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Review

Mast cell progenitors: Origin, development and migration to tissues☆

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ABSTRACT

Mast cells in tissues are developed from mast cell progenitors emerging from the bone marrow in a process highly regulated by transcription factors. Through the advancement of the multicolor flow cytometry technique, the mast cell progenitor population in the mouse has been characterized in terms of surface markers. However, only cell populations with enriched mast cell capability have been described in human. In naïve mice, the peripheral tissues have a constitutive pool of mast cell progenitors. Upon infections in the gut and in allergic inflammation in the lung, the local mast cell progenitor numbers increase tremendously. This review focuses on the origin and development of mast cell progenitors. Furthermore, the evidences for cells and molecules that govern the migration of these cells in mice *in vivo* are described.

1. Introduction

The origin of mast cells was an unresolved mystery for decades. Most other hematopoietic cells are released into the blood stream mainly in an identifiable mature state. However, mast cells are derived from the bone marrow but mature from mast cell progenitors (MCp) in peripheral tissues. During hematopoiesis, the development of MCp is highly regulated by transcription factors. After the commitment, the MCp circulate and home to the tissues in an immature state. Most of the MCp in tissues express the stem cell factor (SCF) receptor c-kit and the high-affinity IgE receptor FcεRI, just as mature mast cells. However, they are less- or non-granulated and were therefore largely unidentifiable with traditional histochemical staining techniques. The migration of MCp to tissues is a regulated process that is stimulated by inflammation and leads to an increase in tissue MCp. The increase in murine lung MCp upon allergic inflammation is largely thought to occur due to recruitment.

2. Quantification of mast cell progenitors

The immature nature of MCp with none or few stainable granules has made the quantification of MCp in different organs to mainly rely on indirect techniques such as limiting dilution assays. To perform this assay, a cell suspension is prepared from the organ and mature mast cells are removed by gradient centrifugation. In early experiments, the purified cells were then injected in different concentrations into the skin of mast cell-deficient W/W^v mice (Sonoda et al., 1982). After several weeks, the injection sites were examined for colonies of toluidine blue-stained mature mast cells. The sites with mast cells had at least one MCp at the time of injection, originating from the transferred cells. The frequency of MCp in the initial cell suspension was thereafter estimated using the Poisson distribution (Sonoda et al., 1982). This technique was later developed into an *in vitro* assay (Crapper and Schrader, 1983). Here, the purified mononuclear cells from an organ are cultured in different concentrations in wells with interleukin 3 (IL-3)-containing medium alone or supplemented with SCF in the presence of a feeder cell layer to support colony growth. After about 2 weeks, mast cells colonies are easily identified as clusters of small to medium-sized cells. These cells display metachromatic granules when stained with May-Grünwald–Giemsa and are detected as c-kit$^+$ FcεRI$^+$ cells by flow cytometry (Crapper and Schrader, 1983; Dahlin et al., 2012, 2013). The frequency of MCp is then estimated with the Poisson distribution as before.

More recently, quantification of MCp in different organs is possible using multicolor flow cytometry. Contrary to the limiting dilution assay that is used to quantify the frequency of cells with mast cell-forming capacity, the flow cytometric method is often

Abbreviations: BMMC, bone marrow derived mast cell; BMCP, basophil/mast cell progenitor; CCR, CC chemokine receptor; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; CXCR, CXC chemokine receptor; IL, interleukin; Lin$^-$, lineage$^-$; MAdCAM-1, mucosal vascular addressin cell adhesion molecule 1; MCp, mast cell progenitor(s); MEP, megakaryocyte erythrocyte progenitor; MPP, multipotent progenitor; OVA, ovalbumin; PI3Kγ, phosphoinositide 3-kinase γ; SCF, stem cell factor; TNF, tumor necrosis factor; VCAM-1, vascular cell adhesion molecule 1.

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used to quantify committed MCp. Verification of the correct population identified with flow cytometry needs to be performed as the phenotype of the MCp varies between organs and mouse strains (Dahlin et al., 2013; Chen et al., 2005; Arinobu et al., 2005; Jamur et al., 2005). This verification is often performed by fluorescence-activated cell sorting of the probable MCp population. The sorted cells are cultured in a cytokine cocktail containing *e.g.* IL-3, IL-5, IL-6, IL-7, IL-9, IL-11, SCF, granulocyte-macrophage colony-stimulating factor, thrombopoietin, and erythropoietin (Arinobu et al., 2005). These conditions allow the sorted cells to differentiate into any myeloerythroid lineage. The cell types of the cultured cells are then determined usually by a combination of May-Grünwald–Giemsa staining and flow cytometry. The initially sorted cells are considered to be committed MCp if they exclusively differentiate into mast cells. Verification of commitment to the mast cell lineage can also be determined by transferring the prospective MCp obtained from CD45.1 wild type mice into mast cell deficient mice (CD45.2) and analyzing what cells the CD45.1 donor cells became *in vivo* (Chen et al., 2005). When the cell surface markers that identify the MCp population in a particular organ are verified, flow cytometric analysis using the same markers can be subsequently used to easily quantify the MCp.

3. What is the origin of mast cells?

Bone marrow-derived hematopoietic stem cells lose their self-renewing capacity when they develop into multipotent progenitors (MPPs). These MPPs differentiate into either common lymphoid progenitors (CLPs) or common myeloid progenitors (CMPs) (Kondo et al., 1997; Akashi et al., 2000). Classically, the CMPs branch into megakaryocyte/erythrocyte progenitors (MEPs) and granulocyte/monocyte progenitors (GMPs) during their development (Akashi et al., 2000). To what lineage the mast cells belong is still debated. In an early study, single cells picked using a micromanipulator from mouse spleen cultured for 7–16 days were giving rise to only (1) mast cells and macrophages; (2) mast cells, neutrophils and macrophages; and (3) mast cells, erythrocytes and macrophages, agreeing with a position of mast cells on the CMP or the GMP branch (Suda et al., 1983). In 2005, three independent groups published papers related to the development of MCp in the bone marrow (Chen et al., 2005; Arinobu et al., 2005; Jamur et al., 2005). Jamur and colleagues identified a committed MCp, even though the developmental relationship to other cells was not determined (Jamur et al., 2005). Chen et al. found a committed MCp within the CMP population defined by flow cytometry (Chen et al., 2005). However, using the classical definition of CMPs (Lin^- IL-7Rα^- c-Kit^+ $Sca\text{-}1^-$ $CD34^+$ FcγRII/III^{lo} cells), no single CMP giving rise to both mast cells and another myeloid cell type was found. Thus, MCp were concluded likely to originate from MPPs. In a follow-up study, Franco et al. redefined the CMP population to Lin^- $Sca\text{-}1^{lo}$ c-Kit^+ $CD27^+$ Flk-2^- cells (Franco et al., 2010). Then mast cell potential was identified within the CMP population and gene expression profiling of single cells suggested that MCp are more similar to MEPs than GMPs (Franco et al., 2010). In contrast, Arinobu et al. found single cells giving rise to neutrophils, basophils and mast cells that were found within the classically defined GMP population (Lin^- IL-7Rα^- c-Kit^+ $Sca\text{-}1^-$ $CD34^+$ FcγRII/III^{hi})(Arinobu et al., 2005). Their findings verify that mast cells belong to the granulocyte/monocyte lineage and that MEPs branch off from CMPs (Arinobu et al., 2005). Moreover, bipotent basophil/mast cell progenitors (BMCPs) were identified within the spleen of C57BL[1] mice, suggesting a close developmental relationship between the basophil and the mast cell lineage (Arinobu et al., 2005; Iwasaki et al., 2006; Qi et al., 2013). In agreement with these results, Qi and colleagues recently isolated single progenitors capable of giving rise to both basophils and mast cells within the GMP fraction in the bone marrow (Qi et al., 2013). Most of these cells were found among the Fc$\varepsilon$$RI^+$ GMPs (called pre-BMPs), even though they were also present in the Fc$\varepsilon$$RI^-$ GMP fraction (Qi et al., 2013). Thus, the data taken together suggest that committed MCp originate from bipotent progenitors with both mast cell and basophil capacity within the GMP population (Fig. 1).

3.1. Transcription factors regulating mast cell development

A complex network of transcription factors regulates the differentiation of hematopoietic stem cells into mast cells. The requirement of PU.1 for lineage development has been studied in mouse embryos lacking PU.1. This transcription factor is required for the *in vivo* and *in vitro* development of mast cells but also for lymphoid and most myeloid progenitors apart from megakaryocyte and erythroblasts (Scott et al., 1994; Walsh et al., 2002). *In vitro* differentiation of fetal liver hematopoietic progenitors from PU.1-deficient embryos did not generate mast cells when cultured in IL-3 and SCF (Walsh et al., 2002).

Formation of mast cells *in vivo* is also dependent on STAT5. STAT5-deficient mice virtually lacked mast cells in the peritoneum, skin, stomach and spleen (Shelburne et al., 2003). *In vitro* however, mast cells could be generated from bone marrow of STAT5 deficient mice when cultured together with IL-3 and SCF. Medium containing IL-3 alone supported mast cell growth from wild type but not from STAT5 deficient bone marrow (Shelburne et al., 2003). In another study, inducible STAT5 knockout mice were used to delineate the role of STAT5 for mast cell development (Qi et al., 2013). These mice still had GMPs and pre-BMPs when STAT5 was ablated. Since the loss of STAT5 could have been induced at any time of the hematopoiesis in this study, the presence of GMPs and pre-BMPs does not explain when STAT5 needs to be expressed in the developing mast cells. However, mast cells could not be induced when the GMPs were cultured in IL-3-containing medium (Qi et al., 2013). These results support the fact that IL-3 is not sufficient to induce mast cell development in STAT5-deficient cells as previously described (Shelburne et al., 2003). This is not surprising since STAT5 is downstream of the IL-3 receptor signal transduction pathway (Steelman et al., 2004).

The differentiation from GMPs into mast cells is controlled by CCAAT/enhancer binding protein α (C/EBPα), MITF and GATA-2. When GMPs sorted from wild type bone marrow were cultured together with a myeloerythroid cytokine cocktail, the vast majority of the cells differentiated into neutrophils and monocytes, even though mast cells, basophils and eosinophils were also generated (Arinobu et al., 2005; Iwasaki et al., 2005, 2006). Disruption of C/EBPα in GMPs led to an increase in developing mast cells, but not basophils (Iwasaki et al., 2006). To further support the phenomenon that absence of C/EBPα resulted in increased number of mast cells, the lineage potential of C/EBPα-deficient fetal liver CMPs was compared to wild type fetal liver CMPs. After one week in culture, the number of mast cells colonies was fourfold higher in C/EBPα-deficient CMPs than in the corresponding wild type cells (Iwasaki et al., 2006). Furthermore, deletion of the gene coding for C/EBPα in BMCPs led to a loss of basophil potential and generation of pure mast cells (Arinobu et al., 2005). On the other hand, overexpression of C/EBPα in BMCPs resulted in skewing into basophil lineage differentiation (Arinobu et al., 2005). This can be explained by the fact that C/EBPα binds to *mitf*'s promoter, thus repressing its expression (Qi et al., 2013). The expression of MITF was important for the development of mast cells in the gut, spleen and skin (Stevens and Loutit, 1982; Stechschulte et al., 1987; Ebi et al., 1990). MITF also affected mast cell development *in vitro* (Qi et al., 2013).

[1] In the discussed articles several different kinds of C57BL-strains have been used, for simplicity this abbreviation is used throughout the text.

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