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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.

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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.

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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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 (Crappier 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 cell 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 (Crappier 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

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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 ($\text{Lin}^- \text{IL-7}\alpha^- \text{c-Kit}^+ \text{Sca-1}^- \text{CD34}^+ \text{Fc}\gamma\text{RII/III}^{\text{lo}}$ 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 $\text{Lin}^- \text{Sca-1}^{\text{lo}} \text{c-Kit}^+ \text{CD27}^+ \text{Flk-2}^-$ 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 ($\text{Lin}^- \text{IL-7}\alpha^- \text{c-Kit}^+ \text{Sca-1}^- \text{CD34}^+ \text{Fc}\gamma\text{RII/III}^{\text{hi}}$) (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 C57BL¹ 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 $\text{Fc}\epsilon\text{RI}^+$ GMPs (called pre-BMPs), even though they were also present in the $\text{Fc}\epsilon\text{RI}^-$ 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/EBP α), 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/EBP α 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/EBP α resulted in increased number of mast cells, the lineage potential of C/EBP α -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/EBP α -deficient CMPs than in the corresponding wild type cells (Iwasaki et al., 2006). Furthermore, deletion of the gene coding for C/EBP α in BMCPs led to a loss of basophil potential and generation of pure mast cells (Arinobu et al., 2005). On the other hand, over-expression of C/EBP α in BMCPs resulted in skewing into basophil lineage differentiation (Arinobu et al., 2005). This can be explained by the fact that C/EBP α 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.

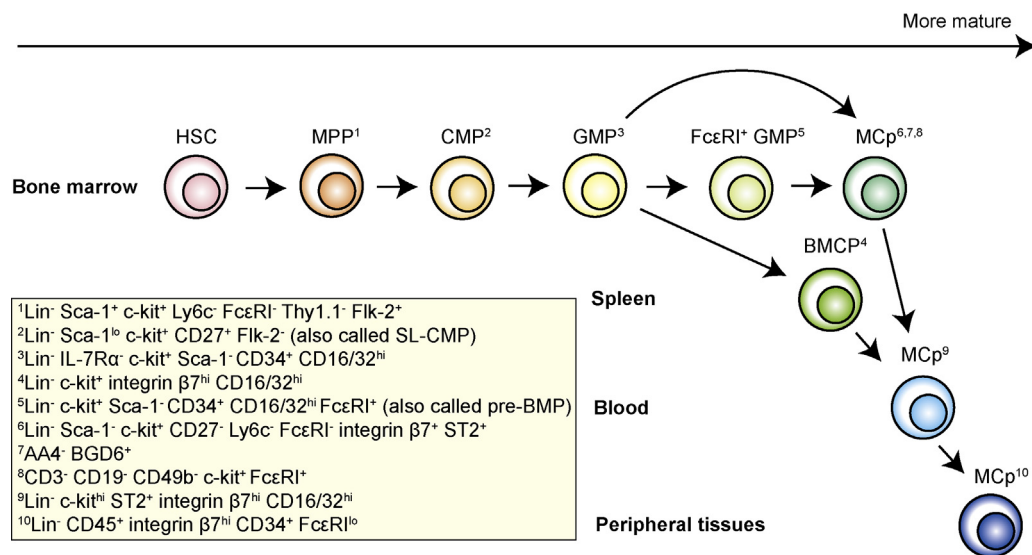


Fig. 1. Proposed model for the development of MCp. Mast cells originate from the bone marrow where they develop from the hematopoietic stem cells (HSC) via multipotent progenitors (MPP), common myeloid progenitors (CMPs) and granulocyte/monocyte progenitors (GMPs). Progenitors giving rise to both mast cells and basophils have been isolated from bone marrow within the GMP fraction. The majority of these progenitors are found among the FcεRI⁺ GMPs. However, progenitors that give rise to mast cells and basophils are also found within the FcεRI[−] GMPs. Bipotent basophil/mast cell progenitors (BMCPs) in the spleen are largely negative for FcεRI expression in C57BL mice, consistent with the FcεRI[−] GMPs in bone marrow. Committed MCp can either lack or express FcεRI in the bone marrow. Therefore, they likely develop from both FcεRI[−] and FcεRI⁺ GMPs. MCp exit the bone marrow as FcεRI[−] and FcεRI⁺ cells that can be found in the blood from BALB/c and C57BL mice as committed MCp. In the peripheral tissues MCp express FcεRI at low levels in C57BL mice. The numbers in superscript at each progenitor stage is repeated in the yellow box to describe the surface markers used to define the particular progenitor stage.

This was shown by IL-3-culturing of bone marrow cells carrying mutant MITF, which binds poorly to mast cell-specific genes. These cells developed into mast cells and cells with several characteristics of basophils. Qi et al. further showed that MITF regulate C/EBPα expression in an antagonistic fashion, thus preventing basophil differentiation (Qi et al., 2013). *In vitro*, it was possible to reprogram committed MCp from the intestine into basophils by retrovirally transducing the cells with C/EBPα (Arimitsu et al., 2005). Conversion of basophils into mast cells could similarly be accomplished by inactivating C/EBPα in the basophils (Qi et al., 2013). Recently, one study even claims that mast cells can be generated from basophils *in vitro* without neither transducing genes nor inactivating genes by manipulation of the cells (Metcalf et al., 2013).

In vitro differentiation of embryonic stem cells have clarified the role of GATA-2 for the generation of mast cells (Tsai et al., 1994). GATA-2-deficient embryonic bodies can differentiate into erythroid and monocytic precursor cells when cultured, although at lower frequency compared to the wild type cells. SCF-responsive MCp are absent in GATA-2-deficient embryonic bodies in contrast to wild type embryonic bodies. However, few MCp colonies were found after culturing GATA-2-deficient embryonic bodies in IL-1, IL-3 and SCF. The frequency was nevertheless only a fraction of that of wild type cells. In another study, GATA-2 expression was enforced in GMPs sorted from bone marrow (Iwasaki et al., 2006). Even though the GMPs express C/EBPα, which is critical for the development of basophils from BMCPs, all GATA-2⁺ GMPs generated 100% pure eosinophil colonies. Iwasaki and colleagues then showed that C/EBPα needed to be downregulated before GATA-2 was induced for mast cells to develop (Iwasaki et al., 2006). This was done by comparing C/EBPα-deficient fetal liver CMPs with or without enforced GATA-2 expression. Mast cells developed under both conditions, though the frequency of mast cell colonies was substantially higher when GATA-2 was enforced. In addition, the transduction of GATA-2 into otherwise lineage-restricted CLPs led to the development of BMCPs whereas the enforced expression of GATA-2 followed by C/EBPα led to development of basophil progenitors. These data indicate how important

the regulation of GATA-2 and C/EBPα is for the development of mast cells.

Hes-1, a downstream transcription factor turned on by Notch signaling, has been shown to negatively regulate the transcription of C/EBPα (Sakata-Yanagimoto et al., 2008). Transducing CMPs and GMPs with Hes-1 alone does not induce the development of mast cells. However, in combination with transduced GATA-3, the frequency of mast cells forming *in vitro* from CMPs and GMPs increase dramatically (Sakata-Yanagimoto et al., 2008). In fact, GATA-3 transduction can even reprogram fetal thymocytes into mast cells (Taghon et al., 2007).

GATA-1 is another transcription factor that is involved in mast cell development. MCp can however develop from cells with low GATA-1 levels (Migliaccio et al., 2003; Cantor et al., 2008; Sugiyama et al., 2008). In fact GATA-1 in mast cells regulated the maturation from MCp into mature mast cells rather than regulating early differentiation (Migliaccio et al., 2003; Harigae et al., 1998). The maturation from MCp to metachromatic mast cells is highly regulated by multiple factors in complex networks and to discuss these factors is beyond the scope of this review. We have summarized the major transcription factors involved in the development from the GMP to committed MCp in Fig. 2.

3.2. Mast cell development in adult mice

Mast cells originate from bone marrow cells in adult mice (Kitamura et al., 1977). In the bone marrow of BALB/c mice, committed MCp have been isolated with magnetic beads conjugated to the antibodies AA4 and BGD6 (Jamur et al., 2005). These antibodies were raised against a rat leukemia cell line with a mast cell-like phenotype. AA4 recognizes ganglioside GD1b on the surface of mature mast cells, whereas BGD6 binds to CD32 with the Fc part and to an undetermined 110 kDa protein present on both immature and mature mast cells with the Fab portion (Jamur et al., 2005; Guiraldelli et al., 2008; Guo et al., 1989). AA4[−] BGD6⁺ cells, which were also characterized as c-kit⁺ FcεRI[−] cells, could only give rise to c-kit⁺ FcεRI⁺ mast cells when cultured in IL-3 and SCF. Cytokines

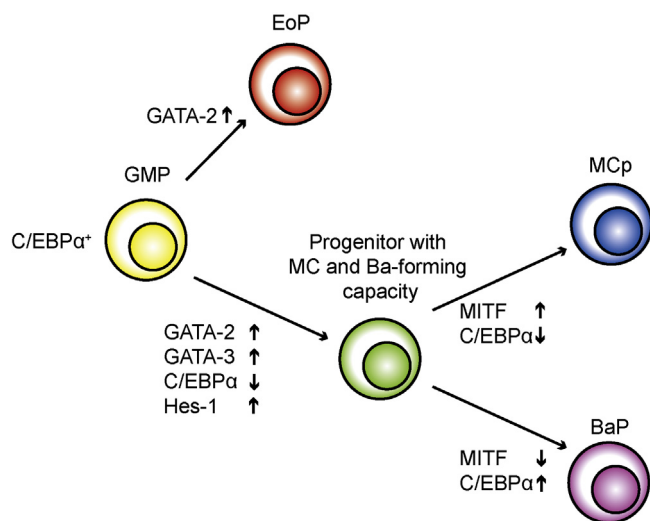


Fig. 2. Representation of transcription factors involved in the mast cell lineage commitment. GMPs, which express C/EBPα, turn on GATA-2 to become eosinophil progenitors (EoP). To form a progenitor with mast cell (MC) and basophil (Ba) potency, C/EBPα is downregulated in the GMP along with upregulation of GATA-2, GATA-3 and Hes-1. For MCp commitment, MITF is upregulated and C/EBPα is further downregulated whereas for basophil progenitor (BaP) commitment MITF is downregulated and C/EBPα is upregulated.

stimulating the erythroid, granulocytic or monocytic lineages did not lead to development of cells other than mast cells when the AA4[−] BGD6⁺ cells were cultured. In the study by Chen et al., Lin[−] c-kit⁺ Sca-1[−] Ly6c[−] FcεRI[−] CD27[−] integrin β7⁺ T1/ST2⁺ progenitors isolated from bone marrow of C57BL mice gave rise to pure mast cells *in vitro* and *in vivo* (Chen et al., 2005). CD3[−] CD19[−] CD49b[−] c-kit⁺ FcεRI⁺ cells were found in bone marrow of BALB/c mice, consistent with cells committed to the mast cell lineage (Liu et al., 2013). Following the exit of MCp from the bone marrow, committed MCp were recognized as Lin[−] c-kit^{hi} ST2⁺ integrin β7^{hi} CD16/32^{hi} cells in both C57BL and BALB/c mice (Fig. 1) (Dahlin et al., 2013). Furthermore, the expression of FcεRI was used as a maturation marker as it is upregulated during mast cell development. Interestingly, the majority of the MCp in blood was FcεRI⁺ in BALB/c mice whereas the majority was FcεRI[−] in C57BL mice (Dahlin et al., 2013). This phenomenon suggests that the MCp in blood are more mature in Th2-prone BALB/c mice than Th1-prone C57BL mice. When the blood MCp migrate into the peripheral tissues such as the intestine, the committed MCp could be identified as Lin[−] CD45⁺ CD34⁺ integrin β7^{hi} FcεRI^{lo} cells in C57BL mice (Arinobu et al., 2005).

If a population of committed MCp can be identified in humans is yet to be determined. Mast cell-forming potential was found in CD34⁺ cells from adult bone-marrow (Kirshenbaum et al., 1991). In human peripheral blood the mast cell-forming potential was mainly in the CD34⁺ c-kit⁺ CD13⁺ cell fraction (Kirshenbaum et al., 1999). However, only a small fraction of these cells gave rise to pure mast cell colonies when cultured *in vitro*. The progenitors forming mast cells from peripheral blood of humans have further been characterized as FcεRI[−] cells (Rottem et al., 1994).

3.3. Mast cell development in prenatal stages

In mouse ontogenesis, cells with mast cell-forming capacity were found in the yolk sac on gestation day 9.5 and peak day 11 (Sonoda et al., 1983). The decline in frequency of these cells was associated with an increase in MCp in the fetal liver, followed by the appearance of skin MCp (Sonoda et al., 1983; Hayashi et al., 1985). In a seminal study, Rodewald et al. found progenitors committed to the mast cell lineage in fetal mouse blood from gestation

day 14.5 (Rodewald et al., 1996). The frequency of these Thy-1^{lo} c-kit^{hi} FcεRI[−] cells reached a maximum day 15.5 and declined until birth.

In rats, the appearance of organ-specific MCp populations have been studied in more detail. The earliest committed MCp were found in the aorta-gonad-mesonephros (AGM) (Guiraldelli et al., 2013). It is likely that this followed the formation of long-term-reconstituting hematopoietic stem cells in the AGM. AA4[−] BGD6⁺ cells from the AGM were c-kit⁺ FcεRI[−] cells that developed into c-kit⁺ FcεRI⁺ metachromatic mast cells when cultured. After generation of MCp in the AGM, appearance of AA4⁺ BGD6⁺ MCp in the fetal liver, lungs and skin, intestine and finally bone marrow followed in the specified order (Guiraldelli et al., 2013). These cells were suggested to be more developed MCp as AA4⁺ BGD6⁺ cells in adult rats correspond to c-kit⁺ FcεRI⁺ MCp (Jamur et al., 2010).

MCp have been found in human umbilical cord blood (Kempuraj et al., 1999). CD34⁺ cells were divided into four groups: CD38⁺, CD38[−], HLA-DR⁺ and HLA-DR[−] cells. Single cells sorted from each group were then cultured with SCF and IL-6. Colonies consisting of mast cells were found mainly in the CD34⁺ CD38⁺ and CD34⁺ HLA-DR[−] fraction, suggesting that MCp were CD34⁺ CD38⁺ HLA-DR[−] cells. However, when the cells from the four groups were cultured with IL-3, SCF and IL-6, pure mast cell colonies were found in all fractions (Kempuraj et al., 1999).

4. Mast cells and their progenitors in tissues

Mast cells in tissues are very long-lived (Padawer, 1974) and even after degranulation they re-granulate and live on (Walker, 1961; Kobayasi and Asboe-Hansen, 1969; Xiang et al., 2001). Many studies report on organ-specific increases in mast cell numbers in human disease (Patella et al., 1998; Terada and Matsunaga, 2000; Molin et al., 2002; Ribatti et al., 2000; O'Sullivan et al., 2000; Ammit et al., 1997; Brightling et al., 2002; Sugamata et al., 2005; Nakajima et al., 1997). The same pattern is also seen in disease models in mice (Dahlin et al., 2012; Yu et al., 2006; Hallgren et al., 2007; Kakizoe et al., 1999; Chang et al., 2011). The increase in mature mast cells in tissues could theoretically be due to cell division of mature mast cells or maturation of MCp that either are newly recruited or a result of cell division from a pool of MCp (Fig. 3). However, mature mast cells are generally not thought to undergo cell division and are considered terminally differentiated. Nevertheless, mature connective tissue mast cells in mice may have an ability to proliferate *in vivo* (Sonoda et al., 1984). In this study, peritoneal mast cells that were picked up individually based on morphology could give rise to a mast cell cluster (in one case out of 17) when injected into the skin of mast cell deficient W/W^v mice. Also human skin mast cells were reported to have potential to proliferate *in vitro* (Kambe et al., 2001). However, the selection of disialylganglioside GD3⁺, c-kit⁺ single cells by a combination of immunoaffinity magnetic enrichment and cell sorting does not prove that proliferation occurred from mature mast cells since human MCp may also express these markers.

Murine MCp have an enormous capacity to divide at least *in vitro* (Arinobu et al., 2005). However, the potential importance of cell division of MCp *in situ* warrants further investigations. The major mechanisms for the increase in mast cells in tissues are likely due to the maturation of dividing MCp that have entered *via* the vascular system due to a continuous low-level homing or because of induced recruitment (Fig. 3).

4.1. Mast cell progenitors in the gut

In naïve mice, peripheral tissues like the lung contain very few MCp (Abonia et al., 2006). The intestine is an exception because it

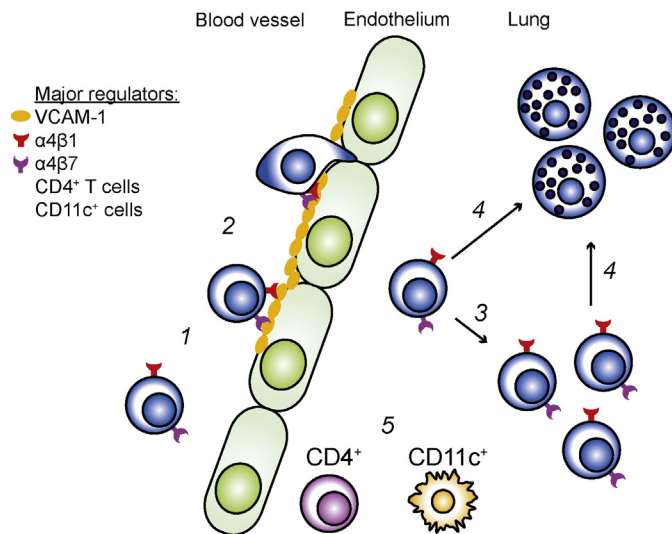


Fig. 3. The major mechanisms involved in the increase of lung MCp during allergic inflammation. 1, Committed MCp circulating in the blood express $\alpha 4$ -integrins. 2, Upon allergic inflammation in the lung VCAM-1 is induced on the lung endothelium enabling $\alpha 4$ -intergrin-expressing MCp to adhere to the lung endothelium and enter the tissue. 3, MCp in the lung are influenced by locally produced factors that may stimulate them to divide *in situ*. 4, Eventually most of the MCp will mature into long-lived mast cells. 5, The increase in lung MCp numbers are strictly regulated by CD4⁺ T cells and CD11c⁺ cells.

contains a higher density of cells with mast cell capacity than even the bone marrow (Gurish et al., 2001). The high number of MCp in intestine is present even in germ-free mice, which are not exposed to antigenic stimuli (Guy-Grand et al., 1984). This innate nature of intestinal MCp implies a homing process and is further strengthened by the finding that mice lacking T, B, NK and NKT-cells or athymic mice all have normal numbers (Gurish et al., 2001).

The relatively high number of MCp in the intestine and the fact that these cells are very sensitive to irradiation were taken advantage of to study the mechanisms of homeostatic homing to the intestine. When intestinal MCp were depleted by irradiation and followed by bone marrow reconstitution it took approximately 11 days to reconstitute the intestinal MCp pool back to normal levels (Gurish et al., 2001). By administration of anti-integrin $\alpha 4$ and $\alpha 4 \beta 7$ blocking antibodies to irradiated mice, the reconstitution of the intestinal MCp was inhibited by 70%. In agreement with these data, mice lacking integrin $\beta 7$ virtually lacked intestinal MCp whereas the frequencies in lung, spleen and bone marrow were normal. These results suggested that integrins $\alpha 4$ and particularly $\alpha 4 \beta 7$ were important for MCp homing to the intestine. The ligands of integrin $\alpha 4 \beta 7$ are mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1) and vascular cell adhesion molecule 1 (VCAM-1). Both anti-MAdCAM-1 and anti-VCAM-1 blocking antibodies were shown to suppress the intestinal MCp influx one by one (Abonia et al., 2005) indicating that these cell adhesion molecules were used in parallel. Using a mouse strain lacking VCAM-1 on endothelial cells confirmed the involvement of VCAM-1 for the homing of MCp to the intestine (Abonia et al., 2005). The responsible chemokine receptor responding to the chemotactic gradient and/or regulating the integrin affinity required for homing of MCp has also been investigated. Several lines of evidence suggested that autologous expression of CXCR2 on the MCp was required for homing to the intestine (Abonia et al., 2005).

The number of intestinal MCp was increased in several rodent infection models (Kasugai et al., 1995; Khalil et al., 1996; Pennock and Grecnis, 2004; Dillon and MacDonald, 1986). In addition, *Trichinella spiralis* infection triggered the appearance of MCp in mesenteric lymph nodes, and these cells could be depleted by

anti-Fc ϵ RI antibodies (Liu et al., 2013). There is some evidence for the role of integrins involved in the increase in mucosal mast cells in the gut. Mice lacking integrin $\beta 6$ infected with the nematode *Nippostrongylus brasiliensis* had a strikingly impaired induction of jejunal mucosal mast cells (Knight et al., 2002). The same integrin $\beta 6$ knockout mice had reduced numbers of mucosal mast cells in the intestinal epithelium upon *Trichinella spiralis* infection albeit with increased numbers of mucosal mast cells in the lamina propria (Brown et al., 2004; Knight et al., 2007). The explanation behind the disturbed mast cell distribution in the mice deficient of integrin $\beta 6$ is still lacking and the mechanisms that drive the recruitment and cell division of the already large pool of intestinal MCp needs to be addressed.

4.2. Mast cell progenitors in the peritoneum

Rodents have a large pool of connective tissue-type of mast cells in the peritoneum. If mast cells were depleted by injection of water into the peritoneum, MCp repopulated this site (Jamur et al., 2010). Two studies report reductions in peritoneal mast cells in specific integrin-knockout mice, but in these studies no attempts to quantify MCp were made (Rosenkranz et al., 1998; Berlanga et al., 2005). Mice lacking macrophage-1 antigen (Mac-1)/ $\alpha M \beta 2$ integrin showed a 70% reduction in peritoneal mast cells but had intact numbers in intestine and spleen (Rosenkranz et al., 1998). In addition, mice lacking glycoprotein IIb (GPIIb), which is the α -subunit of the $\alpha IIb \beta 3$ integrin heterodimer, had around 50% reduction in the percentage of peritoneal mast cells (Berlanga et al., 2005). These data suggest that integrins $\alpha M \beta 2$ and $\alpha IIb \beta 3$ are involved in MCp homing to the peritoneum. The peritoneal cavity contains white adipose tissue consisting mainly of adipocytes but also of immune/hematopoietic cells. Poglio et al. reported that inguinal white adipose tissue contained MCp (Lin[−] c-kit⁺ ST2⁺ Sca-1[−] Fc ϵ RI[−]) in the stroma-vascular fraction (Poglio et al., 2010). Using adoptive transfer of CD45.2 bone marrow cells co-injected with the stromal-vascular fraction of white adipose tissue from CD45.1 mice into lethally irradiated CD45.2 mice, they demonstrated that adipose tissue MCp homed preferentially back to the white adipose tissue or to other peripheral organs whereas the bone marrow MCp homed to the bone marrow (Poglio et al., 2010).

4.3. Mast cell progenitors in the skin

The mobilization of MCp to the skin has been studied by intravenously injecting two weeks old labeled c-kit⁺ BMMC. The migration of the labeled BMMCs was followed after injection of stimuli into the dorsal skin (Weller et al., 2005). Labeled BMMCs accumulated at the injection site when leukotriene B₄ was injected, suggesting that leukotriene B₄ is chemotactic for BMMCs *in vivo*. Since the two weeks old BMMC cultures showed a strong expression of BLT1, which is the receptor of leukotriene B₄, the migration of BMMCs was likely mediated through this receptor. Using the same strategy, Weller and colleagues showed that two weeks old BMMCs accumulated in prostaglandin E₂-injected skin in a dose-dependent manner (Weller et al., 2007). Expression analysis of prostaglandin receptors and *in vitro* studies of chemotaxis suggested that the receptor mediating this effect was the EP₃ receptor. Indeed, also peritoneal mast cells expressed the EP₃ receptor (Sakanaka et al., 2008). Using a similar setup, intradermal injection of CCL2, a chemokine that binds to the chemokine receptor CCR2, into skin could also promote i.v.-injected two weeks old BMMC to move to the injection site (Collington et al., 2010).

IgE alone (without antigen) promotes mast cell survival and increases the expression of Fc ϵ RI (Asai et al., 2001; Kalesnikoff et al., 2001; Furuichi et al., 1985; Yamaguchi et al., 1997). This suggests that IgE in itself have the capability to activate mast cells to

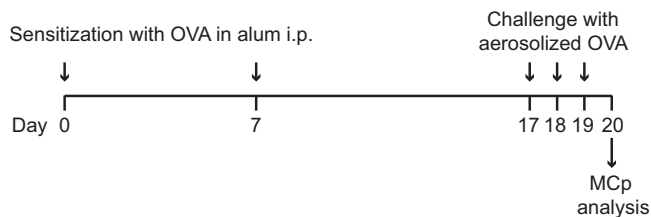


Fig. 4. The protocol used for investigating the mechanisms involved in MCp recruitment to lung during allergic inflammation. Mice were sensitized intraperitoneally (i.p.) days 0 and 7 with OVA in complex with alum. Starting ten days later (day 17), the mice were subjected to three daily treatments lasting for 30 min each with 1% OVA-aerosol. The quantification of MCp was done 24 h after the final challenge.

some degree. Interestingly, intradermal injection of IgE or topical application of gauze strips coated with IgE increased the number of mast cells in the skin after 24–48 h (Collmann et al., 2013; Kitauro et al., 2005). This increase was dependent on functional phosphoinositide 3-kinase- γ (PI3K γ) (Collmann et al., 2013). *In vitro*, endothelial cells upregulated VCAM-1 when stimulated by supernatants from IgE/antigen-activated BMMCs from wild type mice but not from mice lacking functional PI3K γ . The VCAM-1-upregulation was mediated by tumor necrosis factor α (TNF- α), as neutralizing antibodies against TNF- α inhibited the effect (Collmann et al., 2013). In an *in vivo* model, adhesion of BMMCs to the endothelium was stimulated by TNF- α injection into the cremaster muscle. The adhesion of BMMCs to the “inflamed” endothelium was dependent on functional PI3K γ on BMMCs suggesting that PI3K γ was necessary for inflammation induced activation of α 4-integrins (Collmann et al., 2013). Thus, these data support a mechanism where IgE or IgE/antigen in the skin activate mast cells to TNF- α release. TNF- α is necessary for VCAM-1 upregulation and activation of the endothelium to release factors that stimulate activation of α 4-integrins on the MCp. These events are required for the recruitment of MCp to this site. As a complementary mechanism, it is also possible that IgE binding to MCp stimulate the proliferation and maturation of mast cells in the skin.

4.4. Mast cell progenitors in the lung

The lungs of naïve mice contain roughly 20–150 MCp/10⁶ mononuclear cells (Gurish et al., 2001). The low continuous homing of MCp to lung is T cell independent because the levels were intact in RAG-2/IL-receptor common γ -chain double-deficient mice (Gurish et al., 2001). The increase in mast cell number in lungs upon the induction of allergic airway inflammation suggested that the increment could be due to the recruitment of MCp. Indeed, a tremendous increase in the frequency and total numbers of MCp in the lung was observed in ovalbumin (OVA)-sensitized mice one day after three daily challenges with OVA-aerosol (Fig. 4) (Abonia et al., 2006). Using intranasal challenge, but the same sensitization dose and the same timing for challenges, IgE-immune complexes in comparison to the same dose antigen alone was demonstrated to increase the number of lung MCp in a FcR γ -dependent manner (Dahlin et al., 2011).

In the following discussion, the same basal protocol has been used to induce OVA-induced recruitment of MCp (Fig. 4). The recruitment of MCp was highly dependent on the endothelial expression of VCAM-1 because mice lacking endothelial expression of VCAM-1 had similar levels of MCp as naïve or only sensitized mice after OVA-challenge (Abonia et al., 2006). The major importance of VCAM-1, but not MAdCAM-1, was also confirmed using blocking antibodies during the challenge phase. The major integrin ligands for VCAM-1 were evaluated for their involvement in the OVA-induced MCp recruitment. Integrin β 7 knockout mice were shown to have strong reduction in the frequency and total number

Table 1

Chemokine and cytokine receptors or their ligands involved in OVA-induced mast cell progenitor recruitment to the lung.

Deficient strain	MCp frequency (% of WT)	References
CCR2	45*	Collington et al. (2010)
CCL2	41*	Collington et al. (2010)
CCR3	139	Hallgren et al. (2007)
CCR5	64	Hallgren et al. (2007)
CXCR2	47*	Hallgren et al. (2007)
IL-4R α	63	Jones et al. (2009)
IL-4	142	Jones et al. (2009)
IFN- γ	170	Jones et al. (2009)
IL-12p40	71	Jones et al. (2009)

The presented percentages are derived from dividing the average MCp frequency from the indicated deficient mice with the corresponding value for wild type (WT) strains in response to OVA sensitization and challenge.

* Significant reduction in comparison to the wild type control mice.

of MCp. However, the reduction was not as pronounced as in the VCAM-1 deficient mice. In accordance with these results, blocking antibody experiments demonstrated that also β 1-integrins were involved in the MCp recruitment. Blocking α 4-integrins, which associate with both integrins β 1 or β 7, was required for inhibiting the MCp recruitment fully (Abonia et al., 2006). In conclusion, the recruitment of MCp to the allergic lung is dependent on the expression of α 4 β 1 and α 4 β 7 integrins on the MCp and their interaction with endothelial VCAM-1 for the diapedesis to occur (Fig. 3). The rapid increase in lung MCp and the strong dependence on adhesion molecules and integrins suggested that recruitment is the major cause for the strong increase in lung MCp. However, the possibility that MCp division *in situ* still could occur in addition to the OVA-induced recruitment was not investigated.

Recruitment of leukocytes is regulated by chemokine receptors, which mediate chemotaxis and influence the expression or affinity for integrins expressed by the leukocytes. Several chemokine receptor knockouts were investigated to elucidate the potential role for these chemokine receptors in regulation of the OVA-induced recruitment of MCp (Table 1). These studies indicated that CXCR2 and CCR2 were playing a role. The mechanism of action was further investigated using sublethal irradiation (to deplete the host MCp) and adoptive transfer of bone marrow cells. However, these experiment unexpectedly demonstrated that CXCR2 and CCR2 were dispensable on bone marrow derived cells but required on the stromal cells (Hallgren et al., 2007; Collington et al., 2010). CXCR2, which is normally expressed by endothelial cells were required for the OVA-induced upregulation of VCAM-1 to occur, which was a prerequisite for MCp recruitment (Hallgren et al., 2007). This mechanism could not account for the reduction in lung MCp in CCR2-deficient mice or CCR2^{−/−} mice reconstituted with wild type bone marrow (Collington et al., 2010). However, CCL2 seemed to be the major ligand involved since CCL2-deficient mice also had similarly inhibited OVA-induced MCp recruitment as the CCR2-deficient mice (Collington et al., 2010). Using similar bone marrow reconstitution experiments, CCL2 needed to be expressed by both stromal and bone marrow derived cells in order for optimal OVA-induced MCp recruitment to occur. Nevertheless, the exact mechanism of the CCR2-dependent regulation of OVA-induced MCp recruitment to lung remains obscure.

Allergic inflammation in the lung is a process highly dependent on T cells (Robinson, 2010; Lloyd and Hessel, 2010). Thus, the involvement of T cells for the OVA-induced recruitment of MCp to the lung was studied (Jones et al., 2009). As nude mice and RAG2-deficient mice had a severely impaired MCp recruitment compared to wild type mice, the mechanism was T cell dependent. Moreover, wild type mice treated with anti-CD4 but not anti-CD8 blocking antibodies during the challenge phase had a severe decline in lung

MCp compared to control-treated mice. These phenomena together support the idea that CD4⁺ T cells are required for MCp recruitment to the lung. Mice lacking cytokines or cytokine receptors involved in the Th2 development were evaluated for their possible role in the OVA-induced MCp recruitment (Table 1). The results suggested that a classical Th2 response was not required for MCp recruitment. In a similar manner, MCp recruitment was intact in mice with a defect Th1-response (Table 1). These results were also confirmed by the use of blocking antibodies (Jones et al., 2009).

The cytokine IL-9 is known to activate mast cells and support their growth (Noelle and Nowak, 2010). In the study by Jones et al., IL-9 was shown to be important for an optimal OVA-induced increase in lung MCp because IL-9-deficient and wild type BALB/c mice treated with anti-IL-9 antibodies had major reductions in this cell population (Jones et al., 2009). In the same study, similar reductions in the number of OVA-induced lung MCp were seen in CD1d-deficient and anti-CD1d blocking antibody treated mice but not in J α 18^{-/-} mice. Thus, type 2 NKT cells were also implicated as regulators for the OVA-induced increase in lung MCp. Strikingly, anti-CD1d blocking antibody administration to IL-9-deficient mice or anti-IL-9 blocking antibody administration to CD1d-deficient mice did not further reduce the partial reduction in lung MCp (Jones et al., 2009). These results suggested that NKT cells and IL-9 regulate the increase in lung MCp through the same pathway.

The dependence on CD4⁺ T cells for OVA-induced MCp recruitment was also seen in Th1-prone C57BL mice (Jones et al., 2010). However, CD1d-deficient mice on this strain had no diminution of OVA-induced MCp recruitment. Furthermore, blocking with anti-IL-9 antibody did not regulate the OVA-induced increase in lung MCp in C57BL mice. Thus, the NKT cell/IL-9-axis was only required for MCp recruitment in Th2-prone BALB/c mice. Blocking TGF- β 1 or IL-10 and the absence of TGF β RII on T-cells led to significant reductions in the lung MCp population in C57BL mice, suggesting a role for T regulatory cells (Jones et al., 2010). Indeed, treatment with anti-CD25 blocking antibodies during the challenge phase inhibited the increase in lung MCp. These results suggest that although the major events e.g. the dependence on α 4-integrins and VCAM-1 (Fig. 3) agree between the BALB/c and the C57BL strains, details in the requirements for OVA-induced MCp recruitment differ between the strains. A plausible explanation for the difference in regulation of the OVA-induced MCp recruitment to lung is that BALB/c mice have MCp in the circulation that are more mature than MCp in C57BL mice (Dahlin et al., 2013).

The critical requirement of T cells during the challenge phase for OVA-induced increases in lung MCp implicated that CD11c⁺ dendritic cells are a part of the mechanism. To study this, transgenic mice expressing a high-affinity diphtheria toxin receptor under the CD11c promoter was used (Dahlin et al., 2012). When these mice were transiently depleted of lung CD11c⁺ cells during the challenge phase the OVA-induced increase in lung MCp was abolished. These results indicated that the recruitment of MCp was indeed highly dependent on CD11c⁺ cells. To confirm that the MCp recruitment was truly inhibited and to rule out a delayed influx, the mature mast cells were quantified one week after the last OVA treatment. While CD11c⁺ cell-sufficient mice had increased numbers of mature mast cells, mice depleted of CD11c⁺ cells during the challenge phase had low numbers of mast cells, with levels comparable to only sensitized or naïve mice. In accordance with this, the OVA-induced VCAM-1 upregulation on the lung endothelium seen in wild type mice was reduced in the CD11c⁺ cell-ablated mice (Dahlin et al., 2012). The CD11c⁺ cells produced TNF- α , which is a known VCAM-1 inducer as described earlier in this review. Thus, TNF- α was a likely but perhaps not the only mediator from CD11c⁺ cells having a stimulatory effect on the upregulation of endothelial VCAM-1 (Dahlin et al., 2012).

5. Concluding remarks

Mature mast cells activated by numerous stimuli can release a diverse array of mediators, which have a variety of important functions e.g. recruitment of immune cells, smooth muscle contraction and increased vascular permeability. Demonstrating the nature of the progenitor cells giving rise to this cell population and the mechanisms of trafficking into tissues is therefore of immense importance to fully understand the biology of the mast cells *in vivo*. MCp have until recently been regarded as a pool of inert cells with mast cell forming capacity. Using intracellular flow cytometry and IL-4/enhanced GFP reporter mice, Liu et al. showed that MCp induced in the mesenteric lymph nodes by *Trichinella spiralis* infection could produce IL-4 and IL-6 (Liu et al., 2013). This study highlights the possibility that MCp besides being the progenitor pool from which mature mast cells arise from, may have some important functions themselves.

Conflict of interest

The authors declare no financial or commercial conflict of interest.

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