Genetic Evidence for Critical Roles of P38α Protein in Regulating Mast Cell Differentiation and Chemotaxis through Distinct Mechanisms*

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Background: Mast cell development is the foundation for mast cell-involved diseases.

Results: P38α modulates both mast cell differentiation from progenitor cells and SCF-induced migration in fully committed mast cells.

Conclusion: P38α plays a critical role in mast cell development and function.

Significance: P38α is a novel regulator of mast cell development, and interfering with the P38α pathway may present a new therapeutic strategy for mast cell-mediated diseases.

Mast cells mediate a range of immune responses. However, the mechanisms that contribute to their development remain poorly understood. Here, using a P38α conditional knockout system, we provide evidence to suggest that P38α plays critical roles in regulating mast cell differentiation and migration via distinct mechanisms. Induced deletion of P38α in bone marrow cells retards the maturation of mast cells in part by inhibiting the activation of cAMP response element-binding protein and expression of microphthalmia-associated transcription factor, which encourages the generation of basophils over mast cells. In fully differentiated mast cells, absence of P38α inhibits stem cell factor-induced activation of Akt and ERK, which is associated with reduced chemotaxis. In vivo, conditional deletion of P38α results in reduced numbers of mast cells in certain tissues and a failure to reconstitute these cells in W64a mice transplanted with P38α−/−, Lin−, c-kit+Sca−1+ (LKS+) cells. Our findings suggest that P38α plays a dual role in mast cell development by regulating IL-3-induced differentiation of mast cell progenitor cells as well as by regulating stem cell factor-induced migration of fully differentiated mast cells.

Mast cells are critical effectors in allergic disorders and T helper type 2-mediated immune responses. Activation of mast cells results in the secretion of various biological mediators, including histamine, tryptase, leukotrienes, prostaglandins, and numerous growth factors, cytokines, and chemokines (1). Recent studies suggest that mast cells may also be important players in the regulation of innate immunity, in enhancing resistance to toxicity of snake venom, and in facilitating tumor progression and angiogenesis (2–4). Collectively, mast cells influence many aspects of human health, which justifies the importance of studying the mechanisms by which their development and distribution in various tissues is regulated.

In adults, mast cells are derived from pluripotent hematopoietic stem cells in the bone marrow. Mast cells feature two cell surface markers, including c-Kit, which binds to stem cell factor (SCF)2 and high-affinity IgE binding receptor FcεRI. There are several transcription factors and signaling pathways that mediate mast cell development, including PU.1, MITF, c-Kit/SCF, and PI3K (5–8).

The P38 MAPKs belong to a family of highly conserved kinases that convert extracellular signals to intracellular responses. There are four isoforms of p38 (α, β, γ, and δ) in mammals (9). P38α was originally identified as a major mediator of inflammatory responses. P38α regulates the production of key inflammatory mediators by cells of the innate immune system, including TNF-α, IL-1β, and Cox-2. In addition, P38 also acts downstream of cytokines such as TNF-α and mediates some of its effects. The P38 pathway plays a central role in inflammatory diseases like rheumatoid arthritis and Crohn’s disease (10). The role of P38α, which is the most ubiquitously expressed isoform of the P38 family in the development and function of mast cells, is not known. Efforts to determine how P38α contributes to mast cell development and function under either physiologic or pathologic conditions have been curbed by limited target specificity of the P38 inhibitor and early death of P38α-null mouse embryos (11, 12).

In this study, using a P38α conditional knockout mouse model (13, 14), we found that P38α plays an important role in regulating multiple aspects of mast cell differentiation and function. P38α impairs the differentiation of mast cells by regulating the activation of CREB and expression of MITF, respect-
Bone Marrow Transplantation and Reconstitution of Mast Cells in Wsh Mice—Wsh mice were irradiated with a total of 11 gray $\gamma$ radiation on the day of transplantation. LSK$^+$ cells isolated and pooled from P38$\alpha^{+/+}$ and P38$\alpha^{-/-}$ mice were transplanted into Wsh recipients. The presence of mast cells in Wsh recipients was examined 3 months after transplantation.

Examination of Mast Cells in the Peritoneal Cavity and Tissues—5 ml PBS was injected into the peritoneal cavity for 5 min. The fluid containing peritoneal cells was aspirated. After centrifugation, the pellet was resuspended and stained for FACS analysis to determine mast cell percentages. Organs dissected from mice were fixed and embedded in paraffin. To observe tissue mast cells, 5-µm paraffin sections were stained with toluidine blue (Sigma-Aldrich, St. Louis, MO). Purple mast cells were counted in 3 to 10 fields under $\times100$ magnification using a Leica microscope. Data presented are the average numbers of mast cells per field.

Immunoblotting—Cells were lysed in a lysis buffer solution that consisted of 20 mM Tris-Hcl, 150 mM NaCl, 1 mM $\beta$-glycerophosphate, 1 mM EDTA, 1% Triton X-100, 1 µg/ml leupeptin (Cell Signaling Technology). The lysates were obtained by centrifugation at 10,000 $\times g$ for 30 min. Protein concentration was quantified by bicinchoninic acid kit (Pierce). Cell lysates were boiled in sample buffer (187.5 mM Tris-buffered saline containing Tween 20, and then incubated with indicated antibodies. The Supersignal West Dura extended duration detection system (Pierce) was used to expose the membrane to film.

Apoptosis and Cell Death Assay—P38$\alpha^{+/+}$ and P38$\alpha^{-/-}$ BMMC cells were cultured with or without growth factor for 24 to 36 h as indicated, washed with calcium and magnesium-free PBS, and stained with annexin V and 7AAD according to the instructions of the manufacturer instructions (BD Biosciences). The percentage of apoptotic cells was analyzed by flow cytometry.

Cell Cycle Analysis—P38$\alpha^{+/+}$ and P38$\alpha^{-/-}$ BMMC were cultured with IL-3 (5 ng/ml) for 24 h. After being washed with PBS, the total DNA content was stained with propidium iodide solution. Cell cycle status was analyzed by flow cytometry.

Transwell Migration Assay—For migration studies, P38$\alpha^{+/+}$ and P38$\alpha^{-/-}$ BMMC suspended in IMDM were placed in the upper chambers of transwell plates (pore size 0.8 $\mu$m, Costar) with or without SCF (20 ng/ml) added to the lower chambers. After 3 h, migrated cells were removed from the wells and counted.

Statistical Analysis—Two-group comparisons were done with Student’s $t$ tests. Intergroup differences were considered significant at $p < 0.05$.

RESULTS

P38$\alpha$ Regulates Mast Cell Maturation in Vitro—The role of mitogen-activated protein kinase P38$\alpha$ in mast cell regulation is largely unknown. To analyze whether P38 functions during
the differentiation of mast cells from its progenitors in the BM, we assessed whether P38 is activated in BM cells cultured in the presence of IL-3. IL-3 is a potent inducer of mast cell maturation in vitro (5). Strong phosphorylation of P38 was observed in these cells in response to IL-3. ATF2, a downstream target of P38, was also phosphorylated in response to IL-3 (Fig. 1A).

Next, the maturation of BM cells into mast cells cultured in IL-3 was analyzed in the presence of SB203580, a P38 inhibitor. Inhibition of P38 reduced the maturation of BM-derived mast cells as assessed by the decrease in the frequency of c-Kit+/FceRI+ double-positive (DP) cells in these cultures (Fig. 1B, upper right quadrants). These data suggest that P38 path-

FIGURE 1. Inactivation of P38α inhibits the differentiation of BM-derived mast cells in vitro. A, cells were stimulated with IL-3 (10 ng/ml) for the indicated interval. Activated and total P38 and ATF2 were analyzed by Western blotting. B, IL-3 induced BM-derived mast cell maturation in the presence of dimethyl sulfoxide (DMSO) or SB203580 (10 μM). Representative dot plots show the percentage of c-Kit and FceRI double-positive cells. C, after 2 weeks of culture in IL-3, cells were treated with dimethyl sulfoxide or SB203580, and the protein level of MITF, GATA-2, and PU.1 was detected by immunoblotting. D, excision of P38α alleles and loss of P38α protein in cells from Mx-Cre+P38αfl/fl and Mx-Cre+P38αfl/fl mice 8 weeks after polyIC administration was measured by genomic PCR and immunoblotting, respectively. E, BM cells derived from P38α+/+ and P38α−/− mice were cultured in IL-3, and the frequency of c-Kit+/FceRI+ DP cells was measured at the indicated times by flow cytometry. F, quantitative analysis of the average percentage of mast cells (n = 4 per group). *, p < 0.05. Data are mean ± S.D. G, after 2 weeks of culture in IL-3, the protein level of GATA-2, PU.1, and P38α in P38α+/+ and P38α−/− cells was detected by immunoblotting. H, BM cells from P38α+/+ and P38α−/− mice were infected with retrovirus containing MIEG3 or MIEG3-P38α. After culturing the cells in IL-3 for 2 weeks, percentage of c-Kit+/FceRI+ DP cells within the GFP+ population was detected by flow cytometry, and expression of P38α was measured by immunoblotting. I, quantification of the average percentage of mast cells (n = 5 per group). *, p < 0.05. Data are mean ± S.D. J, P38α+/+ and P38α−/− BM cells were incubated with M-CSF for 6 days, and the CD11b+F4/80+ population was analyzed by flow cytometry. Data in A–D are representative of three independent experiments. Signals in G were quantified by densitometric scanning.
way may be involved in the differentiation of mast cells from its predecessors in the BM. Among the transcription factors associated with mast cell differentiation, including MITF, GATA2, and PU.1, inhibition of P38 reduced the expression of MITF and GATA2 but enhanced the expression of PU.1 in these cells (Fig. 1C).

To further characterize the role of P38α in mast cell development, a P38α conditional knockout mouse model was utilized (13, 14). By crossing mice harboring floxed P38α alleles to mice bearing the Mx-Cre allele, we derived mice in which the deletion of P38α could be induced in the BM cells. Eight weeks after treatment of polyIC, P38α alleles were excised, and P38α protein was deleted completely in BM cells from Mx-Cre−/P38αfl/fl (P38α−/−) mice but not from the heterozygous Mx-Cre+/P38αfl+/− (P38α+/−) mice (Fig. 1D). Because we did not observe a difference in the phenotype of cells derived from P38α+/− or P38α−/− mice (data not shown), we utilized cells derived from P38α−/− mice as controls for all the studies described in this manuscript. Compared with P38α+/−, a significant reduction in the percentage of c-KitFcreR1+ DP cells was observed in P38α−/− cultures at every point during maturation (Fig. 1, E and F). The reduction in the frequency of c-KitFcreR1+ DP cells was observed despite similar survival of cells in these cultures (data not shown). Similar to inhibitor results, deletion of P38α decreased expression of MITF and GATA2 and moderately increased the expression of PU.1 (Figs. 1G and 4C). Importantly, the mast cell maturation defect observed in P38α−/− cultures was rescued by introducing an exogenous P38α cDNA into P38α-deficient cells (Fig. 1, H and I). These data suggest that P38α contributes to the development of BM-derived mast cells.

We next investigated whether P38α was also involved in the differentiation of other types of hematopoietic cells in addition to mast cells. M-CSF was used to induce the differentiation of macrophages. CD11b+/F4/80+ cells were identified as macrophages. Flow cytometry results demonstrated that P38α−/− and P38α+/− BM-derived cells differentiated equally well into macrophages (Fig. 1J). It is therefore likely that P38α does not play a prominent role in the differentiation of bone marrow-derived macrophages.

P38α Regulates Mast Cell Development in Vivo—Next, we examined the role of P38α in the development of mast cells in vivo. First, we examined mast cells in different tissues from adult P38α+/− and P38α−/− mice after polyIC treatment. We observed a marked reduction in the frequency of c-KitFcreR1+ DP mast cells in the peritoneal cavity of P38α−/− mice relative to P38α+/− controls as assessed by flow cytometry (Fig. 2, A and B). These findings are similar to those reported in mice expressing a mutant form of MITF (6). Toludine blue staining demonstrated a reduced number of mast cells in the lung and spleen of P38α−/− mice compared with P38α+/− (Fig. 2, C and D). No significant difference in the number of mast cells in the back dermis was observed between P38α−/− and P38α+/− mice (Fig. 2, C and D).

**FIGURE 2.** P38α modulates mast cell development in vivo. A, c-KitFcreR1+ mast cells from the peritoneal cavity were analyzed by flow cytometry in P38α+/− and P38α−/− mice 24 weeks after PolyIC treatment. B, the percentage of peritoneal cavity mast cells from P38α+/− and P38α−/− mice (n = 5 per group). *, p < 0.05. C, tissue mast cells in the lung, spleen, and back dermis were detected in P38α+/− and P38α−/− mice with toluidine blue staining. Scale bar = 25 μm. D, number of mast cell from spleen, lung, and back dermis of P38α−/− and P38α+/− mice (n = 5). *, p < 0.05. Data in B and D are mean ± S.D.
P38α Regulates Mast Cell Development

P38α-deficient LKS+ Cells Fail to Reconstitute Mast Cells in Wsh Mice—To further confirm whether the observed differences in the number of mast cells because of P38α deficiency in vivo were cell intrinsic, we took fixed numbers of Lin− c-kit+Sca-1+ cells (LKS+) sorted, respectively, from P38α+/− and P38α−/− mice and transplanted them into mast cell-deficient Wsh mice. After 3 months, tissues were collected and fixed, and tissue mast cells were visualized by toluidine blue staining, whereas mast cells from the peritoneal cavity were assessed by flow cytometry. Compared with controls, P38α+/− LKS+ cells failed to restore peritoneal cavity mast cells in Wsh mice (Fig. 3, A and B). A reduction of mast cells in spleen and lung was also observed (Fig. 3, C and D). We noticed that there were more mast cells in spleen and lung from reconstituted Wsh mice than in non-reconstituted baseline mice. This was also observed in a previous study (15). We think that the amount of transplanted cells and irradiation may cause this difference. LKS+ cells from P38α−/− mice efficiently restored mast cells in back skin dermis and ears, and there was no significant difference compared with LKS+ cells from P38α+/− mice (data not shown). These findings indicate that P38α regulates normal homeostatic generation of mast cells in certain tissues and that impaired ability of P38α-deficient LKS+ cells to restore mast cells in some tissues is due to a cell-autonomous defect rather than environmental impairments.

P38α Modulates Mast Cell Maturation through MITF—Next, we sought to study the mechanisms underlying the role of P38α in mast cell development. From the flow cytometry data, we discovered that inactivation of P38α during mast cell differentiation primarily impacted the c-Kit-positive population. We focused our studies on the transcription factor MITF on the basis of the facts that c-Kit is a direct target of MITF and that MITF is required for mast cell development (6, 16). As shown in Fig. 4A, overexpression of MITF induced robust c-kit expression in BM cells. Next, we wondered whether IL-3 regulates MITF expression. Here, we show that MITF may be a new target of IL-3 in BM-derived cells. Treatment of IL-3 increased the mRNA (data not shown) and protein expression of MITF (Fig. 4B). We further found that the level of MITF protein in these cells depended on the presence of IL-3, as withdrawal of IL-3 led to a rapid decline in the expression of MITF (Fig. 4B).

Next, we determined whether P38α mediates IL-3-induced MITF expression in these cells. A significant decrease in the expression of MITF was observed in response to IL-3 in P38α−/− cells relative to P38α+/− cells (Fig. 4C). Similar results were observed with the P38 inhibitor SB203580 (data not shown). Reconstitution of P38α in P38α−/− BM-derived cultured cells enhanced the protein level of MITF (Fig. 4D). We further found that forced expression of exogenous MITF in P38α−/− BM-derived cultured cells restored the maturation of mast cells (Fig. 4E, F and G, upper right quadrant). These results suggest that MITF functions downstream of P38α and can efficiently reconstitute the impaired maturation of mast cells because of P38α deficiency.

MITF and P38α Regulate Differentiation of Basophils versus Mast Cells—It has been reported that IL-3 can induce both mast cells and basophils from cultured bone marrow cells (17). Mast cells and basophils share several common features (18, 19). In mice, only mast cells and basophils are known to constitutively express high-affinity IgE receptor FceRI. One key difference between mast cells and basophils is that most mast cells express c-Kit, whereas basophils do not (19, 20). A recent study shows that basophils and mast cells are siblings that share a common progenitor stage (20). However, the relationship between mast cell and basophil differentiation under the control of IL-3 is still largely unknown. In Fig. 4E, we observed that overexpression of MITF significantly reduced the
c-kit− FceRI+ population, whereas it increased c-kit+ FceRI+ population. To further characterize the c-kit− FceRI+ population, we stained cells with an anti-CD11b antibody. As shown in Fig. 5A, right panel, over 90% of c-kit− FceRI+ cells are CD11b-positive. This indicates that most of them are basophils (c-Kit− FceRI+CD11b+) (19, 20). When we observed a decrease in the percentage of c-Kit− FceRI+CD11b− cells because of MITF overexpression, we simultaneously observed an increase in the content of c-Kit+ FceRI+ mast cells (Fig. 5, A and B). These results indicate a critical role for MITF in commitment of mast cells versus basophils. To further confirm these observations, c-Kit+ FceRI+ cells and c-Kit− FceRI+ CD11b+ cells were sorted and assessed by Giemsa staining. c-Kit− FceRI+ cells possessed purple granules in cytoplasm and exhibited small nuclei in relation to their cytoplasm, whereas c-Kit− FceRI+CD11b+ cells showed lobular nuclei and reduced granules in cytoplasm, which are consistent with the morphological characteristic of mast cell and basophil, respectively (Fig. 5C). As expected, MITF and c-Kit protein was expressed in c-Kit+ FceRI+ cells but was absent in c-Kit− FceRI+CD11b+ population (Fig. 5C). We further studied the kinetics of the expression of MITF during mast cell maturation. As shown in Fig. 5D, MITF protein levels are low at the beginning of BM-derived cultures. However, its expression increases with time. To our surprise, the level of expression of MITF at the fourth weeks of culture surged dramatically. These results suggest that committed BM-derived mast cells may also utilize MITF to perform additional functions in fully differentiated mast cells. P38α deficiency reduced the c-Kit+ FceRI+ population, whereas it increased the c-Kit− FceRI+CD11b+ basophil population (Fig. 5, E and F) which is consistent with the result that P38α mediates MITF expression during mast cell differentiation. These results suggest that the P38α/MITF pathway modulates the content of mast cells versus basophils during differentiation in vitro.

CREB Plays a Role in Mast Cell Maturation, and CREB Phosphorylation Is Regulated by P38α—cAMP/CREB signaling modulates MITF expression in melanocytes (21), although its role in the maturation of mast cells is unknown. Here, we found that forced expression of CREB increased MITF expression in BM-derived cultures (Fig. 6A). Next, we sought to determine the kinetics of CREB expression during mast cell maturation. We found that CREB protein was predominantly elevated around the second week of BM culture (Fig. 6B), suggesting that CREB may function early during the maturation of mast cells. Forced expression of CREB accelerated the maturation of mast cells (Fig. 6C). A retroviral CREB shRNA was used to further study the role of CREB in the differentiation of mast cells. Knockdown CREB in these cells reduced the expression of MITF (Fig. 6D) and reduced the maturation of mast cells and enhanced the differentiation of basophils (E and F). Consistent with the differentiation result, CREB levels were higher in the sorted c-Kit− FceRI+ cells. Similar to MITF, CREB protein was
also less in c-Kit$^{-}$FcεR1$^{-}$CD11b$^{+}$ cells (Fig. 6G). MSK1, a direct target of P38, controls CREB activity by regulating its phosphorylation (22). We wanted to determine whether P38 regulates CREB activity during mast cell differentiation. IL-3-induced phosphorylation of CREB and MSK1 in P38$^{-}$/H11002/ cells was reduced relative to P38$^{-}$/H11001/ counterparts (Fig. 6H). Pre-treatment of SB203580 significantly inhibited CREB phosphorylation (Fig. 6I). Thus, CREB is important for the maturation of mast cells, and its activity is controlled by P38.

**Characteristics of P38$^{-}$/H11002/ BMMC Cells**—Fully differentiated mast cells leave the bone marrow and enter blood circulation. Intrinsic characteristics of committed mast cells are important for their function. Thus, we examined the functions of IL-3-induced 6-week-old fully differentiated P38$^{-}$/H11002/-deficient mast cells (sorted c-Kit$^{+}$FcεR1$^{+}$ double-positive BMMCs). The P38 pathway mediates antiproliferation or proproliferation in different kinds of cells (23, 24). We wondered whether P38$^{-}$/H11002/$^{-}$ plays an important role in the regulation of proliferation of BMMCs in response to IL-3. A cell cycle profile analysis showed that inactivation of P38$^{-}$/H11002/$^{-}$ cells almost doubled the percentage of S phase cells (Fig. 7A). The P38 pathway is also a critical mediator of apoptosis under certain types of stresses (25, 26). To determine the role of P38$^{-}$/H11002/$^{-}$ in regulation of apoptosis in BMMCs, P38$^{-}$/H11002/$^{-}$ and P38$^{-}$/H11002/$^{-}$ BMMCs were cultured in the absence of growth factor, and apoptotic cells were detected by annexin V staining. As shown in Fig. 7B, the absence of P38$^{-}$/H11002/$^{-}$ failed to prevent BMMCs from growth factor withdrawal-induced apoptosis. These results suggest that dele-
Disruption of P38α Disturbs the Output of SCF/c-Kit Signaling, and P38α Is Required for SCF-induced Chemotaxis in Matured BMMC Cells—SCF/c-Kit mainly functions as a chemotaxis stimulant during mast cell development. In SCF-deficient SI/Sld mice, local injection of SCF efficiently restores mast cell numbers (27). We wondered whether deficiency of P38α in fully committed mast cells impairs the SCF/c-Kit pathway and interferes with mast cell migration function. Treatment of wild-type BMMCs with SCF induced marked phosphorylation of P38 (Fig. 8A). Consistent with this result, forced expression of a constitutively active form of c-Kit (KitD814V) also induced a strong activation of P38 (Fig. 8B). On the basis of the observation that P38 can be activated via the SCF/c-Kit signal axis, P38 is likely to be an essential component in the SCF/c-Kit pathway. The well known downstream targets of SCF/c-Kit include PI-3K/Akt, ERK, and JNK. Each of these signaling molecules plays an essential role in SCF-induced proliferation, survival, synthesis of cytokines, and migration of mast cells (28). To explore the effect of P38α on these pathways, we first examined the expression level of c-kit on the surface of 6-week-old P38α+/− and P38α−/− BMMCs. As shown in Fig. 8C, the fluorescent intensity of c-kit was comparable between P38α+/− and P38α−/− BMMCs. After stimulation with SCF, inactivation of P38α significantly inhibited SCF-mediated phosphorylation of Akt and ERK but promoted a modest but significant increase in the activation of JNK (Fig. 8D). Similar results were obtained with the P38 inhibitor SB203580 (Fig. 8E).

FIGURE 6. CREB is involved in mast cell differentiation and P38α modulates CREB activity. A, cells were infected with MIEG3 or MIEG3-CREB retrovirus. MITF and CREB expression was measured by immunoblotting. B, total cell lysates from IL-3 treated BM cells for 0, 1, 2, 3, and 4 weeks were prepared, and CREB protein levels were measured by immunoblotting. C, cells were infected with MIEG3 or MIEG3-CREB retrovirus. Actin was used as a loading control. D, total cell lysates from sorted mast cells and basophils were analyzed by immunoblotting for CREB expression. E, P38α+/− and P38α−/− BM cells were treated with IL-3 and phosphorylation of CREB was measured by immunoblotting. F, after pretreatment with dimethyl sulfoxide or SB203580 (10 μM), BM cells were stimulated with IL-3. Phosphorylation of CREB was measured by immunoblotting. All the above data are representative of two to three independent experiments. Signals in D were quantified by densitometric scanning.
The response to chemokines in BMMCs is dependent on the level of maturation. LTB4 induces highly potent chemotactic activity in mast cell progenitors but not in 6-week-old BMMCs (29). However, the chemotactic effect of SCF is more potent in matured 6-week-old BMMCs than in mast cell progenitors. To investigate the role of P38 in SCF-induced chemotaxis, 6-week old P38/H9251/H11001 and P38/H9251/H11002 BMMCs were subjected to SCF stimulation. Utilizing fixed numbers of c-Kit/FcR1 cells, a transwell assay showed that deletion of P38/H9251 inhibits the migration of BMMCs in response to SCF (Fig. 8F). Together, these results demonstrate that P38/H9251 affects the outcome of multiple signaling components of the c-Kit pathway and is required for SCF-mediated chemotaxis.

DISCUSSION

Mast cells mediate a range of adaptive and innate immune responses. Here, we provide genetic evidence to demonstrate that P38/H9251 imposes multiple effects on mast cell development and biological function(s). Deletion of P38/H9251 impairs IL-3-induced differentiation of mast cells in vitro by promoting an increase in the frequency of basophils by modulating MITF and CREB. Inactivation of P38/H9251 also disturbs SCF/c-Kit signaling and inhibits SCF-induced chemotaxis in fully differentiated BMMCs. Both inhibitory effects affect the physiologic development of mast cells in vivo and in a transplant model (Fig. 8G).

Mast cell populations in certain tissues under physiologic or pathologic conditions are dependent not only on migration of mast cells/mast cell precursors into these sites but also on pro-survival and proliferation signals. Both IL-3 and SCF are major regulators of apoptosis and proliferation in hematopoietic cells. MITF is a critical regulator of cell cycle progression (30, 31). MITF also plays an important role in anti-apoptosis (32, 33). In addition to regulating the development of mast cells, MITF also promotes the production of prostaglandin D2, mast cell proteases, granzyme B, and tryptophan hydroxylase TPH (34–36). In a complex environment of inflammation, it is important for mast cells to survive and migrate. Antiapoptosis proteins bcl-2 and Dia1, which promote cell mobility, are among the targets of MITF (32, 37). These conditions justify higher MITF levels in committed mast cells. During mast cell maturation, MITF expression seems be regulated by a positive feedback through profound epigenetic changes that occur at the MITF locus. In contrast to MITF, CREB protein levels peak at the second week of IL-3-induced mast cell differentiation. Because the second week is a critical phase during mast cell commitment, CREB may act as an initiation signal to pave the way for later effectors such as MITF. In response to IL-3 treatment, the absence of P38/H9251 partially inhibits MITF expression. One possibility is that other pathways utilized by IL-3 induce MITF expression.
Another possibility is that other members of the P38 family, such as P38/H9252, compensate for the loss of P38/H9251 function. A recent study has shown redundant roles for P38/H9251 and P38/H9252 during mouse development (38).

Along with IL-3 and SCF, several other cytokines, including IL-4 and IL-9, also regulate mast cell development and tissue homeostasis (8). Because different tissues produce different amounts of these cytokines, it is conceivable that they utilize the p38α pathway to different degrees in regulating mast cell homeostasis in vivo. Signals emanating via these cytokines in distinct tissues may overlap or compensate and, therefore, may explain why the distribution of mast cells in certain tissues is not impaired in the absence of p38α.

Cross-talk between members of MAPK family has been reported. In mouse embryonic fibroblasts, disruption of P38α increases JNK activity and enhances proliferation (9). We also...
observe that inactivation of P38α promotes BMMCs to enter the cell cycle in response to SCF and induces higher JNK activation. These results suggest that the P38α-JNK-c-Jun pathway may also function in mast cells. Downstream of the SCF/c-Kit pathway, activated PI3 kinase/Akt and ERK provide survival signaling by phosphorylation of FOXO3a and Bim (39). We show that inactivation of P38 inhibits SCF-induced activation of Akt and ERK, which may sensitize P38α−/− mast cells to apoptosis. However, P38 itself mediates apoptosis in response to certain kinds of stress, and deletion of P38α may impose resistance to certain apoptotic stimuli. We did not observe a significant difference in apoptosis between P38α+/+ and P38α−/− BMMCs in response to growth factor withdrawal. This may be a result of the lack of involvement of P38α in growth factor withdrawal-induced cell death in mast cells, or perhaps reduced apoptotic signaling because of the absence of P38α is offset by decreased survival signaling because of inhibition of Akt and ERK. Cross-talk between P38, Akt, and ERK presents a negative feedback to balance proapoptotic and antiapoptotic signaling within mast cells, which may be important to maintain mast cell homeostasis in tissues.

Although the dynamics of mast cell involvement in various diseases is poorly understood, it is safe to speculate that mast cells are consumed and supplemented, especially in chronic diseases. Because bone marrow is the ultimate source of mast cells, we believe that the impact of P38α on mast cell development should be of some consequence on mast cell-involved diseases. Inhibition of P38α-mediated mast cell development may be a useful long-term therapeutic strategy.

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