New Aspects of Mast Cell Progenitors in Health and Disease

MAYA SALOMONSSON
Abstract


Mast cells are tissue-resident immune cells that are well known for their involvement in asthma and allergy. They originate in the fetal yolk sac, or in the bone marrow. From the bone marrow, they are released as immature progenitors that are transported via the blood circulation to the peripheral tissue, where they mature. Human mast cells accumulate at certain sites in the airways of individuals with asthma, and in a mouse model of allergic airway inflammation, an influx of mast cell progenitors (MCp) to the lung has been demonstrated. We quantified MCp frequency by flow cytometry to determine if there is a correlation between MCp frequency and reduced lung function among allergic asthmatics and controls. We found that individuals with reduced lung function had an increased MCp frequency in the blood circulation. Additionally, women had a higher MCp frequency than men.

In the second study, we identified a bone marrow counterpart to the human blood MCp. These two MCp populations had similar mast cell-related gene expression, morphology and proliferation capacity. However, the blood MCp expressed higher levels of the integrin β7 receptor, which in mice has been shown to be required for MCp transmigration into peripheral tissues.

Mast cells can be activated by antigen crosslinking of IgE bound to FceRI. Both human and mouse MCp express FceRI, but it is unknown whether they can be activated via this receptor. In the third study, we demonstrated that human and mouse MCp can become activated in vitro via FceRI by measuring tyrosine kinases phosphorylation by flow cytometry. Furthermore, we showed the activation in vivo by measuring IL-13 production in MCp in an allergic airway inflammation model.

In the final study, we investigated the chemokine receptor expression on mouse MCp. We found that MCp in the peritoneal cavity express CCR5 whereas bone marrow MCp express CCR1.

To summarize, my thesis has provided novel insights into the significance of circulating human MCp, and reveled an MCp population in human bone marrow. Moreover, the data presented suggest that MCp can be activated and that their chemokine receptor expression depends on tissue localization.

Keywords: Mast cells, Mast cell progenitor, Lung function, Bone marrow, Blood, Activation, Chemotactic receptors

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“Eventually is a wonderful time of the day. This was a cross-that-bridge-when-we-get-to-it situation for me. And here we are. We’re at the bridge.”

The Newsroom
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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### Abbreviations

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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ACT</td>
<td>Asthma control test</td>
</tr>
<tr>
<td>BLT1</td>
<td>Leukotriene B receptor 1</td>
</tr>
<tr>
<td>BMCP</td>
<td>Basophil/mast cell progenitor</td>
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<td>BMMC</td>
<td>Bone marrow derived mast cells</td>
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<tr>
<td>CTMC</td>
<td>Connective tissue mast cells</td>
</tr>
<tr>
<td>DCBLD2</td>
<td>Discoidin, CUB and LCCL domain-containing protein 2</td>
</tr>
<tr>
<td>EMP</td>
<td>Erythroid/myeloid progenitor</td>
</tr>
<tr>
<td>EP3</td>
<td>Prostaglandin receptor 3</td>
</tr>
<tr>
<td>EoBP</td>
<td>Eosinophil/basophil progenitor</td>
</tr>
<tr>
<td>FeNO</td>
<td>Fractional exhaled nitric oxide</td>
</tr>
<tr>
<td>FEV₁</td>
<td>Forced expiratory volume in one second</td>
</tr>
<tr>
<td>FGF-21</td>
<td>Fibroblast growth factor 21</td>
</tr>
<tr>
<td>GMP</td>
<td>Granulocyte-macrophage progenitor</td>
</tr>
<tr>
<td>HSC</td>
<td>Hematopoietic stem cells</td>
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<tr>
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<td>A human mast cell line</td>
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<tr>
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<td>Immunoreceptor tyrosine-based activation motifs</td>
</tr>
<tr>
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<td>Lineage</td>
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<td>Mast cell progenitors</td>
</tr>
<tr>
<td>MEP</td>
<td>Megakaryocyte/erythroid progenitors</td>
</tr>
<tr>
<td>MMC</td>
<td>Mucosal mast cells</td>
</tr>
<tr>
<td>mMCP</td>
<td>Mouse mast cell proteases</td>
</tr>
<tr>
<td>MMP</td>
<td>Multipotent myeloid progenitors</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger RNA</td>
</tr>
<tr>
<td>OVA</td>
<td>Ovalbumin</td>
</tr>
<tr>
<td>p38 MAPK</td>
<td>p38 mitogen-activated protein kinase</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PEF</td>
<td>Peak expiratory volume</td>
</tr>
<tr>
<td>PR8</td>
<td>Influenza strain A/Puerto Rico/8/34</td>
</tr>
<tr>
<td>SCF</td>
<td>Stem cell factor</td>
</tr>
<tr>
<td>SL-CMP</td>
<td>Sca&lt;sup&gt;+&lt;/sup&gt; common myeloid progenitors</td>
</tr>
<tr>
<td>Syk</td>
<td>Spleen tyrosine kinase</td>
</tr>
<tr>
<td>TNP</td>
<td>Trinitrophenyl</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>Vascular cell adhesion molecule 1</td>
</tr>
</tbody>
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Introduction

The prevalence of asthma is increasing worldwide. The most common type of asthma is allergic asthma, i.e., when an individual experiences asthmatic symptoms, such as wheezing, coughing and shortness of breath, due to repeated exposure to an antigen. Human mast cells have been shown to accumulate at specific sites in the lung tissue of individuals with asthma. However, due to the localization of mature mast cells, they have been difficult to study without invasive methods for sample collection. Therefore, the discovery of human mast cell progenitors (MCp) in peripheral blood allowed translational studies of the significance of circulating MCp in humans.

In the immune system, mast cells are known for their dual role, both the detrimental role in allergy and their protective role as part of the innate immune system. Mast cells are potent cells that, when activated release various mediators that have been shown to be protective in the defense against e.g., parasites, bacteria and certain toxins. They can also contribute to the immune system by releasing mediators that recruit other immune cells that are part of the adaptive immune system.

Mast cells are tissue resident cells that originate in the fetal yolk sac, or in the bone marrow, from which they are released as immature MCp and are transported via the blood circulation to the peripheral tissue, where they mature. The most well-known mechanism for the activation of mast cells is by the crosslinking of IgE, to the high-affinity receptor for IgE (FcεRI) by an antigen. Although both human and mouse MCp are known to express FcεRI, it is unknown whether they can be activated via this receptor. In mouse models, it has been shown that MCp are recruited and accumulate in the mouse lung in allergic airway inflammation, and also during the acute phase of influenza infection.

The aim of this thesis is to increase the knowledge about MCp in healthy individuals as well as in patients with allergic asthma.
Background

Mast cells

Mast cells are potent cells that, when activated can release a cascade of mediators, e.g., proteases, cytokines and lipid mediators. Depending on the setting, mast cells can either be protective or detrimental. They are known for their protective role in contributing to host defense against parasite infection, toxins, venoms and, to some extent, bacterial infection. However, they are just as known for their detrimental role in allergy, asthma and anaphylaxis.

There are different subtypes of mast cells that can be found in various types of tissue. In mice, mast cells can be divided into mucosal mast cells (MMC) and connective tissue mast cells (CTMC) depending on the types of proteases they are storing in their granules. The CTMC stores both tryptase and chymase in their granules, whereas the MMC predominantly store chymase in their granules.

The two subtypes of mast cells have also been shown to develop from different compartments. CTMC are mainly derived from the fetal yolk sac and reside as mature mast cells in the skin and peritoneal cavity. The MMC are predominantly originate from the bone marrow and reside in the intestine and the lungs as mature mast cells (Figure 1). In humans, mast cells can also be divided into two subtypes, MC\textsubscript{TC}, tryptase- and chymase-expressing mast cells that reside in the skin and intestinal submucosa, and the MC\textsubscript{T}, tryptase-expressing mast cells that mainly reside in the alveoli and intestinal mucosal.

![Image](https://via.placeholder.com/150)

Figure 1. Mast cells are tissue-resident cells that originate from the fetal yolk sac or bone marrow. They are granulated cells with histamine and protease-containing granules.
Mast cell progenitors (the origin of mast cells)

Mast cells originate from mast cell progenitors (MCp). CTMC have been shown to mainly originate from the fetal yolk sac⁴,⁵, whereas MMC mainly originate from the bone marrow stem cells⁶-⁸. The development of committed MCp from hematopoietic stem cells (HSC) and the identification of MCp in different organs have been described in mice by many different investigators⁶-¹³ and reviewed by Dahlin et al¹⁴, as summarized in Figure 2A and Figure 3. However, in humans the development of mast cells is less characterized, although there are some studies that have tracked the development using mainly genetic analyses¹⁵,¹⁶ as summarized in Figure 2B.

Figure 2. A model of the development of mast cell progenitors (MCp) from the bone marrow of mouse (A) and human (B). (A) Mouse MCp are developed from hematopoietic stem cells (HSC) via (multipotent progenitors) MPP into Scalo common myeloid progenitors (SL-CMP) directly to a committed MCp. Alternatively, the SL-CMP may develop into a common basophil/mast cell progenitor (BMCP) which could develop into MCp. (B) Human MCp are most likely developing from HSC via multipotent myeloid progenitors (MMP) to erythroid/myeloid progenitors (EMP) to eosinophil/basophil progenitors (EoBP) which might also hold mast cell potential and develop into MCp. Alternatively, the MCp may develop from MMP via megakaryocyte/erythroid progenitors (MEP) to Eo/Baso/Mast=Eosinophil/basophil/mast cell progenitors to a committed MCp.
Mouse mast cell progenitors

The development of mouse mast cells and identification of MCp in different organs has been studied by many groups (Figure 2A). Kitamura and colleagues were first to demonstrate that mouse mast cells are derived from bone marrow\(^1\). They irradiated C57BL mice and transplanted bone marrow from C57BL-Bg\(^{-}\)/Bg\(^{-}\) “Beige” mice, which have mast cells with abnormal granules that can be distinguished from the C57BL mast cells, into the irradiated mice. After 63 days, they demonstrated that mast cells in the intestine of the transplanted mice were bone marrow-derived to a high extent, whereas mast cells in the skin were predominantly from the host\(^1\). Using knockout mice in combination with irradiation and cell transfer, it has been shown that CTMC are developed from the fetal yolk sac, which was first shown by Sonoda et al\(^1\). In accordance with this, also using knockout mice in reconstitution experiments and transgenic mice with reporter genes to track different subsets of stem cells, Li et al\(^5\) and Gentek et al\(^5\) showed that CTMC originate from the fetal yolk sac. They also showed using transcriptomic analysis that the yolk sac-derived mast cells are slowly replaced by mast cells originating from the bone marrow, and when comparing the embryonic yolk sac cells to the adult skin mast cells, they showed different gene expression patterns\(^5\).

The first MCp population was described in fetal blood\(^11\). Later, other investigators identified committed MCp in the bone marrow\(^7,8\) and a basophil/mast cell progenitors (BMCP) in intestine\(^6\). In the granulocyte-macrophage progenitors (GMP) pool of stem cells, Qi et al found that Fc\(\varepsilon\)RI\(^+\) GMP had a high potential of developing into a bipotential BMCP\(^19\). They also confirmed the importance of signal transducer and activator of transcription 5 (STAT5) for mast cell development, as shown by Shelburne et al previously\(^20\). Another transcription factor that have been shown to be important for the differentiation of mast cells and basophils for GMP is CCAAT/enhancer-binding protein alpha (C/EBP\(\alpha\))\(^21\). Qi et al found that the fate decision was regulated by the transcription factors C/EBP\(\alpha\) and also microphthalmia-associated transcription factor (MITF). Upregulation of C/EBP\(\alpha\) was shown to promote the development of basophils, and MITF promoted the development of the BMCP into mast cells\(^19\). Using RNA sequencing, it was confirmed that the already established BMCP population\(^6\) from the HSC pool, here identified as lineage\(^-\) (Lin\(^-\)) Sca-1\(^-\) c-kit\(^+\) integrin \(\beta\)^7hi CD16/32\(^+\)hi cells, indeed had dual mast cell and basophils lineage commitment\(^22\). Sorting of these cells and culturing in presence of various cytokines also confirmed the existence of bipotent BMCP\(^22\).

A rare population of MCp (0.0045% of mononuclear cells) identified as Lin\(^-\) c-kit\(^hi\) ST2\(^+\) integrin \(\beta\)^7hi and CD16/32\(^hi\) cells have also been detected in blood\(^10\). In the same paper, the authors demonstrated that there is a strain difference in the maturation of MCp between the C57BL/6 and the BALB/c mice\(^10\). They found that BALB/c mice had a higher percentage of Fc\(\varepsilon\)RI\(^+\) MCp.
in the blood circulation compared to the C57BL/6 mice\textsuperscript{10}. In naïve mice, a small population of MCp identified as CD45\textsuperscript{+} c-kit\textsuperscript{hi} Lin\textsuperscript{−} T1/ST2\textsuperscript{+} integrin β7\textsuperscript{hi} CD16/32\textsuperscript{+} FcεRI\textsuperscript{+} cells can be found in the lung\textsuperscript{12}. Upon influenza infection, the population of MCp in the lung increases, most likely due to recruitment from the bone marrow\textsuperscript{12}. Another model, that causes the recruitment of MCp to the lung is sensitization with ovalbumin (OVA)/alum followed by OVA aerosol challenge\textsuperscript{23}.

MCp can also be detected in the peritoneum where they are identified as c-kit\textsuperscript{hi} Lin\textsuperscript{−} SSC\textsuperscript{lo} integrin β7\textsuperscript{hi} CD16/32\textsuperscript{int} FcεRI\textsuperscript{+} cells\textsuperscript{9}. Mouse MCp from the peritoneum have an immature mast cell phenotype; compared to mature mast cells, they are smaller in size and have none or few granules. However, after fourteen days in culture with IL-3 and SCF, the MCp mature, i.e., they start to fill up the granules and increase in size\textsuperscript{9}. The various markers for identifying MCp in the different organs are summarized in Figure 3.

**Figure 3.** Overview of the mast cell progenitor (MCp) populations in mice. Committed MCp have been identified in different organs by different investigators. MCp have been identified by flow cytometry in lung\textsuperscript{12}, spleen\textsuperscript{6}, peritoneum\textsuperscript{9}, intestine\textsuperscript{6}, bone marrow\textsuperscript{7,8}, fetal blood\textsuperscript{11} and peripheral blood\textsuperscript{10} using antibodies against different surface receptors. Lin; lineage and SSC; side-scattered light.
Human mast cell progenitors

Human mast cells have been derived from CD34+ cells from peripheral or cord blood in vitro for many years. However, to develop into mast cells, CD34+ cells need to be kept in culture with mast cell-promoting cytokines for a long time. Human mast cells originate from the bone marrow, which was first described in a study by Kirshenbaum et al, where CD34+ bone marrow cells were shown to develop into mast cells when cultured in the presence of IL-3. Later, mast cells were shown to also be derived from CD34+ FcεRI- cells from peripheral blood after six weeks in culture together with recombinant human stem cell factor (SCF) and/or IL-3. Additionally, CD34+ c-kit+ CD13+ peripheral blood progenitors were shown to readily develop into mast cells when cultured in the presence of IL-3 and SCF.

The development of human mast cells from HSC is still mostly unknown. However, two studies have investigated the origin of human mast cells using a genetic approach. (Figure 2B). Most likely, mast cells, like other myeloid cells, are derived from multipotent myeloid progenitor cells (MMP). Velten and colleagues suggested the existence of a common progenitor for basophils/eosinophils/mast cells (Eo/Baso/Mast) that are derived from Lin- CD34+ CD38+ IL-3α- CD45RA- megakaryocyte/erythroid progenitor cells (MEP). Another study suggested the existence of a common eosinophil/basophil progenitors (EoBP) derived from CD133lo CD34+ CD38+ CD45R- erythroid/myeloid progenitor (EMP). The authors in this study speculated that such EoBP might also have the capability to form committed MCp (Figure 2B).

Recently, a population of MCp was identified in peripheral blood as CD4- CD8- CD19- CD14- CD34+ CD117+ FcεRI+ cells, and in a microarray analysis, these cells had higher expression of mast cell-related genes compared to sorted peripheral blood basophils, implying that they are committed to the mast cells lineage. The human MCp constituted approximately 0.005 % of Ficoll-enriched blood mononuclear cells. In the same study, a connection between reduced lung function and a high frequency of MCp could be established in a small number of individuals. Human MCp contains none or very few granules, and the nuclei occupy most of the cytoplasm. However, after seven days in a myelo-erythroid cytokine cocktail, the mast cell granules could be detected by May-Grünwald Giemsa staining.

Mast cell activation and migration

Activation of mast cells

Mast cells can become activated in various ways via different receptors. The level of activation varies from the production of cytokines or other mediators
to full degranulation and the release of a cascade of granule-stored proteases and mediators\textsuperscript{3}.

The most well-known activation mechanism is via the high affinity receptor for IgE, FcεRI. However, mast cells can become activated by other mechanisms, such as, complement and complement receptors and through pathogen-associated molecular patterns (PAMPs) that are recognized by pattern recognition receptors (PRRs), e.g., toll-like receptors that are expressed by mast cells\textsuperscript{28}.

Activation through IgE crosslinking of FcεRI by antigen occurs in two steps. The sensitization phase, when due to a combination of environmental factors and genetic predisposition, an individual is exposed to an antigen that stimulates the B-cells to produce antigen-specific IgE. When the individual is re-exposed to the antigen, it can bind to the IgE already bound to the FcεRI on the mast cell, causing IgE crosslinking. FcεRI contains of three subunits: one α-subunit, one β-subunit and two γ-subunits (αβγ\textsubscript{2})\textsuperscript{29}. The α-subunit binds with high affinity to the Fc portion of the IgE molecule, whereas the intracellular signaling occurs when IgE is crosslinked to FcεRI and is mediated through the β-subunit, and two γ-subunits containing immunoreceptor tyrosine-based activation motifs (ITAM)\textsuperscript{30,31}. Phosphorylation through ITAM starts a signaling cascade in which a number of tyrosine kinases are phosphorylated, including the early protein kinase, spleen tyrosine kinase (Syk) and the downstream protein kinase mitogen-activated protein kinase (MAPK), e.g., p38 MAPK\textsuperscript{32,33}.

The activation of the mast cell via the signaling cascade eventually leads to degranulation, where the cell releases stored mediators e.g., histamine and proteases\textsuperscript{29}. Mast cells produce lipid mediators and cytokines, which also are released after activation\textsuperscript{29}.

In addition to mast cells, mouse and human basophils also express a tetrameric form of FcεRI. However, mouse superior cervical ganglion neurons\textsuperscript{34} and human airway smooth muscle cells\textsuperscript{35} have also been shown to express this form of the receptor. Moreover, a variant of the FcεRI receptor with a α-chain and two γ-chains has been shown to be expressed on a variety of human cells, including dendritic cells\textsuperscript{36}, eosinophils\textsuperscript{37}, monocytes\textsuperscript{38} and neutrophils\textsuperscript{39}.

The CCL3/MIP-1α that binds to CCR1 has also been shown to be important for mast cells to become activated and degranulate\textsuperscript{40,41}. By injecting MIP-1α into the skin of mice and analyzing the tissue with electron microscopy, Alam et al, demonstrated a cutaneous response of mast cell degranulation \textit{in vivo} two hours post injection\textsuperscript{40}. Moreover, results similar to those obtained by Alam et al\textsuperscript{40} have been demonstrated using MIP-1α knockout mice that were subjected to a cat allergy model\textsuperscript{41}. The authors of this study observed that MIP-1α knockout mice had lower amounts of specific IgE, fewer clinical symptoms and fewer degranulated mast cells than the wildtype mice\textsuperscript{41}. Similar results were shown with blocking antibodies for MIP-1α\textsuperscript{41}. 
Migration

**Chemokine receptors and adhesion molecules**

Many different types of leukocytes have been shown to migrate in response to chemokine receptor activation by their ligands\textsuperscript{42}. For example, T-cell progenitors require CCR7 and CXCR4 to enter the thymus\textsuperscript{42}. The CXCR4/CXCL12 interaction has also been shown to regulate the release of different cell types, i.e., monocytes, B-cells and neutrophils from the bone marrow into the blood circulation\textsuperscript{42}. However, the expression and function of chemokines and their receptors on mast cells have been explored mainly \textit{in vitro}. Mouse mast cells developed \textit{in vitro} express mRNA or demonstrate the protein expression of several chemokine receptors, as described in Table 1. Stimulation of bone marrow-derived mast cells (BMMC) with IgE and SCF causes the upregulation of CCR1-3, and CCR5 mRNA\textsuperscript{43}. Furthermore, when the BMMC were exposed to IgE and SCF, directed migration was induced in an \textit{in vitro} chemotaxis assay\textsuperscript{43}. BMMC have also been shown to express mRNA for chemokine receptors CCR1\textsuperscript{44}, CXCR4\textsuperscript{44} and CX\textsubscript{3}CR1\textsuperscript{43,44}. In a transcriptomic analysis of embryonic yolk sac-derived mast cells, CX\textsubscript{3}CR1 was detected; however, the authors did not find expression on sorted skin mast cells from adult mice in the same study\textsuperscript{5}. Studying human mast cells, it has been shown that isolated mast cells from neonatal foreskin express mRNA for CX\textsubscript{3}CR1\textsuperscript{44}.

Human mast cells developed \textit{in vitro} express mRNA or demonstrate surface expression of the chemokine receptors described in Table 2. \textit{In vitro} derived human mast cells from cord blood display directed migration towards ligands to CCR3 and CXCR4 when assayed in a chemotaxis assay\textsuperscript{45}. In a study where mast cells were isolated from human lung tissue, the surface expression of chemokine receptors CCR1, CCR3, CCR4, CCR6, CCR7, CXCR1, CXCR3 and CXCR4 was demonstrated by flow cytometry. They also found that the most abundantly expressed chemokine receptor was CXCR3\textsuperscript{46}.

### Table 1. Chemokine receptor expression in mouse mast cells.

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<th>mRNA</th>
<th>Surface expression</th>
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<td></td>
<td>CCR1\textsuperscript{41}</td>
</tr>
<tr>
<td>BMMC</td>
<td>CCR1\textsuperscript{43,44}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CCR2\textsuperscript{43}</td>
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<td>CX\textsubscript{3}CR1\textsuperscript{43,44}</td>
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<tr>
<td><strong>Yolk sac derived embryonic mast cells</strong></td>
<td>CX\textsubscript{3}CR1\textsuperscript{5}</td>
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Table 2. Chemokine receptor expression in human mast cells.

<table>
<thead>
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<th>mRNA</th>
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<tr>
<td>Isolated mast cells from neonatal foreskin</td>
<td>CX3CR1^{44}</td>
</tr>
<tr>
<td>Isolated lung mast cells</td>
<td>CCR1^{46}</td>
</tr>
<tr>
<td></td>
<td>CCR3^{46}</td>
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<td>Mast cells derived from cord blood</td>
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<td>CXCR2^{45}</td>
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<td>CXCR4^{45}</td>
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</tbody>
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Recruitment of mast cell progenitors in vivo

Homing of MCp has been investigated in mice, but due to the rareness of the cells, it has been difficult to study. In mouse models of allergic asthma and influenza, MCp have been demonstrated to be recruited to the lung^{12,13,23,47,48}. Both influenza infection and airway inflammation induce maturation of a fraction of the MCp into mast cells, a process that proceeds through an intermediate maturation state of mast cells (immature mast cells) in the lung^{12,13,49}. While MCp express a high level of integrin β7 and the mature mast cells lack or express low levels, the immature mast cells have an intermediate expression of integrin β7^{12,13,49}.

In a mouse model of allergic asthma, mice lacking the vascular cell adhesion molecule (VCAM-1) on endothelial cells were unable to recruit MCp to the lung^{23}. In addition, a partial block of the antigen-induced recruitment of MCp to the lung was achieved by the administration of blocking antibodies toward VCAM-1 or α4-, β1- and β7-integrins during the challenge phase^{23}. Together, these data suggest that MCp use their expression of α4-integrins (α4β1 and α4β7) to firmly attach to VCAM-1 on the lung vascular endothelium and that this interaction is necessary for the recruitment of MCp into the inflamed lung.

The chemokine receptors CXCR2 and CCR2 regulate the recruitment of MCp to the lung in mice with allergic airway inflammation^{48,50}. However, the effects were indirect because the expression of CXCR2 was necessary for the inflammation-induced upregulation of VCAM-1 on the endothelium, which, as mentioned before, is required for the antigen-induced recruitment of MCp.
to the lung\textsuperscript{23}. In the same experimental set-up, CCR2 expression was required both on stromal- and bone marrow-derived cells for the optimal recruitment of MCp to the allergic lung to occur\textsuperscript{50}.

In a mouse model of acute influenza infection, using the PR8 virus, MCp were recruited to and accumulated in lung tissue\textsuperscript{12,13}. After irradiation of wild-type mice (CD45.2) and bone marrow transfer from congenic CD45.1 mice, it was observed that MCp accumulation in the lung tissue was mainly from the donor mice and not the recipient mouse\textsuperscript{12}. This experiment suggests that the influx of MCp is mainly due to recruitment of MCp from the bone marrow and not proliferation. They also demonstrated that up to 21 days post infection, the dominant mast cell type was the MCp, followed by immature mast cells and, lastly, the mature mast cells\textsuperscript{12}.

**Leukotrienes and prostaglandins**

Lipid mediators could be potential candidates for the mediating MCp migration. More specifically, leukotrienes and prostaglandins have been investigated for their potential role in inducing the migration of mast cells\textsuperscript{51,52}. BMMC express the leukotriene receptor 1 (BLT1)\textsuperscript{51} and the prostaglandin receptor 3 (EP3)\textsuperscript{52} and migrate towards concentration gradients of leukotriene B\textsubscript{4}\textsuperscript{51} and prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) \textit{in vitro}\textsuperscript{52}. \textit{In vivo}, BMMC injected intravenously in mice migrated towards an intradermal injection of leukotriene B\textsubscript{4}\textsuperscript{51,52} and PGE\textsubscript{2}\textsuperscript{52}.

In a human mast cell line (HMC-1), the leukotriene receptors BLT1 and leukotriene receptor 2 were expressed at the mRNA level, and intracellular antibody staining demonstrated BLT1 protein expression\textsuperscript{53}. Furthermore, stimulation of HMC-1 cells with leukotriene B\textsubscript{4}-induced migration of the cells in an \textit{in vitro} chemotaxis assay\textsuperscript{53}.

**Allergic asthma and lung function**

Asthma is becoming an increasing health issue of the worldwide, and causes a reduced quality of life for many people. According to the global health initiative for asthma (GINA), common symptoms among asthmatic patients include coughing, difficulty breathing, chest tightness, shortness of breath and wheezing\textsuperscript{54}. These symptoms are tightly connected with bronchoconstriction, thickening of the airway wall and mucus production\textsuperscript{54}. There are many different cell types involved in asthma, e.g., mast cells, eosinophils, neutrophils and T-cells that contribute to the symptoms. There are several different phenotypes of asthma, for example allergic and non-allergic asthma. Non-allergic individuals with asthma have asthma symptoms but are not allergically sensitized to a specific antigen and have normal or low levels of specific IgE antibodies in serum\textsuperscript{55}. 

20
Allergic asthma is mediated by IgE, and IgE-mediated mast cell activation is thought to be a major mechanism of this disease. The most common mechanism for mast cell activation is IgE crosslinking of FcεRI with antigen. Allergic asthmatics often have high levels of allergen-specific IgE, and also high levels of total serum IgE compared to healthy controls\textsuperscript{56,57}. Nevertheless, the level of total serum IgE alone is rarely used as a biomarker for asthma, as reviewed by Froidure and colleagues\textsuperscript{55}.

An asthmatic attack can occur due to e.g., respiratory virus infection, commonly rhinovirus, or an allergic reaction in the airways. These episodes cause e.g. bronchoconstriction, mucous production, and in worst-case acute respiratory failure. Allergic asthma is more common among boys, but during puberty there is a shift, and asthma becomes more common among women\textsuperscript{58}. To measure the lung function of a patient, several different lung function parameters can be used. Two of the most common, FEV\textsubscript{1} (forced expiratory volume in one second) and PEF (peak expiratory flow) (Table 3), were analyzed in manuscript I. To determine whether the FEV\textsubscript{1} and PEF measurements are normal or signs of reduced lung function, the percent predicted FEV\textsubscript{1} or PEF can be calculated. A standard value depending on gender, age and length are compared to the measured value of each patient. Usually, a value <80\% of predicted in PEF or FEV\textsubscript{1}, is considered to be a sign of impaired lung function among asthmatic individuals\textsuperscript{59,60}.

FeNO (fractional exhaled nitric oxide) is a measurement of the nitric oxide concentration in exhaled air (Table 3). In asthma patients, a high FeNO correlates with eosinophilic airway inflammation\textsuperscript{61}. FeNO is also used to determine if the patient is likely to respond to steroid treatment of the airway inflammation symptoms\textsuperscript{62}. To evaluate how much control the patients have of their asthma symptoms in their daily life, questionnaires such as the ACT (asthma control test) are used (Table 3). In this test, the patients will answer five questions about their symptoms, such as e.g., “In the past four weeks, how often did you have shortness of breath?” The patients use a 1-5 scale to answers the questions based on their symptoms. The maximum score is 25, and a score >19 indicates well-controlled asthma according to guidelines from the American Thoracic Society.

<table>
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<th>Unit</th>
<th>Description</th>
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<td>FEV\textsubscript{1}</td>
<td>L or % of predicted</td>
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<tr>
<td>The largest volume of air that is exhaled during the first second of measurement</td>
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<td>PEF</td>
<td>L/min or % of predicted</td>
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<td>The maximum rate of expiration</td>
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<tr>
<td>FeNO</td>
<td>ppb (parts per billion)</td>
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<td>The concentration of nitric oxide in exhaled air</td>
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<td>ACT</td>
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<td>Asthma control test</td>
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Mast cells in allergic asthma

Mast cells are known to accumulate at certain sites in the airways of asthmatics. For example, asthmatic patients have an increased number of mast cells in airway smooth muscles than do healthy controls or patients with eosinophilic bronchitis. Asthmatics also have an increased density of mast cells in the bronchial epithelium and the lamina propria when compared to healthy controls.

In human lung tissue from allergic asthmatics, both subtypes of mast cells, MC\(_T\) and MC\(_{TC}\), can be found. Compared to healthy controls, patients with atopic uncontrolled asthma have an altered distribution of the two mast cell subtypes in the alveolar parenchyma. Uncontrolled asthmatics were shown to have a higher density of total mast cells as well as the MC\(_{TC}\) subtype of mast cells. In the same study, the mast cells from the uncontrolled asthmatics were shown to have a significantly higher expression of FceRI on the cell surface. There are reports of mast cells residing in the alveolar parenchyma with no or very low expression of FceRI. The same authors have shown that the FceRI expression and IgE binding on mast cells are much higher in allergic asthmatics with allergic rhinitis than in control groups with or without allergic rhinitis.

Moreover, asthmatics who died due to factors unrelated to their asthma have a higher number of mast cells in the submucosal mucous gland of their lungs compared to individuals without asthma. In the same study, patients with fatal asthma had a higher percentage of degranulated mast cells in the submucosal glands. In another study, an increased density of mast cells was found in the alveolar parenchyma of patients with uncontrolled asthma compared to healthy controls. There are also studies demonstrating the release of mast cell mediators upon allergen provocation in allergic asthmatics. For example, the levels of histamine, prostaglandin D\(_2\) and its metabolites are increased in the bronchoalveolar lavage fluid of allergic asthmatics after allergen provocation.
Present investigation

Aim
The general aim was to increase our knowledge about mast cell progenitors (MCp) in healthy individuals as well as in patients with allergic asthma. Specifically, the aims were to determine whether the frequency of blood MCp was related to disease parameters in asthma. We also wanted to elucidate whether MCp can be activated through IgE crosslinking of FcεRI, and what receptors that are expressed and that may be involved in the homing and recruitment of the cells to peripheral tissues.

Manuscript I
The aim of this study was to determine whether there is an increased frequency of circulating MCp in allergic asthmatics compared to controls. Further we wanted to investigate whether there is a relationship between reduced lung function and a high frequency of MCp. Finally, we quantified 180 different plasma proteins to determine whether there was a correlation between any of these proteins and the frequency of MCp.

Manuscript II
The aim of this study was to determine whether there is a bone marrow counterpart to the human peripheral blood MCp. Since such a population could be detected, we investigated similarities and differences between bone marrow and blood MCp.

Manuscript III
In this manuscript, the aim was to determine whether MCp in humans and mice are immunologically functional cells that can be activated via the high affinity receptor for IgE, FcεRI.

Manuscript IV
The aim of this study was to determine what chemokine receptors are expressed by mouse MCp.
Experimental setup

Analysis of human samples
In manuscripts I, II and III, human MCp from peripheral blood and bone marrow were analyzed.

Study populations

Manuscript I: Minimally Invasive Diagnostic Procedures in Allergy, Asthma or Food Hypersensitivity Study (MIDAS)
The MIDAS cohort included 411 young adults in Uppsala, both allergic asthmatic and healthy controls. In our study, a fraction of these subjects, 29 controls and 38 allergic asthmatics, were donated blood for a frequency analysis of MCp.

Manuscript II: Healthy bone marrow donors
To determine if there is a population of MCp in the bone marrow of healthy donors, 10 individuals donated bone marrow and/or peripheral blood samples. In addition, buffy coats from the blood center at the Akademiska hospital were used for the Ki-67 expression analyses.

Manuscript III: Respiratory Health In Northern Europe, Switzerland, Spain and Australia (RHINESSA)
The RHINESSA cohort, focuses on investigating lung status, allergy and other related diseases among the included subjects. To investigate if MCp can become activated fresh blood was obtained from donors within the RHINESSA cohort.

Mononuclear cell enrichment
Mononuclear cells from blood and bone marrow were enriched by Ficoll-gradient centrifugation and platelets were removed by centrifugation.

Flow cytometry
Cells were incubated with fluorescent-labeled antibodies and analyzed by flow cytometry to determine the frequency of MCp. The gating strategy first described by Dahlin et al27 was used (Figure 4). MCp were gated as negative for the lymphocyte markers CD4, CD8 and CD19 and the monocyte marker CD14. Furthermore, the MCp were gated as positive for the expression of the progenitor marker CD34, the stem cell factor receptor CD117 and the high affinity receptor for IgE, FceRI.
**Figure 4.** Flow cytometry gating strategy for mast cell progenitors from human peripheral blood.

**Statistical analysis**
Statistical differences between groups were determined by Student’s t-test. Correlation analysis was tested with Spearman correlation and adjustment for multiple analysis was performed with the false discovery rate and Bonferroni correction. A p-value less than 0.05 was considered significant.
Analysis of mouse samples

**Mononuclear cell preparation**
Lung, spleen, bone marrow and peritoneal cells were collected from euthanized BALB/c mice. The lungs were flushed with phosphate buffered saline (PBS) before they were carefully removed. The lungs were enzymatically and mechanically digested and red blood cells were lysed. Centrifugation in 44% percoll was used to remove debris.

**Flow cytometry**
Cells were incubated with fluorescent-labeled antibodies to detect MCp. MCp are a rare cell population among the CD45-positive, hematopoietic cells. Therefore, the negative selection of lineage markers expressed by other cell types is part of the gating strategy; CD8b, CD3, CD4 (T-cells), CD19, B220 (B-cells), CD11b, Gr-1 (granulocytes and macrophages) and TER119 (erythrocytes) are discarded in the second step of the gating. Next, the stem cell factor receptor, c-kit positive cells are gated. MCp are also positive for the IL-33 receptor, ST2/T1, integrin β7 receptor, the Fcγ receptor III/II (CD16/32) and the high affinity receptor for IgE, FcεRI. To further distinguish the MCp from the mature mast cells forward and side scatter can be used, MCp are smaller in size and have less granularity compared to the mature mast cells, and thus they have lower forward and side scatter. An example of a gating strategy for mouse peritoneal mast cells and MCp is shown in (Figure 5).

![Flow cytometry gating strategy for mast cell progenitors (black) and mast cells (gray) from the mouse peritoneal cavity.](image)

**Figure 5.** Flow cytometry gating strategy for mast cell progenitors (black) and mast cells (gray) from the mouse peritoneal cavity.

**Allergic airway inflammation model**
In the both the experimental group and the control group, mice were sensitized with OVA intraperitoneally on days 0 and 7. On days 17-19, the mice in the experimental group were challenged with OVA aerosol, and 30 min after the last challenge, the mice were euthanized. After that, the lungs were flushed with PBS and carefully removed.
**In vivo sensitization and in vitro challenge protocol**
Mice were sensitized with OVA/alum intraperitoneally at days 0 and 7. After 20-30 days, the mice were euthanized and cells were extracted from the peritoneal cavity. The peritoneal cells were stimulated *ex vivo* with OVA before analysis by flow cytometry.

**In vitro sensitization and challenge protocol**
To analyze peritoneal cells *in vitro*, mice were euthanized and peritoneal cells were harvested. After extraction, the cells were sensitized with TNP (trinitrophenyl)-specific IgE. After washing, the cells were challenged with OVA-TNP.

**Influenza model**
Mice were anesthetized with isoflurane and infected intranasally with 4x10⁴ tissue culture infectious dose 50 (TCID₅₀) of the H1N1 influenza A/Puerto Rico/8/34 strain. The control mice were administered an equal intranasal volume of sterile PBS (pH 7.4). The weight of the mice was monitored and mice that lost more than 15 % of their initial weight were terminated. Depending on the experiment, on days 6, 8 or 9 post infection, the mice were euthanized. After that, the lungs were flushed with PBS and carefully removed.

**Statistical analysis**
Statistical differences between groups were determined by Student’s t-test. A p-value less than 0.05 was considered significant.
Results, discussion and future perspectives

Manuscript I

Circulating mast cell progenitors correlate with reduced lung function in asthma

Mast cells are known to have a detrimental role in allergic asthma. However, due to the peripheral tissue location of mature mast cells, they have been difficult to study without invasive methods for sample collection. Recently, a population of MCp was identified by our group in human peripheral blood. Using flow cytometry, the MCp can be identified as CD4⁻ CD8⁻ CD19⁻ CD34⁺ CD117⁺ FcεRI⁺ cells. This method of identifying MCp in blood has allowed us to study whether there were differences in the frequency of MCp in peripheral blood between allergic asthmatics and controls. We found that there was no difference in the MCp frequency in the blood circulation between allergic asthmatics and controls. However, this might be explained by the fact that the allergic asthmatics were very well-controlled in their asthma, i.e., they had a good asthma control test scores and on average, a close to normal lung function. Still, we found that subjects with reduced lung function, determined by FEV₁ and PEF (% of predicted), had an increased frequency of MCp in their blood circulation. We also found that women with allergic asthma had a higher frequency of MCp in the blood compared to men, also after adjustment for differences in FEV₁ (% of predicted).

In mice, the binding of IgE enhances the expression of FcεRI on mast cells. We found that although there was no correlation between the MCp frequency and the total IgE level, there was a correlation between FcεRI-expression (the geometrical mean fluorescence) on MCp and the total level of IgE in the controls but not in the allergic asthmatics. This finding suggests that the binding of IgE might also stabilize the expression of FcεRI in human MCp, similar to what has been shown in mice, at least up to a saturation point.

Lastly, using a proximity extension assay, we analyzed 180 different plasma proteins that can be measured in plasma. We found that after adjustment for multiple analyses, there was one protein, fibroblast growth factor 21 (FGF-21), that was significantly correlated with the frequency of MCp both among allergic asthmatics and all subjects together. There is not much known about FGF-21 and asthma; therefore, further studies are necessary to determine the significance of the protein in relation to MCp frequency. Another protein that was positively correlated with the MCp frequency among allergic asthmatics but that did not pass the correction for multiple analysis was discoidin, CUB and LCCL domain-containing protein 2 (DCBLD2). DCBLD2 is a transmembrane protein expressed in endothelial and smooth muscle cells. There are different haplotypes of DCBLD2. In one study, the authors found that among 9 out of 12 common haplotypes of DCBLD2, there was a decline in FEV₁ among patients with aspirin-exacerbated respiratory disease when
challenged with aspirin. Therefore, we find the possible relation between DCBLD2 and MCp frequency interesting.

One aspect that should be investigated in the future is whether the MCp frequency in allergic asthmatics or acute or uncontrolled asthma is increased compared to the frequency in controls. To follow-up our findings, blood samples will be collected from allergic asthmatics who are sensitized to birch pollen during the birch pollen season and after the pollen season. We will analyze blood samples from these patients to determine whether they have a higher frequency of MCp during the pollen season or out of season and whether the MCp frequency is correlated with different lung function parameters.

In the future, it would be of interest to further investigate if women have higher frequency of MCp in other mast cell-related diseases. For example, it would be interesting to study mastocytosis patients who do not experience any respiratory symptoms to investigate whether the increased MCp frequency is driven by the reduced lung function. In this type of study, it could be relevant to determine if women have a higher frequency of MCp compared to men. Additionally, one could continue exploring the mediators found in plasma that correlate or had a tendency to correlate with the MCp frequency. This would provide insights as to whether the increased frequency of MCp among women or the increased levels of plasma protein is related to reduced lung function.

Finally, an additional option to study the relevance of FGF-21 and DCBLD2 is to translating our human data to a mouse model. By implementing such a model, it would be possible to perform allergic airway models with various antigen. This would allow us to study MCp in the lung or other tissue that requires the more invasive methods to obtain the samples.

Manuscript II

Demonstration of human mast cell progenitors in the bone marrow

In this study, we were interested in investigating whether a population similar to blood MCp identified as, CD4− CD8− CD19− CD34hi CD117+ FcεRI+ cells could be found in bone marrow from healthy donors. For culture experiments, CD4− CD8− CD19− CD34hi CD117+ FcεRI+ cells from blood and bone marrow of the same donor were sorted and cultured in a myelo-erythroid cytokine cocktail for seven days before they were reanalyzed by flow cytometry. The reanalysis of the cells revealed that the blood and bone marrow cells were very similar in proliferation capacity; they divided 4-5 times per cell. It also showed that the progenies expressed similar levels of CD117 and FcεRI after culture. Next, the progenies were cytocentrifuged on to glass slides and stained with May-Grünwald Geimsa to determine the morphology of the cells. Alternatively, the cells were incubated with a fluorescently labeled anti-tryptase monoclonal antibody to determine the tryptase content. These analyses showed that the sorted cells from both blood and bone marrow were similar in both
morphology and tryptase content, suggesting that the bone marrow population consisted of MCp. The only noticeable difference between the cell populations was the 13-fold higher frequency of CD4- CD8- CD19- CD34hi CD117+ FcɛRI+ cells in bone marrow compared to the blood. However, the cell population found in the bone marrow is still rare, composing of approximately 0.06% of the mononuclear cells. To further investigate the CD4- CD8- CD19- CD34hi CD117+ FcɛRI+ cells from bone marrow and blood, 100 cells from the same donor were sorted and lysed, and the cDNA was pre-amplified to analyze the mRNA level of six mast cell-related genes: the stem cell factor binding receptor (KIT), the high affinity IgE binding receptor (FCER1A), carboxypeptidase A3 (CPA3), hematopoietic prostaglandin synthetase D (HPGDS) serglycin (SRGN) and tryptase alpha/beta I/II (TPSAB1/TPSAB2). All these targets were equally expressed by the CD4- CD8- CD19- CD34hi CD117+ FcɛRI+ cells from both bone marrow and blood. Therefore, combining our findings from sorted and cultured CD4- CD8- CD19- CD34hi CD117+ FcɛRI+ cells and the mRNA expression in the cells, we concluded that the MCp in the bone marrow of healthy donors are similar to the population previously described in the blood circulation.

To compare the MCp population in bone marrow with the corresponding MCp population in blood, we investigated surface receptor expression of the IL-3 receptor (IL-3R), the IL-33 receptor ST2 and integrin β7; the last two are receptors known to be expressed by mouse MCp9,10,12,13. We found that the expression of integrin β7 was increased on the blood MCp compared to the bone marrow MCp. We found this especially interesting since it has previously been determined that integrin β7 is critical for the transmigration of mouse MCp from the blood circulation to the lung23. The higher integrin β7 expression on blood MCp compared to that on bone marrow MCp might be an indication that the blood MCp are at a more mature stage, as summarized in (Figure 6).

We also investigated the proliferation capacity of MCp from bone marrow and blood using two different methods. First, we cultured the cells in a myeloid-erythroid cytokine cocktail for seven days before determining the number of progenies. In another set of experiments, we measured the intracellular signal of the proliferation marker Ki-67 and found that, on average, 58% of the blood MCp, and 87% of the bone marrow MCp, expressed this protein. Both analyses demonstrated similar proliferation capacities between the two populations. Therefore, we conclude that the MCp from both blood and bone marrow have high proliferation capacities but that the culture conditions are insufficient to support optimal MCp proliferation.
Figure 6. Mast cell progenitors (MCp) in the blood circulation express higher levels of integrin β7 compared to MCp in bone marrow. This might indicate that blood MCp are more mature than their bone marrow counterpart and are getting prepared for transmigration into peripheral tissue.

In the future, it would be interesting to investigate the culture conditions for MCp to determine a more optimal cytokine cocktail to support the cells in culture. In the myeloid-erythroid cytokine cocktail used in the above study, there are eleven different cytokines to support the differentiation of the progenitors into different types of myeloid-erythroid lineage cells. By adding all these different cytokines, the aim was to determine if the sorted MCp were committed to the mast cell lineage. However, the high expression of Ki-67 in the MCp in combination with poor proliferation in vitro, suggests that this myeloid-erythroid cytokine cocktail is insufficient to support optimal MCp proliferation. To further investigate what cytokines are necessary for optimizing the culture conditions of MCp, it would be interesting to culture the cells with classical cytokines to support mast cells in culture, e.g., IL-3, IL-6, and SCF. Another option would be to culture the sorted MCp with different cytokine cocktails and in the presence of human IgE, which has been shown to stabilize the FceRI expression in CD34-derived mast cells in culture.

Finally, sorting MCp from bone marrow and blood and performing a genetic analysis, i.e., microarray analysis or RNA sequencing, could provide more insight into the differences and similarities between the two MCp populations.
Manuscript III

**Mast cell progenitors can be activated by IgE crosslinking**

In this study, the aim was to determine if MCp are immunologically functionally activated cells. Mast cells are known to express the FcεRI, as are MCp. Thus, we hypothesized that MCp can be activated via the crosslinking of IgE with antigen on FcεRI. Because both mature mast cells and MCp are very rare cells, we started by investigating mast cells and MCp from the peritoneal cavity, where these cells are more abundant compared to other organs. In the peritoneal cavity, MCp can be identified as Lin⁻ c-kit⁺ SSClow/FSClow integrin β7hi CD16/32int cells and mast cells can be identified as Lin⁻ c-kit⁺ SSChi/FSChi integrin β7low CD16/32hi cells. In the first experimental setup, peritoneal cells from sensitized mice were stimulated *ex vivo* before intracellular detection of total tyrosine phosphorylation by flow cytometry. Using an *in vitro* model, we also investigated the phosphorylation of a protein kinase involved in the FcεRI signaling pathway. In an *ex vivo* model, we found that compared to the baseline, the stimulated MCp exhibited increased tyrosine phosphorylation. This was confirmed in an *in vitro* stimulation model in which we found that MCp had a higher degree of Syk phosphorylation compared to unstimulated MCp. These data suggest that mouse MCp can become activated upon IgE sensitization and challenge by antigen.

Additionally, we studied whether human MCp also demonstrated signs of being activated by IgE crosslinking. To investigate this, peripheral blood samples were separated using ficoll to enrich for mononuclear cells, which were stimulated with anti-IgE for 30 seconds to 3 minutes, depending on the target. Next, the cells were incubated with fluorescently labelled antibodies for detection of MCp and basophils, which were used as positive controls for the activation. We found that anti-IgE-stimulated MCp exhibited increased total tyrosine phosphorylation after 30 seconds and 1 minute of stimulation compared to unstimulated cells, and basophils exhibited increased total tyrosine phosphorylation after 30 seconds compared to the unstimulated samples. We also investigated whether anti-IgE treatment could stimulate the phosphorylation of two protein kinases involved in the FcεRI-mediated signaling pathway, Syk and p38 MAPK. The phosphorylation of the protein kinase Syk was analyzed after 30 seconds of stimulation with anti-IgE. Although there were patients with increased phosphorylation of Syk in both basophils and MCp after stimulation, there were no significant differences between the unstimulated and stimulated groups. We also investigated the p38 MAPK phosphorylation after 3 minutes of stimulation with anti-IgE. However, induced phosphorylation could only be detected among the basophils and not the MCp. It is known from basophil activation tests that there are patients who are “non-responders” when stimulated with anti-IgE, which might be a possible explanation if there are individuals who have non-responding MCp. Although, since the basophils did respond, another possibility is that there is a timing issue, and that
the time point for the phosphorylation of p38 MAPK in MCp differs for the time point for the phosphorylation of this kinase in basophils. However, due to the rareness of the MCp in peripheral blood, it is challenging to study several time points and to add extra controls to circumvent these issues.

In a final experiment, we investigated whether lung MCp from mice sensitized and challenged in an allergic airway inflammation model in vivo could become activated. MCp in the lung can be identified as CD45⁺ c-kit⁺ ST2⁺ FcεRI⁻ CD16/32⁻ integrin β7hi cells, and mast cells can be identified as CD45⁺ c-kit⁺ ST2⁺ FcεRI⁺ CD16/32⁺ integrin β7int cells. To determine if the MCp could be activated, the lung cells from the OVA challenge group and the control group were incubated with an antibody to detect intracellular IL-13. In these experiments, we found that the mice subjected to the OVA challenge had a higher percentage of lung MCp expressing IL-13 than the control group. Based on these data, we conclude that MCp from both the peripheral blood of humans and peritoneal and lung MCp from mice can become activated.

In the future, different types of experiments could be used to confirm that MCp can become activated. For example, one way of confirming these data would be to sort MCp from peripheral blood from humans, or the lung or peritoneal cells from mice, stimulate the cells in vitro and measure different mediators secreted from the cells when activated. Another approach would be to sort the MCp from either humans or mice and to measure the calcium flux. Since the cells are rare, a fluorescence microscope in combination with a dye that emits a fluorescent signal when the cells become activated could be used to determine the calcium flux from few or even single cells.

To further determine if the activation in the mouse models is specific to the FcεRI-mediated pathway, FcεRI knockout mice could be utilized. Using a mouse in which FcεRI is knocked out and repeating the experimental design with the in vivo model in mice, this could give us a better understanding of whether the activation is FcεRI-dependent.

Lastly, it could be interesting to investigate the gene expression similarities and differences between stimulated and unstimulated MCp. To address this, MCp could be sorted and stimulated in vitro or left untreated and be analyzed either by microarray analysis or RNA sequencing. This approach might be possible both for the human peripheral blood MCp and the mouse peritoneal MCp.

**Manuscript IV**

**Differential expression of CCR1 and CCR5 on murine mast cell progenitors depending on tissue localization**

MCp are known to be recruited to the infected or inflamed lung in allergic airway inflammation and influenza infection. However, the receptors required for the migration and homing of MCp are mostly unknown. Therefore,
we screened for the expression of chemokine receptors in mast cells and MCp. Because the MCp are a rare population of cells and only make up approximately 0.005 % of the mononuclear cells in the blood of mice, we decided to perform the first screening of chemokine receptors on mast cells and MCp from the peritoneal cavity. At this site, MCp can be identified as Lin− c-kithi SSClo integrin β7hi CD16/32int cells and mast cells as Lin− c-kithi SSCint/hi integrin β7int CD16/32hi cells using flow cytometry. Mast cells and MCp were screened for CXCR2-5, CX3CR1, CCR1-3, CCR5-7 and CCR9 expression. We found that in the peritoneal cavity, the only chemokine receptor expressed by mast cells and MCp was CCR5.

Because our main focus is lung MCp, we next investigated whether the lung MCp expresses CCR5. MCp in the lung can be identified as CD45+ c-kitST2+ FcεRI− CD16/32+ integrin β7hi cells. We found that neither the MCp from naïve or influenza-infected mice expressed CCR5. However, it has been shown that peritoneal mast cells of the CTMC subtype have a different origin, the fetal yolk sac, compared to the mast cells found in lung and that originates from the bone marrow stem cells. Therefore, the lack of CCR5 expression on lung MCp during influenza infection could indicate the requirement for a different mechanism or could be a result of the different origin of the lung MCp compared to the peritoneal MCp.

Because MCp are such rare cells, we were also interested in determining whether there was a difference in commitment to the mast cell lineage between the Lin− c-kit+ ST2+ CD16/32+ integrin β7hi FcεRI+ cells (previously defined MCp markers in mouse blood) and the Lin− c-kit− ST2+ CD16/32+ integrin β7hi FcεRI− cells found in the bone marrow. To address this, 50 cells were sorted into wells with media containing a myelo-erythroid cytokine cocktail and cultured for four or eight days. After culture, the progeny cells were reanalyzed with flow cytometry to determine their FcεRI, c-kit and DX5 expression. After four days of culture, 70 % of the cells expressed FcεRI and c-kit, indicating that FcεRI− and FcεRI+ progenitors were equal and not fully committed to the mast cell lineage. The few cells that had low c-kit expression did express the basophil marker DX5. Similar results were observed for cells cultured for eight days. Of the progenies from both the FcεRI+ and FcεRI− cell populations approximately 90 % of the cells were double positive for FcεRI and c-kit. Therefore, we concluded that independent of the FcεRI expression, Lin− c-kit+ ST2+ CD16/32+ integrin β7hi cells from the bone marrow are MCp, which retain a small basophil potential.

We also investigated the CCR5 expression in MCp from spleen and bone marrow; CCR5 was no expressed by MCp in either of these organs.

To determine which chemotactic receptors that might be expressed on lung mast cells and MCp, we screened for the mRNA expression of chemotactic receptors. MCp were sorted from the bone marrow of naïve mice, and the bone marrow and lungs from influenza-infected mice. Using qPCR, the mRNA expression levels of the chemokine receptors CXCR2-5, CX3CR1, CCR1-3,
CCR5-7, and CCR9, the leukotriene receptor BLT1, and the prostaglandin receptor EP3 were studied. The only two chemokine receptors that were expressed at mRNA level in all three organs were CCR1 and CX3CR1. In comparison, when verifying the primers, all of the investigated chemokine receptors except CXCR4 were expressed by BMMC at a mRNA level. This we believe highlights an issue when translating *in vitro* data into *in vivo* models. There are clear benefits with *in vitro* systems that allow testing of multiple targets at the same time, or without sacrificing a higher number of animals. However, our data indicate the importance of confirming data *in vivo*. The protein expression of CCR1 and CX3CR1 on lung MCp was subsequently investigated by flow cytometry. Our data show that CCR1 protein is expressed by MCp from naïve bone marrow but not by lung MCp from influenza-infected mice. However, CX3CR1 protein was not expressed by bone marrow MCp from naïve mice or by lung MCp from influenza-infected mice.

In summary, we found that CCR5 is expressed on MCp from the peritoneum of naïve mice and that CCR1 is expressed on MCp from the bone marrow of naïve mice. Interestingly, both of these chemokine receptors bind the same ligand, CCL3 (MIP-1α).

Recent studies have shown that CTMC, a subtype of mast cells in the peritoneum, originates from the fetal yolk sac. Furthermore, previous studies have shown that the MMC subtype of mast cells originates from the bone marrow. Therefore, we find it interesting that MCp in the bone marrow and peritoneum both express different chemokine receptors; however, both receptors can bind the same ligand, CCL3. Miyazaki et al demonstrated that the CCL3 is involved in the regulation of mast cell degranulation *in vivo* in an allergy model. They also showed that when knocking out CCL3 or administering a blocking antibody, the mast cells degranulated to a lesser extent. Furthermore, the mice lacking CCL3 had less severe allergy symptoms compared to the wildtype mice treated in parallel. Thus, it is possible that the protein expression of CCR5/CCR1 is also mediating a mode of activation.

To continue exploring different receptors that might mediate the migration of MCp, microarray analysis could be an option. By sorting MCp from the bone marrow of naïve mice and lung and bone marrow MCp from influenza-infected mice, or mice subjected to an asthma model, a broader screening of receptors that potentially regulate the migration of MCp would be possible. Since microarray analysis allows for relative expression comparisons between different groups, this would be highly relevant.

Another possibility would be to explore the CCL3 ligand and the interaction between CCL3 and CCR1 and CCR5 on MCp. This could be done by administrating CCL3 to mice, and analyzing MCp at the site of injection.

Lastly, there are studies that demonstrating that BMMC that have been injected intravenously migrate towards the injection site of intradermally in-
jected leukotriene $B_4^{51,52}$ and prostaglandin $E_2^{52}$. Therefore, it would be relevant to study the protein expression of the leukotriene $B_4$ receptor BLT1 and also the prostaglandin $E_2$ receptor EP3 on MCp from different sources.
Förekomsten av astma och allergi ökar världen över. Astmatiska symptom uppstår ofta i samband med allergi när en genetiskt mottaglig person utsätts för upprepad exponering av ett allergen via luftvägarna. En typ av immunologiska celler som är välkända för sin inblandning i astma och allergi är mastceller och det finns studier som påvisar en ökad mängd mastceller i lungorna hos astmatiker jämfört med friska kontroller. Mastceller utvecklas från en typ av förstadiecell som kallas mastcellsprogenitorer. Genom att studera mastcellsprogenitorer hoppas vi kunna bidra med kunskap om mastcellens uppkomst och beteende. Potentiellt skulle också mängden mastcellsprogenitorer kunna användas som biomarkör inom vården. Tidigare studier visar att mastcellprogenitorer rekryteras och ansamlas i lungorna hos möss som har experimentell allergisk astma eller influensainfektion.

För några år sedan identifierade vår forskningsgrupp en population av mastcellsprogenitorer i blod hos allergiska astmatiker och kontroller. Denna upptäckt har gjort det möjligt att studera mastceller i människa utan invasiva metoder för att tillhandahålla prover. Genom att utnyttja denna kunskap har vi i delarbete I undersökt skillnaden i förekomst av mastcellsprogenitorer hos allergiska astmatiker och kontroller, samt undersökt samband mellan patienternas lungfunktion och förekomsten av mastcellsprogenitorer. Vi fann att det inte var någon skillnad mellan allergiska astmatiker och kontroller i mängden mastcellsprogenitorer. Däremot fann vi att de individer som hade en sämre lungfunktion, oberoende på om de tillhörde astma- eller kontrollgruppen, hade en högre förekomst av mastcellsprogenitorer i blodet. Vi fann också att kvinnor hade en ökad frekvens mastcellsprogenitorer i blodet.

I delarbete II var målet att undersöka om liknade mastcellsprogenitorer som tidigare hittats i blod finns även i benmärgen hos friska donatorer. Vi fann att det finns en population mastcellsprogenitorer även i benmärg som är mycket lik de i blodet. Dock fann vi också att cellerna i blodet skilde sig från cellerna i benmärgen genom en ökad koncentration av en yt-molekyl som i mus påverkar cellernas förmåga att lämna blodet och transporteras in i ett organ. Detta skulle kunna tyda på att cellerna i blodet är mer mogna och gör sig redo för att transporteras ut i vävnaden.

I delarbete III undersökte vi om mastcellsprogenitorer är funktionella celler som kan aktiveras via IgE-korslänkning, ett av de mest klassiska exemplen på mastcellsaktivering. I detta arbete fann vi att mastcellsprogenitorer från både mus och människa kan aktiveras. Slutligen har vi i delarbete IV undersökt om...
mastcellsprogenitorer hos möss har yt-molekyler som skulle kunna reglera transporten av mastcellsprogenitorer från benmärg via blodcirkulationen och ut i vävnaden. Här fann vi att cellerna hade olika komposition av yt-molekyler beroende på vilket organ de befann sig i.

Sammanfattningsvis har vi undersökt mastcellsprogenitorer i både mus och människa för att bidra till ny information om hur dessa celler förflyttar sig och vilken betydelse de har hos patienter med allergisk astma. Våra studier syftar till att öka förståelsen kring mastcellers betydelse vid astma.
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