

Molecular Mechanisms of Mast Cell Development

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Mast cells are progeny of multipotential hematopoietic stem cells (MHSCs) [1–5]. MHSCs give rise to common lymphoid progenitors (CLPs) and common myeloid progenitors (CMPs). Although mast cells had been considered to be progeny of CMPs, mast cell-committed progenitors (MCPs) were recently shown to be derived from MHSCs as an independent lineage (Fig. 1) [6]. Although most progeny of CMPs complete their differentiation within the bone marrow, MCPs leave the bone marrow, invade connective or mucosal tissue, and then proliferate and differentiate into morphologically identifiable mast cells [6,7]. The phenotype of mast cells, connective tissue type or mucosal, is determined by each site of final differentiation [4,6]. Most progeny of CMPs lose proliferation potential after maturation, but mature mast cells retain proliferation potential [8]. Even after functional activation and degranulation, mast cells can proliferate and reacquire the original morphology [9,10]. The developmental process of mast cells is unique, and we describe here molecules that are used for the process.

Mast cell-committed progenitors

Morphologically identifiable mast cells were observed in the skin of mouse embryos as early as day 15 of the pregnancy [11,12]. In spite of their development in the skin of embryos, morphologically identifiable mast cells

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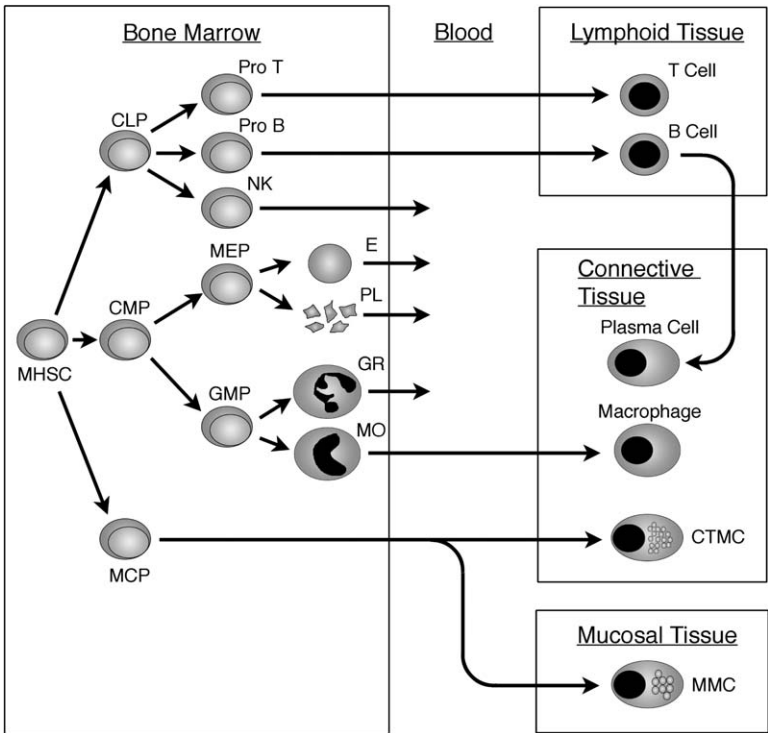


Fig. 1. Development of mast cells from the MHSC. CTMC, connective tissue-type mast cell; E, erythrocyte; GMP, granulocyte/macrophage progenitor; GR, granulocyte; MEP, megakaryocyte/erythrocyte progenitor; MMC, mucosal mast cell; MO, monocyte; NK, natural killer cell; PL, platelet.

never developed in other tissues of mice before birth. Mast cells developed 4 weeks after birth in the glandular stomach and 6 weeks after birth in the peritoneal cavity [13].

Although mast cell progenitor activity was demonstrated in 9.5-day yolk sacs and 13-day livers of mouse embryos [11,14], the activity may represent the presence of MHSCs rather than that of MCPs. Rodewald and colleagues [15] isolated MCPs from peripheral blood of mouse embryos as a Thy-1^{low} KIT^{high} cell population. The fetal MCPs contained basophilic granules and could not differentiate to erythrocytes, neutrophils, macrophages, B cells, or T cells. Moreover, the Thy-1^{low} KIT^{high} fetal MCPs expressed mast cell-specific proteases (ie, mast cell carboxypeptidase A [MC-CPA]), mouse mast cell protease (mMCP)-2 and mMCP-4, at least at the reverse transcriptase polymerase chain reaction (RT-PCR) level. The expression of mast cell-specific proteases seemed to precede their immigration into peripheral tissues. In spite of the expression of mast cell-specific proteases, the Thy-1^{low} KIT^{high} fetal MCPs did not express Fcε receptors. After culturing with interleukin (IL)-3 and KIT ligand (KITL), however, they expressed the functional Fcε receptors [15].

Recently, Chen and coworkers [6] isolated MCPs from the bone marrow of adult mice. They are phenotypically $\text{Lin}^- \text{KIT}^+ \text{Sca-1}^- \text{Ly6c}^- \text{Fc}\epsilon \text{ receptor I (RI)}\alpha^- \text{CD27}^- \beta 7^+ \text{T1/ST2}^+$. Although whether MCPs in the peripheral blood of adult mice have the same phenotype as MCPs of the bone marrow remains to be determined, the phenotype of the adult MCPs is different from that of the fetal MCPs isolated by Rodewald and colleagues [15]. The adult MCPs did not contain basophilic granules, but the fetal MCPs contained a small number of basophilic granules and thus seemed to be more mast cell-like than the adult MCPs. In the experiments of Rodewald and colleagues [15], blood was collected from late embryos undergoing a rapid increase in the number of mast cells in the skin [11,12]. The difference may simply reflect the difference in the ages of the animals used in the two studies. It may be plausible that MCPs identified in the adult bone marrow represent a steady-state pool of MCPs, however.

Chen and coworkers [6] used $\text{Lin}^- \text{KIT}^+ \text{Sca-1}^-$ cells as a pool of hematopoietic progenitors, and $\text{Fc}\epsilon \text{RI}\alpha^+$ mature mast cells were removed. As a negative selection marker for MCPs, they used CD27 that is expressed by MHSCs and CMPs. The T1/ST2, which was recently identified to be the receptor of an IL-1-like cytokine (IL-33) [16], and $\beta 7$ integrin subunit were used as positive selection markers. The $\beta 7$ had been considered to be necessary for the development of only mucosal mast cells. Blocking of $\alpha 4$ or $\beta 7$ integrin by antibodies inhibited the increase in mucosal mast cells in the intestinal epithelium of rats that were infected with *Nippostrongylus brasiliensis* [17]. Expression of integrin $\alpha 4 \beta 7$ seemed to be necessary for migration of mast cell precursors into the intestinal epithelium of mice [18]. The results of Chen and coworkers [6] indicate that the $\beta 7$ is expressed by murine MCPs that can differentiate not only to mucosal mast cells but to connective tissue-type mast cells.

Jamur and colleagues [19] used two monoclonal antibodies and isolated "undifferentiated mast cells" in the bone marrow of adult mice. The isolated cells differentiated only to granulated mast cells. The morphology of these cells is similar to that of MCPs isolated by Chen and coworkers [6].

MCPs invade mucosal or connective tissue before maturation. Chemoattractant of MCPs has not been identified, but immature IL-3-dependent mast cells were attracted by leukotriene B_4 (LTB_4), which is released from mature mast cells [20]. This autocrine role of LTB_4 in regulating tissue mast cell number is apparent in vitro, but the actual in vivo significance of LTB_4 remains to be confirmed.

Cytokines and their receptors involved in mast cell development

KIT ligand

We found that W/W^v and Sl/Sl^d mice are deficient in mast cells [21,22]. The W locus was then identified to encode KIT receptor tyrosine kinase [23,24],

and the *Sl* locus was identified to encode the KITL [25–31]. Lack of mast cells in connective and mucosal tissues of *Kit^W/Kit^{W-v}* and *Kitl^{Sl}/Kitl^{Sl-d}* mutant mice directly indicates the essential role of KITL-KIT signals for the development of connective tissue-type and mucosal mast cells. In addition, we found mutant rats with a loss-of-function mutation of the KIT gene [32]. The *Kit^{W^s}/Kit^{W^s}* rats lack mast cells, as in the case of *Kit^W/Kit^{W-v}* mice [33]. In spite of the lack of mast cells in tissues of *Kit^{W^s}/Kit^{W^s}* rats, the number of basophils increased in peripheral blood of *Kit^{W^s}/Kit^{W^s}* rats as well as in that of control *+/+* rats after infection of *N brasiliensis*, indicating the lack of necessity of KITL-KIT signals for the development of basophils [34].

KIT and KITL also play significant roles for adhesion of mast cells. KIT expressed on the surface of mast cells and KITL expressed on the surface of fibroblasts are used for their adhesion. For the adhesion, the tyrosine kinase activity of KIT is not necessary [35]. KIT autophosphorylated by binding of KITL also activates phosphoinositide 3'-kinase (PI3K), and this is essential for adhesion of mouse cultured mast cells (CMCs) to extracellular matrix proteins [36,37].

Interleukin-3

CMCs develop from hematopoietic cells of mice in the presence of IL-3 [4,5]. In this condition, CMCs can develop even from hematopoietic cells of *Kit^W/Kit^{W-v}* mice [4]. Moreover, mast cells developed in tissues of *Kit^W/Kit^{W-v}* mice with local inflammation [38], where cytokines derived from infiltrating T cells may be abundant. In contrast to intact *Kit^W/Kit^{W-v}* and *Kitl^{Sl}/Kitl^{Sl-d}* mice in which mast cells are deficient in connective and mucosal tissues, intact nude athymic mice lack mast cells only within the epithelium of the small intestine [39].

A huge number of mucosal mast cells develop in the intestinal epithelium within 2 weeks after infection of helminths such as *N brasiliensis*, *Trichinella spiralis*, and *Strongyloides venezuelensis*. This intestinal mast cell hyperplasia and the following expulsion of helminths did not occur in nude mice [39]. IL-3 plays a significant role in the development of intestinal mast cell hyperplasia [5]. Its particular contribution was shown by the attenuation of helminth-elicited mast cell hyperplasia in normal mice that received antibodies to IL-3 [40] and in mice with disruption of IL-3 gene. The disruption of the IL-3 gene and the loss-of-function mutation of the KIT gene showed additive effects for prolongation of the *S venezuelensis* infection [41].

In nude mice and IL-3 gene-disrupted mice, mast cells did not decrease in connective tissues. IL-3 supported the development and survival of mouse CMCs [4,5], but it did not support the development of human CMCs because of their poor expression of IL-3 receptors [42]. For development of human CMCs, KITL is indispensable [43–45]. When human hematopoietic cells were cultured with IL-3, development of basophils but not mast cells

was observed [46,47]. The major source of IL-3 seems to be T cells, but other inflammatory cells and some epithelial cells may produce IL-3.

Transduction of KIT ligand and interleukin-3 signals

KITL and IL-3 induce signal transduction through several pathways, including PI3K, phospholipase C, protein kinase C, Ras mitogen-activated protein (MAP) kinase cascade, Janus kinases (JAKs), and signal transducers and activators of transcription (STATs). The sum of the studies suggested the relative importance of PI3K, Ras-MAP kinase cascade, and STAT5 for KITL- and IL-3-induced development and survival of mast cells (Fig. 2) [48–50].

Binding of dimerized KITL induced the dimerization of KIT [51]. This led to the autophosphorylation of KIT on tyrosine and its association with substrates of various kinds. The PI3K/Akt system is one of the major pathways [52]. PI3K is composed of an 85-kd regulatory subunit and a 110-kd catalytic subunit. The 85-kd regulatory subunit is associated with activated KIT through the Src homology 2 (SH2) domain and is then phosphorylated on tyrosine. The binding of IL-3 to its receptor also results in the phosphorylation of the 85-kd subunit of PI3K [48]. The 110-kd catalytic subunit of the activated PI3K produces phosphatidylinositol-3, 4-bisphosphate, which, in turn, is used for phosphorylation of Akt, a serine-threonine kinase. The activated Akt is involved in the inhibition of apoptosis via inactivation of FOXO3a (forkhead box, class O3A) and its transcription target Bim (Bcl-2 interacting modulator of cell death) [53].

Mice bearing a mutant KIT unable to recruit the p85 regulatory subunit of PI3K showed a decrease in peritoneal mast cells [54]. Phosphorylation of

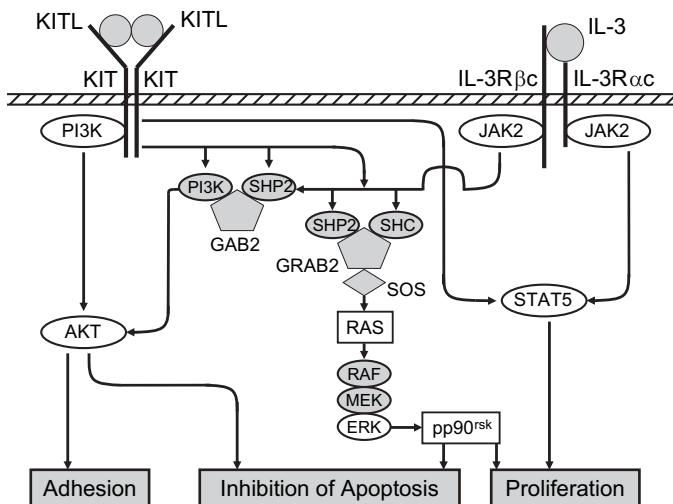


Fig. 2. Major signal transduction pathways downstream of KIT and IL-3 receptor.

a particular tyrosine (Tyr-719 in an insert of mouse KIT kinase domain) was essential for association of KIT with the 85-kd subunit of PI3K [54]. Because activation of PI3K is necessary for adhesion of mast cells to fibronectin, the substitution of Tyr-719 to phenylalanine resulted in abolishment of the adhesion. Conversely, the substitution of another tyrosine (Tyr-821 of mouse KIT) to phenylalanine impaired the KITL-dependent proliferation and survival of CMCs but did not impair the KITL-dependent adhesion [36].

When the gene encoding the 85-kd regulatory subunit of PI3K was disrupted, the mutant mice lacked mast cells in the gastrointestinal canal and peritoneal cavity but possessed mast cells in the skin [55]. As a result, they were highly susceptible to *S venezuelensis* infection and showed impaired resistance against acute bacterial peritonitis. The presence of mast cells is essential for innate immunity against acute bacterial peritonitis [56,57]; however, systemic anaphylactic shock responses normally occurred in the mutant mice because of the presence of skin mast cells [55].

Another major signaling pathway is the Ras/MAP kinase cascade [48–50,58,59]. The activated KIT recruits SH2-containing proteins, such as Grb2, Shc, and SHP2. Grb2 may bind KIT directly or indirectly through the interaction with Shc or SHP2. Because Grb2 is constitutively associated with Sos, a guanine nucleotide exchange factor, the recruitment of Grb2 to the activated KIT resulted in colocalization of Sos and Ras and the subsequent activation of Ras. This promotes the interaction of Ras with Raf serine-threonine kinase and then the activation of MEK (a MAP kinase). MEK phosphorylates extracellular signal-regulated kinase (ERK) (another MAP kinase), and ERK phosphorylates a number of substrates, including pp90^{tsk}. Alternatively KITL and IL-3 signaling may also activate JNK kinase, a MAP kinase activated in response to stress [50,58].

Substitution of tyrosine residues in the juxtamembrane domain of KIT (tyrosines 567 and 569 of mouse KIT) to phenylalanines reduced its potential to activate ERK MAP kinase. This resulted in depletion of mast cells in the tail dermis, peritoneal cavity, and stomach [60].

Gab2 is an adaptor molecule and is tyrosine-phosphorylated on stimulation of KITL or IL-3 [61]. Phosphorylated Gab2 bound SHP2 and the 85-kd regulatory subunit of PI3K. The phosphorylation level of the ERK MAP kinase was lower in Gab2 gene-disrupted mice than in the normal controls. The KITL-induced Akt phosphorylation was also impaired in the Gab2 gene-disrupted mice. The number of mast cells decreased in the stomach and peritoneal cavity of the Gab2 gene-disrupted mice [61].

KITL and IL-3 activated STAT5. Ryan and coworkers [49] studied the effects of STAT5 deficiency on mouse mast cell development and survival. CMCs derived from STAT5 gene-disrupted mice survived in the presence of KITL and IL-3 but did not survive in the presence of either cytokine alone. Moreover, mast cells were not identified in tissues of STAT5

gene-disrupted mice and in tissues of Kit^W/Kit^{W-v} mice that had received bone marrow transplantation from STAT5 gene-disrupted mice.

Other cytokines

IL-3 was sufficient to support the formation of colonies in methylcellulose by mouse CMCs, but IL-3 and IL-4 were necessary for the formation of colonies by mouse peritoneal mast cells [62]. Almost all peritoneal mast cells of mice can form colonies in the presence of IL-3 and IL-4 [63]. IL-4 showed an additive effect on the IL-3-dependent proliferation of mouse CMCs [64], but it suppressed the KITL-dependent proliferation of mouse and human CMCs by downregulating their KIT expression [65,66]. Moreover, IL-4 suppressed the KITL-induced adhesion of human CMCs to extracellular matrix proteins, such as fibronectin, laminin, and collagens [37].

Austen and Boyce and their coworkers [5,67,68] established a common culture system for mouse and human mast cells. They cultured mouse bone marrow or human cord blood cells in the presence of KITL, IL-6, and IL-10. KITL and IL-6 supported the proliferation and differentiation of mast cell precursors, and IL-10 suppressed the competing growth of macrophages. Stimulation of mouse CMCs by IL-10 induced the expression of mMCP-1 and mMCP-2, which characterized mucosal mast cells [5,69].

IL-9 [70,71], IL-10 [72], and IL-15 [73] showed a positive effect on IL-3-dependent development and survival of mouse CMCs, whereas IL-12 demonstrated a negative effect [74]. The administration of exogenous IL-12 suppressed the mucosal mast cell hyperplasia and delayed the expulsion of *Trichinella spiralis* [74]. The effect of IL-18 on mucosal mast cell hyperplasia is controversial. IL-18 gene-disrupted mice are highly resistant to *T spiralis*, suggesting a negative effect of IL-18 on the development of mucosal mast cells [75]. Conversely, the simultaneous administration of IL-2 and IL-18 enhanced the mucosal mast cell hyperplasia in normal mice and accelerated the expulsion of *S venezuelensis* [76].

Cytokines other than these interleukins also influence the development of mast cells. Thrombopoietin augmented the KITL-dependent proliferation of human CMCs [77]. Transforming growth factor (TGF)- β suppressed the IL-3-dependent proliferation of mouse CMCs [78]. It also prevented KITL-mediated rescue of mouse CMCs from apoptosis after deprivation of IL-3 [79]. Conversely, the addition of TGF β induced the expression of mMCP-1 and integrin α E in mouse CMCs [80,81].

Transcription factors that affect development of mast cells

GATA-1 and GATA-2

GATA transcription factors are a highly conserved family of zinc finger proteins [82]. The key role of GATA-1 for differentiation of erythrocytes,

platelets, and eosinophils was shown using GATA-1 gene-disrupted mice [83–85]. In the case of mast cells, however, the key role was not demonstrated by this approach, because GATA-1 gene-disrupted mutants were embryonic lethal and because differentiation of mast cells is better studied in adult animals.

Chimeric mice derived from a mixture of normal and GATA-1^{null} embryonic stem cells contained apparently normal numbers of mast cells deriving from the GATA-1^{null} clone [86,87]. Conversely, the skin tissue of heterozygous female mice carrying a GATA-1 expression mutation (GATA-1 gene locates on the X chromosome) contained mast cells whose granules were stained with Alcian blue but not with berberine sulfate, suggesting a defect in mast cell differentiation [88]. No staining with berberine sulfate indicates the decreased content of heparin in mast cell granules [89].

Using the GATA-1^{low} mice with a targeted deletion of upstream enhancer and promoter sequences of the GATA-1 gene, Migliaccio and colleagues [90] showed that the reduced expression of the GATA-1 gene resulted in amplification of progenitors that may differentiate to mast cells, increased apoptosis of mast cell precursors, and defective differentiation of mature mast cells. Interestingly, the amplified progenitors were abnormally committed to the erythroid, megakaryocytic, and mast cell lineages. Because the impaired mast cell differentiation was reversed by retrovirus-mediated expression of GATA-1 cDNA, GATA-1 seemed to have a direct role for differentiation of mast cells as well as for erythrocytes and platelets [90].

Tsai and Orkin [91] showed the importance of GATA-2 for the differentiation of yolk sac cells to mast cells. Mast cells are not present in long-term cultures of GATA-2 gene-disrupted yolk sac cells. In normal mouse embryos, however, mast cells develop in the skin tissue relatively late in the embryonic period [11,12]. In tissues other than the skin, mast cells develop after birth [13]. The poor differentiation potential of GATA-2^{null} yolk sac cells may not necessarily indicate the indispensability of GATA-2 for development of mast cells in adult tissues.

Mouse CMCs expressed GATA-1 and GATA-2 genes, but mast cells in the skin tissue expressed only GATA-2. Moreover, the expression of GATA-2 was limited to the period when mast cells were proliferating in the skin tissue. When CMCs obtained from normal (+/+) mice were injected into the peritoneal cavity of mast cell-deficient *Kit^W/Kit^{W-v}* mice, their proliferation and GATA-2 expression stopped simultaneously, suggesting the association of these two parameters in peritoneal mast cells [92].

The GATA consensus sequence is present in the promoter region of MC-CPA and T1/ST2 IL-33 receptor genes [16,93,94]. The T1/ST2 IL-33 receptor was used for the isolation of MCPs [6,16]. Ectopic GATA-1 expression activated the expression of the endogenous MC-CPA gene and T1/ST2 gene from the mast cell-specific promoters in immortalized mast cells and CMCs, respectively [93,94]. There are possibilities that GATA factor-dependent

genes may be regulated by one type of GATA in developing mast cells and that the same genes may be regulated by another type of GATA in differentiated mast cells.

PU.1

An Ets family transcription factor, PU.1, is necessary for the development of neutrophils, macrophages, B cells, and T cells, but not for the development of erythrocytes and platelets [95]. Mutation of PU.1 impaired the development of macrophages and B cells more severely than that of neutrophils and T cells [96,97]. When PU.1 was overexpressed in mouse CMCs, they acquired the macrophage-like phenotype [98]. For development of mast cells, the expression of PU.1 and GATA-2 of appropriate levels may be required. Walsh and coworkers [99] showed that PU.1 antagonized GATA-2 expression during macrophage differentiation but functioned cooperatively with GATA-2 to specify the mast cell fate. They proposed a developmental model in which reciprocal antagonism between PU.1 and GATA family transcription factors was essential for differentiation of macrophages, whereas cooperative interaction between PU.1 and GATA-2 was essential for differentiation of mast cells [99].

MITF

MITF is a basic helix-loop-helix leucine zipper-type transcription factor [100]. The *Mitf^{mi}* is the first found mutant allele, and homozygous *Mitf^{mi}/Mitf^{mi}* mice show pleiotropic abnormalities, including microphthalmia, depletion of pigment in hair and eyes, and osteopetrosis. In addition to these abnormalities, Stevens and Loutit [101] and Stechschulte and colleagues [102] reported a decrease in mast cells and deficiency in natural killer (NK) activity in *Mitf^{mi}/Mitf^{mi}* mice. The MITF encoded by the mutant *Mitf^{mi}* allele (hereafter *mi*-MITF) deletes one of four arginines in the basic domain [100]. The normal amount of the abnormal *mi*-MITF was produced in CMCs derived from *Mitf^{mi}/Mitf^{mi}* mice [103]. The mutant *mi*-MITF was defective in DNA-binding potential, and therefore lacked the transcription-promoting activity [104]. In addition, the *mi*-MITF inhibited the transcription-promoting activity of other transcription factors [103,105,106]. Conversely, MITF itself was not transcribed in CMCs derived from *Mitf^{mi-vga9}/Mitf^{mi-vga9}* mice because of the insertion of a transgene at the promoter region of the MITF gene [100,103]. Because of the inhibitory function of *mi*-MITF, the abnormalities were more severe in dermal mast cells of *Mitf^{mi}/Mitf^{mi}* mice than in those of *Mitf^{mi-vga9}/Mitf^{mi-vga9}* mice and more severe in CMCs derived from *Mitf^{mi}/Mitf^{mi}* mice than in those of *Mitf^{mi-vga9}/Mitf^{mi-vga9}* mice. The *MITF^{mi-vga9}* is a null mutation, and the *MITF^{mi}* is an inhibitory mutation [107,108].

Mitf^{mi}/Mitf^{mi} mice die on weaning, because teeth do not erupt as a result of severe osteopetrosis, whereas most *Mitf^{mi-vga9}/Mitf^{mi-vga9}* mice grow up to

adulthood [13]. The severe osteopetrosis of *Mitf^{mi}/Mitf^{mi}* mice may be attributable to defective differentiation of osteoclasts by the inhibitory effect of the mutant *mi*-MITF [109]. Adult *Mitf^{mi-vga9}/Mitf^{mi-vga9}* mice are suitable for studying the effects of MITF on the development of mast cells [107].

A decreased but apparently recognizable number (approximately 30% of normal) of mast cells were present in the dermis of *Mitf^{mi-vga9}/Mitf^{mi-vga9}* mice [13]. In contrast, when bone marrow cells of *Mitf^{mi-vga9}/Mitf^{mi-vga9}* mice were transplanted to adult *Kit^W/Kit^{W-v}* mice, mast cells did not develop in tissues of the *Kit^W/Kit^{W-v}* mice, including the dermis [110]. CMCs developed when bone marrow cells of *Mitf^{mi-vga9}/Mitf^{mi-vga9}* mice were cultured in the presence of IL-3. When *Mitf^{mi-vga9}/Mitf^{mi-vga9}* CMCs were transplanted to the peritoneal cavity of adult *Kit^W/Kit^{W-v}* mice, however, they disappeared within 5 weeks after transplantation [13,111,112]. Because dermal mast cells develop before birth [11], the mechanisms of mast cell development may be different between embryonic and adult tissues.

Although mast cells did not appear after transplantation of *Mitf^{mi-vga9}/Mitf^{mi-vga9}* bone marrow cells into adult *Kit^W/Kit^{W-v}* mice, erythrocytes, neutrophils, macrophages, B cells, and T cells of the donor origin developed. MHSCs of *Mitf^{mi-vga9}/Mitf^{mi-vga9}* mice are defective only in differentiation of mast cells. In addition to the defect of MHSCs, the tissue environment of *Mitf^{mi-vga9}/Mitf^{mi-vga9}* mice for supporting mast cell development seemed to be impaired. When normal (+/+) MHSCs or +/+ CMCs were transplanted to *Kit^W/Kit^{W-v}* and *Mitf^{mi-vga9}/Mitf^{mi-vga9}* mice, the number of mast cells was significantly greater in tissues of the former mice than in tissues of the latter mice [110,113]. Because the expression of KITL was normal in tissues of *Mitf^{mi-vga9}/Mitf^{mi-vga9}* mice [13,113], the molecular abnormalities that are responsible for the environmental defect of *Mitf^{mi-vga9}/Mitf^{mi-vga9}* tissues remain to be identified. The defect of tissue environment in *Mitf^{mi}/Mitf^{mi}* mice has been suggested as well in the development of NK cells and B cells [114].

When CMCs derived from *Mitf^{mi-vga9}/Mitf^{mi-vga9}* mice were compared with +/+ CMCs, transcription of various genes was impaired in the mutant CMCs. The expression of the KIT gene was deficient in *Mitf^{mi-vga9}/Mitf^{mi-vga9}* CMCs [103,115], and their response to KITL was significantly lower when compared with the response of +/+ CMCs [109]. The impaired transcription levels of mMCP-4, -5, and -6 genes were observed in *Mitf^{mi-vga9}/Mitf^{mi-vga9}* CMCs [103,104,116,117]. The transcription of the recently found mast cell adhesion molecule, spermatogenic immunoglobulin superfamily (SgIGSF), was severely impaired in *Mitf^{mi-vga9}/Mitf^{mi-vga9}* CMCs [111]. The deficient differentiation of *Mitf^{mi-vga9}/Mitf^{mi-vga9}* MHSCs to mast cells may, at least in part, be attributable to the impaired expression of KIT and SgIGSF.

Recently, we demonstrated that the expression of SgIGSF is necessary for in vitro adhesion of mouse CMCs to fibroblasts [111,112]. As already mentioned, the transcription of SgIGSF is regulated by MITF. CMCs derived from mutant *Mitf^{mi}/Mitf^{mi}* and *Mitf^{mi-vga9}/Mitf^{mi-vga9}* mice did not

attach to fibroblasts and did not survive in the peritoneal cavity of *Kit^W/Kit^{W-v}* mice. The overexpression of the MITF gene or SgIGSF gene normalized the adhesion of CMCs derived from MITF mutant mice to fibroblasts [111,112]. Because SgIGSF is not expressed by fibroblasts, it cannot be used as a homophilic adhesion molecule between mast cells and fibroblasts [111,112]. The ligand of SgIGSF expressed on the fibroblast remains to be identified. Conversely, Furuno and coworkers [118] cocultured murine CMCs with superior cervical ganglion neurons that express SgIGSF and demonstrated that SgIGSF was used as a homophilic adhesion molecule between CMCs and neurons.

Most transcription factors cannot work alone effectively but rather function in cooperation with other factors. We showed the cooperation of PEB-P2 α B1/AML1b/RUNX1 or MAZR with MITF for the transcription of mMCP-6 gene [119,120]. Razin and coworkers [121] reported the association of protein kinase C-interacting protein 1 (PKCI) with MITF. The association negatively regulated the transcription of mMCP-6 gene promoted by MITF. They also described the association of MITF with HINT, a histidine triad protein, and lysyl transfer RNA synthetase (LysRS). Ap4A, an endogenous molecule consisting of two adenosines linked by four phosphates, which was synthesized by LysRS, bound to HINT, liberated MITF, and thus led to activation of MITF-dependent genes in mast cells [122].

Deficiency of mast cells and melanocytes is observed in all MITF, KITL, and KIT loss-of-function mutant mice. Phosphorylation of MITF by KITL-KIT signaling via ERK MAP kinase was reported in melanocytes [123,124]. Whether this signaling pathway is also acting in mast cells remains to be investigated.

Taken together, GATA-1, GATA-2, PU.1, and MITF are involved in the development of mast cells (Fig. 3). GATA-1, GATA-2, and PU.1 seem to be involved in the relatively early stage of development that determines the

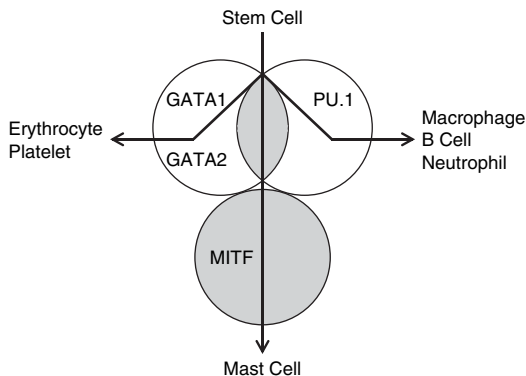


Fig. 3. Transcription factors that are involved in development of mast cells. GATA-1, GATA-2, and PU.1 are required in the early stage, and MITF is required in the late stage.

direction of differentiation. MITF seems to be involved in the relatively late stage of migration of committed precursors, their phenotypic expression within tissues, and survival of differentiated mast cells.

Factors influencing mast cell phenotypes

The phenotypes of connective tissue-type mast cells and mucosal mast cells are different in morphology, types of proteases, and types and contents of other chemical mediators [4,5]. If these three markers are used in combination, there may be too many subpopulations of mast cells. For example, although skin and peritoneal mast cells are usually classified as connective tissue-type mast cells, they have different protease expression phenotypes [125].

Protease expression phenotypes are used frequently in mice, rats, and human beings, because various types of proteases are expressed by mast cells and because the expression levels of proteases can be evaluated rather objectively [4]. Stevens and his coworkers [68,126,127] demonstrated the effect of cytokines on protease expression phenotypes of mouse CMCs. Using in situ hybridization, we demonstrated that the protease expression phenotype of peritoneal mast cells changed to that of mucosal mast cells after transplantation into the muscle layer of the stomach of *Kit^W/Kit^{W-v}* mice [128,129]. Some transplanted peritoneal mast cells migrated from the muscle layer to the epithelium and acquired the protease expression phenotype of mucosal mast cells.

Kambe and colleagues [130] investigated the differentiation of human cord blood CD34⁺ cells in tissues of irradiated immunodeficient mice. They used non-obese diabetes/severe combined immunodeficiency (SCID)/IL-2 receptor γ chain-deficient mice as the recipients. Mast cells of human origin developed in the dermis, lung, and stomach of the recipient mice. The mast cells that developed in the mucosa of the stomach and lung contained only human tryptase and were considered to be mucosal mast cells, whereas those that developed in connective tissues contained human tryptase and human chymase and were considered to be connective tissue-type mast cells. Tissue environments of mice induced development of human mast cells of appropriate phenotype. This is a good in vivo model for studying the development and phenotypes of human mast cells.

Heparin and histamine are important components of mast cell granules. N-deacetylase/N-sulphotransferase-2 (NDST-2) is the key enzyme for production of heparin. The number of mast cells decreased, and their morphology was abnormal in tissues of the NDST-2 gene-disrupted mice [131,132]. The content of histamine and mast cell proteases decreased in CMCs derived from the NDST-2-deficient mice. Conversely, histidine decarboxylase (HDC) synthesizes histamine from histidine. The number of mast cells decreased, and their morphology was abnormal in the HDC gene-disrupted mice [133]. The amount of mast cell proteases was remarkably decreased.

NDST-2 and HDC can affect not only the contents of heparin and histamine but the number and phenotype of mast cells.

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