cells. $\beta 7^+$ T1/ST2 $^+$ cells differentiated only into mast cells. $\beta 7^-$ CMPs and GMPs did not form mast cell colonies, suggesting that MCPs may directly develop from MPPs independent of the myeloid pathway. The

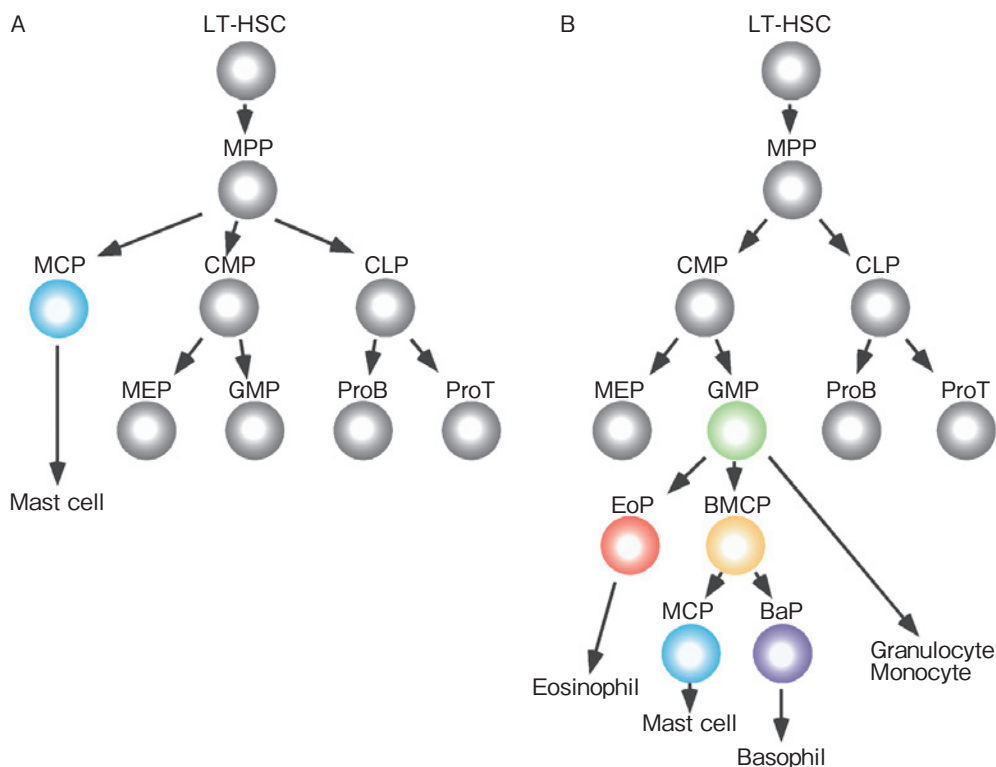


Fig. 4 Proposed basophil/mast cell developmental pathway in adult murine hematopoiesis. **(A)** Chen *et al.* identified MCPs downstream of MPPs. **(B)** Our model does not fit **(A)** because BMCPs, BaPs, MCPs and EoPs are identified downstream of GMPs. BMCP, basophil/mast cell progenitors; BaP, basophil progenitors; MCP, mast cell progenitors; EoP, eosinophil progenitors.

proposed developmental scheme of the mast cell lineage based on these observations is shown in Figure 4 A.

In another report, by using two monoclonal antibodies (AA4 and BGD6) raised against rat mast cell line, immature mast cells were isolatable in adult murine bone marrow as the AA4-BGD6⁺ cells. These cells also expressed CD34, CD13, and c-Kit, but not FcεRI.³⁸ It is unclear whether this AA4-BGD6⁺ progenitor overlaps with the β7⁺T1/ST2⁺ population.

In our hands, a small fraction of GMPs expressed β7-integrin at a low level³⁹ (Fig. 3A left). The purified β7⁺GMPs preferentially differentiated into basophils and mast cells in vitro but still possessed the significant GM potential, suggesting that this population have not fully committed to the basophil and/or mast cell lineages³⁹ (Fig. 3A right). As a result of our intensive search for β7⁺ progenitors in various hematopoietic organs, the Lin⁻c-Kit⁺ cells expressing β7-integrin at a high level were isolatable in the spleen³⁹ (Fig. 3B left). Surprisingly, these β7^{hi} spleen progenitors gave rise only to basophil and mast cell colonies. Furthermore, a significant fraction (~10 %) of them were bipotent for the basophil and mast cell lineages at the single cell level³⁹ (Fig. 3B right). Thus, this newly-isolated population in the spleen was named as

the basophil/mast cell progenitor (BMCP), providing a formal proof for a common origin of the basophil and mast cell lineages. By using another basophil/mast cell lineage specific marker, FcεRIα, monopotential progenitors for the basophil (basophil progenitor; BaP) and the mast cell lineage were isolatable in the bone marrow and the intestine, respectively.³⁹ The BaP and MCP were defined as the Lin⁻CD34⁺FcεRIα^{hi}c-Kit^{-/lo} and the Lin⁻CD34⁺β7^{hi}FcεRIα^{lo} phenotypes, respectively. In vitro cultures, GMPs were capable of generating BMCPs, BaPs, and MCPs irrespective of the expression of β7-integrin, and BMCPs also gave rise to BaPs and MCPs. Furthermore, the murine eosinophil lineage-committed progenitor (EoP) was also identified downstream of GMPs as the Lin⁻CD34⁺IL-5Rα⁺c-Kit^{lo} phenotype.⁴⁰ The lineal relationships among these progenitor populations are schematized in Figure 4B.

TRANSCRIPTIONAL REGULATION AT THE DIVERGING POINTS OF BASOPHIL, MAST CELL, AND EOSINOPHIL LINEAGES DOWNSTREAM OF GMPs

BMCPs, BaPs, MCPs, and EoPs are all derived from GMPs (Fig. 4B). The question is how each lineage specification is made. The numerous gene-inactiva-

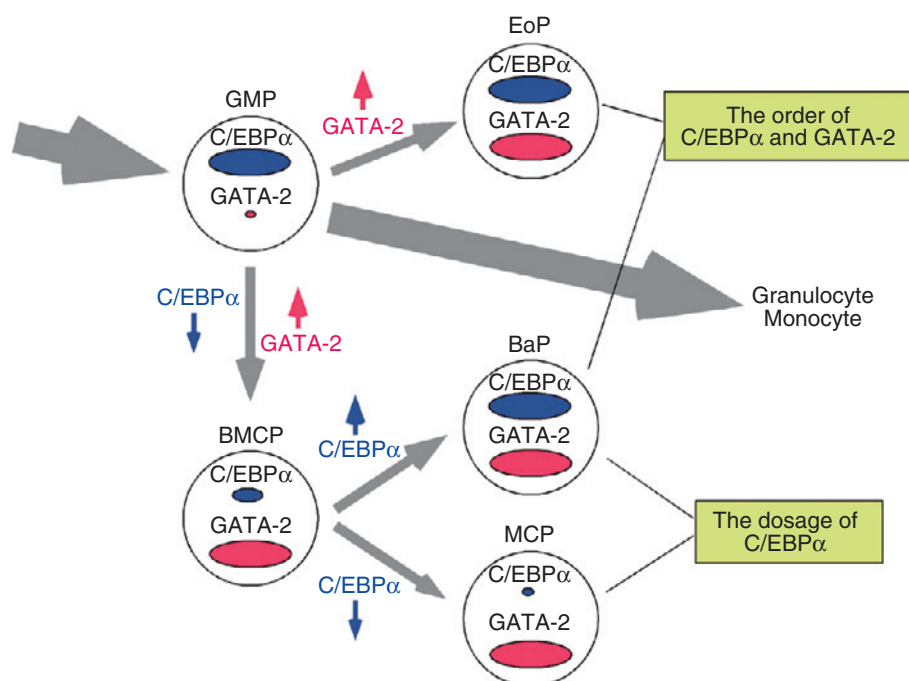


Fig. 5 Schematic presentation of roles of transcription factors in lineage specification to basophils, mast cells, and eosinophils. Up-regulation of GATA-2 instructs GMPs to differentiate into eosinophils. If GMPs down-regulate C/EBP α and up-regulate GATA-2, they become BMCPs. For the basophil development, C/EBP α needs to be reactivated after BMCP stage, whereas the continuous suppression of C/EBP α is required for the mast cell differentiation.

tion or overexpression studies have revealed that the antagonistic or cooperative effects of multiple transcription factors, called a “transcription factor network”, play a pivotal role in establishment of lineage diversity.^{41,42}

The CCAAT enhancer-binding protein α (C/EBP α) is known to be an indispensable transcription factor for the development of GMPs,^{43,44} regulating the transactivation of multiple myeloid genes such as G-CSF receptor and myeloperoxidase.⁴⁵ Along with the basophil/mast cell development downstream of GMPs, C/EBP α was expressed at the highest level in BaPs followed by GMPs, while BMCPs and MCPs expressed lower levels of C/EBP α as compared to GMPs.⁴⁶ The depletion of C/EBP α at the BMCP stage results in their exclusive differentiation into mast cells, whereas BMCPs overexpressing C/EBP α differentiate mainly into basophils. Furthermore, MCPs enforced with C/EBP α are reprogrammed into basophils.³⁹ These data collectively suggest that the dosage of C/EBP α regulates the specification of the basophil versus mast cell lineage.

Not only “dosage” but also “order” of expression of transcription factors might be important.⁴⁶ If we compare the expression profiles of transcription factors and lineage-related genes, BaPs and EoPs display almost similar expression pattern.⁴⁶ The question is the

molecular mechanisms of basophil or eosinophil lineage commitment downstream of GMPs. GMPs express C/EBP α but not GATA-2, which is the major transcription factor for megakaryocyte/erythrocyte development. In contrast, MCPs express GATA-2 but not C/EBP α . GMPs with the enforced expression of GATA-2 differentiate exclusively into eosinophils,⁴⁶ whereas MCPs enforced with C/EBP α generate pure basophil colonies.³⁹ These data led us to hypothesize that the order of expression of C/EBP α and GATA-2 should be critical for lineage specification in basophil versus eosinophil development. CLPs transduced with C/EBP α alone generate granulocyte and monocyte colonies. When CLPs are introduced with C/EBP α and then GATA-2, they give rise to eosinophil colonies. Then, the order is switched. CLPs introduced with GATA-2 generate mast cell or basophil colonies, whereas CLPs introduced GATA-2 then C/EBP α form pure basophil colonies. Collectively, these data show that the dosage and the order of the expression of C/EBP α and GATA-2 simply regulate myeloid developmental programs for granulocytes/monocytes, basophils, mast cells, and eosinophils.⁴⁶ The interplay of C/EBP α and GATA-2 at the fate decision of these myeloid lineages is summarized in Figure 5. It still remains unclear the mechanism as to how these key transcription factors are regulated dur-

ing basophil, mast cell and eosinophil development.

CONCLUSION

New and unique basophil functions are now emerging. The physiological roles of basophils and mast cells must be revised. With this view, newly-identified murine lineage-restricted progenitors should be very useful to understand their developmental machinery. To apply such information in understanding human disorders, it is critical to identify the counterpart precursor populations in human hematopoiesis. Recently, Mori *et al.* successfully identified the human eosinophil progenitors (hEoPs) as the Lin⁻CD34⁺CD38⁺IL-3R α ⁺CD45RA⁻IL-5R α ⁺ phenotype.⁴⁷ Similar news in the field of basophil and mast cell biology is awaited.

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