Mast Cell Migration in Inflammatory Diseases

BY

NICLAS OLSSON

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Abstract

Mast Cells (MCs) are forceful multifunctional effector cells of the immune system. MCs are normally distributed throughout connective and mucosal tissues, but in several pathological conditions accumulation of MCs occur. This accumulation is probable due to directed migration of MCs and they are subjects for migration at least two different occasions: 1) when they are recruited as progenitor cells from the blood into the tissue; and 2) when they as mature MCs are recruited to sites of inflammation. The aim of this study was to investigate MC migration to chemoattractants released in vivo or in vitro (body fluids collected from patients with asthma or rheumatoid arthritis and T_h, T_1- and T_2-cytokines) and to recombinant cytokines (transforming growth factor -β (TGF-β) and CCL5/RANTES).

This thesis shows that bronchoalveolar lavage (BAL) fluid from asthmatic patients and synovial fluid from patients with rheumatoid arthritis contain MC chemoattractants, and that part of the chemotactic activity can be related to the presence of stem cell factor (SCF) and TGF-β. We also show that MC chemotactic activity during pollen season is significantly increased compared to before pollen season. Furthermore, we demonstrate that TGF-β isoforms, CCL5, TNF-α and IL-4 act as MC chemoattractants in a bellshaped dose-dependent manner. TGF-β proved to be an extremely potent attractant giving an optimal migratory response at 40fM and TGF-β3 being the most effective isoform. The chemokine CCL5 induced migration through interaction with the receptors CCR1 and CCR4 expressed on MC. Furthermore, we also found that TNF-α produced by T_h,1-lymphocytes and IL-4 produced by T_h,2-lymphocytes are MC chemoattractants.

In conclusion, with this thesis we have identified six new human mast cell chemoattractants and provide evidence that BAL fluid and synovial fluid from patients with asthma and rheumatoid arthritis, respectively, contain MC chemoattractants. This information provides important clues in understanding the mechanisms behind MC recruitment to sites of inflammation.

Keywords: Mast cells, chemotaxis, inflammation

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To my family with love
PAPERS INCLUDED IN THE THESIS

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


V  Olsson, N., D.D. Taub, and G. Nilsson. Differential regulation of mast cell migration by T_{H1} and T_{H2} cytokines: Identification of TNF-α and IL-4 as mast cell chemotaxins.

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<tr>
<td>BAL</td>
<td>Bronchoalveolar lavage</td>
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<tr>
<td>CBMC</td>
<td>Cord blood derived mast cell</td>
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<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
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<tr>
<td>HMC</td>
<td>Human mast cell line</td>
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<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>MC</td>
<td>Mast cell</td>
</tr>
<tr>
<td>MC_T</td>
<td>Tryptase positive mast cell</td>
</tr>
<tr>
<td>MC_TC</td>
<td>Tryptase and chymase positive mast cell</td>
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<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
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<tr>
<td>RA</td>
<td>Rheumatoid arthritis</td>
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<tr>
<td>SCF</td>
<td>Stem cell factor</td>
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<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
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<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
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<td>TGF</td>
<td>Transforming growth factor</td>
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INTRODUCTION

It has been known for a long time that cells have the capacity to move. This capacity is of pivotal importance for the cells of the immune system. They need to be able to mobilise to different sites of inflammation in order to perform their functions. Cellular movement is a very complex process governed by extracellular signals. Many inflammatory diseases have a great influx of inflammatory cells.

One cell whose functions in inflammatory diseases have been overlooked for many years, and only recently have been more thoroughly investigated is the mast cell. The mast cell (MC) is an important effector cell of the immune system, and has historically been connected to IgE-mediated allergic reactions. During the last decade it has been discovered that MCs are involved in the pathology of autoimmunity and innate immunity [1-3].

Mast cells are of hematopoietic origin and originate from the bone marrow [4]. The mature MC can only be found within tissues and are particularly numerous in the proximity to the external environment, i.e. the skin, gastrointestinal tract and respiratory tract.

Mast cell have been shown to accumulate at a variety of different inflammatory diseases in these tissues. The factors that cause this infiltration in different conditions are poorly described. This thesis investigates the ability of different body fluids and chemoattractants to induce MC migration.
MAST CELLS – A BRIEF OVERVIEW

Introduction

Mast cells (MCs) were discovered 1863 by F. Von Recklinghausen and were named by Paul Ehrlich in the late 1870s [5]. He named them mästzellen (from the German “mastig” = well fed) after their granulation phenotype. MCs are one of the major effector cells of the immune system [6] and in the acute-phase of the IgE-mediated allergic reactions they are also the key cells. In addition they have many important functions in biological responses, apart from pathological disorders related to IgE-dependent immediate hypersensitivity. Tissue remodelling, wound healing, fibrotic conditions, blood vessel formation, host responses to parasites and acute and chronic inflammatory disorders are some of the biological responses they are involved in [7-9]. Due to their ability to release large amounts of preformed inflammatory mediators from granules, they have important roles in both health and disease.

The origin, maturation and localisation of mast cells

In 1991 it was shown that human MCs originate from hematopoietic CD34+ stem cells residing in the bone marrow [4]. Mast cell progenitors leave the bone marrow into the blood circulation from where they enter the tissue and differentiate into mature cells. The phenotype of the bone marrow progenitor is so far poorly characterised. However, the progenitor in peripheral blood express Kit+, CD34+, and CD13+, but lacks expression of FceRI, FceRII, CD14 and CD17 [10-12]. When the progenitors have entered the tissue they undergo final maturation under the influence of growth factors. The most important growth factor for MCs is stem cell factor (SCF) which not only guide the differentiation, but also regulates growth, survival, migration,
adhesion and degranulation of MCs [13, 14]. Mature MCs can normally be found throughout body tissue. They are very long-lived compared to other blood cells and can probably live in the tissue for several months. They are particularly numerous in the respiratory tract, in the gastrointestinal and genitourinary tracts, adjacent to blood or lymphatic vessels, beneath the epithelial surfaces of the skin, and in the close proximity to peripheral nerves [15].

Mature human MCs are divided into two subtypes depending on the proteases expressed in the cells: MC_T, tryptase-containing, and MC_TC, tryptase- and chymase-containing [16]. Which subtype of mast cell the progenitor will become depends on the tissue the mast cell resides in. MC_T are predominantly found in lung tissue and in the intestinal mucosa, whereas MC_TC normally are found in skin and in the intestinal submucosa [15, 17].

Mast cell activation leads to mediator synthesis and release

The classical way for the interspersed MCs in the tissue to be activated is when an allergen interact with its specific IgE-antibody which in turn is bound to the high-affinity IgE-receptor, FcεRI, on the MC. The subsequent crosslinkage of adjacent FcεRIIs results in degranulation and transcription of inflammatory cytokines. During recent years it has become evident that not only IgE but also IgG can activate human MCs to degranulate, by binding to the FcγRI [18-20]. MCs can also be activated by neuropeptides, complement factors, C3a and C5a through C3aR and C5aR (CD88). Nerve growth factor can also activate MCs through TRKA, and lectines by binding to the Fc region of FcεRI and thereby directly crosslinking it [21-23]. Furthermore, toll-like receptors (TLR) have recently been shown to differentially activate MCs. Through interactions between lipopolysacchride (LPS) and peptidoglycan (PGN) and the TLRs, MCs are directly activated by different bacteria and thereby contribute to the first line of host defence against bacterial infections.

One characteristic of MCs is their capability to produce and secrete a large array of inflammatory mediators upon activation. These mediators are released from three different compartments, as shown in Figure 1 [24]. There are (1) the preformed secretory granule-associated mediators, like cytokines,
histamine, serine proteases and proteoglycancs. They are released within seconds to a few minutes after MC activation. Hereby they influence the early phase of an acute inflammation.

Next (2) the lipid derived mediators, such as leukotrienes and prostaglandins, are formed originating from the arachidonic acid metabolism. They also act as important players in the acute phase of inflammation, and secretion starts a few minutes after activation and the production can continue for 30 minutes or more.

After 4-12 hours (3) de novo synthesis and secretion of cytokines, chemokines, and growth factors start and reach the peak between 24-48 hours after activation. At this stage the late phase of the inflammation has begun, and the secreted cytokines and chemokines from MCs recruit and activate other leukocytes. This large array of biologically active mediators capable of many different effects, render a cell capable of orchestrating the inflammatory response, giving them an important role in both health and disease.

**ACTIVATED HUMAN MAST CELLS**

**Figure 1.** Mediators released by activated mast cells.
The role of mast cells in health – they really are good guys!

As already mentioned, MCs are ubiquitously distributed, resident tissue cells and have for many decades been viewed as harmful cells due to their function as effector cells in allergic and anaphylactic reactions. Their contribution to our health was long thought to be limited to the elimination of parasites. However, recently MCs have been shown to exert beneficial functions in the innate and adaptive immune responses. Today mast cells are considered to be involved in a variety of biologic processes. The location of the MCs close to the epithelial surfaces in the skin, the respiratory system, and the gastrointestinal mucosa, makes them extraordinary guardians against environmental attacks.

An essential role for MCs in the innate immunity was described in 1996 by Malaviya et al. [25, 26] and Echtenachter et al. [27]. In in vivo studies using genetically MC-deficient mice they provided evidence that MC-deficient mice where less efficient in clearing and surviving bacterial infections compared to wild type or MC-reconstituted mice. Another study indicated that complement fragment 3 (C3) induced degranulation of MCs and that the following release of TNF-α might be essential for the recruitment of neutrophiles and subsequent bacterial clearance [28].

Our understanding of the interaction between MCs and micro-organisms are slowly emerging, showing an either direct or indirect interaction with MCs. Receptors that have been found to mediate these interactions in MCs are: CD48, recognising Fim H, a 29-kDa mannose-binding lectin expressed by E.coli and other enterobacteria [25, 26], Toll-like receptors [29-32], and complement receptors [28]. The interactions between MC and bacteria lead to activation and mediator release that leads to an inflammatory response or a direct killing of the pathogen and subsequently bacterial clearance.

It has also become evident that MCs function as initiators of acquired immune reactions [33]. By expressing a number of receptors, such as MHC class II antigens, ICAM-1, ICAM-3, CD43, CD80, CD86 and CD40L, MCs can interact directly with T- and B-lymphocytes and endothelial cells. Thereby MCs can, for example, stimulate T-cell proliferation and cytokine release and IgE production by B-cells [34-36].

The emerging evidence points toward a pivotal role of MCs in both innate and adoptive immune responses. However, most of these studies have been
performed in mice, but it is reasonable to believe that the data is applicable on humans as well.

MCs control the key events of the wound healing phases by releasing mediators from the three different compartments discussed earlier. The different phases are initiating and modulating the inflammatory stage, proliferation of connective cellular elements, and the remodelling of the newly formed connective tissue matrix [37].

The role of mast cells in disease – are they bad guys?

Mast cells are activated in a variety of inflammatory diseases as mentioned earlier. In the allergic inflammation it is well known that mast cells accumulate and act as effector cells. Allergy is mostly caused by a prolonged overproduction of IgE in response to antigens. These antigens can be pollen, dust mite, fungal spores and insect venom for example. Cross-linkage of the IgE-receptor (Fc\_RI) by antigen-specific IgE, initiate a signal cascade that leads to release and synthesis of mediators. The released mediators contribute to increased permeability of blood vessels, tissue oedema, leukocyte recruitment, bronchoconstriction and inflammation.

Recently, it has become evident that mast cells are involved in the pathology of many autoimmune diseases and chronic inflammations [9, 38]. Mast cells have been assigned roles in the onset and/or the severity of diseases such as rheumatoid arthritis [1, 39, 40], multiple sclerosis [3, 41], and Sjögren’s syndrome [42], as well as other chronic inflammatory and fibrotic diseases [43, 44]. Their capacity to release a variety of strong pro-inflammatory mediators, such as TNF-\(\alpha\) and IL-1, make them prime candidates for modulating autoimmune diseases.

It is long known that mast cells accumulate at the periphery of tumours, and it has been thought that the MCs function as a response to the neoplasia. However, during more recent years it has been proposed that MCs actually support development of the tumour [45, 46]. The mediators released by MCs can aid the tumour with neovascularisation through release of TNF-\(\alpha\), IL-8, fibroblast growth factor-2 (FGF-2) and vascular endothelial growth factor (VEGF) [47-49]. All of these factors help the endothelial cells to proliferate and differentiate. Angiogenesis can also be assisted by heparin and histamine by facilitation the penetration of the extracellular matrix (ECM) [50, 51].
Mast cells also modulate the ECM by releasing matrix metalloproteinases, tryptase and chymase [52-54]. These enzymes degrade the ECM components and hence facilitate angiogenesis, invasion, and metastasis.

Mast cells accumulate at sites of inflammation

Mast cells have been reported to accumulate at sites of both acute and chronic inflammations [13, 55], including allergy, asthma, and RA [38-40, 56-61]. It has also been found that some human tumours with enhanced growth and invasion are associated with MC accumulation [45, 46]. The mechanisms for this MCs accumulation are not clear. Due to the rapid kinetics for the accumulation of MCs it is most likely that redistribution of neighbouring MCs by directed migration is the major mechanism. Other possible, but more unlikely, mechanisms are proliferation, prolonged survival, and MC differentiation of recruited progenitors from the peripheral blood. Studies of MC migration may provide crucial information on how mast cells are recruited into sites of inflammation.

Regulation of mast cell adhesion and migration

Mast cells are subject for recruitment when they 1) are recruited from the blood stream into the tissue as precursors and 2) when they are recruited into inflamed tissues from the surrounding tissue, see Figure 2. The recruitment of cells depends on adhesion and chemoattractants. Adhesion of MC to the endothelium and/or extracellular matrix by means of adhesion molecules is a prerequisite for migration. This requires that the appropriate adhesion molecules such as integrins, cadherins, immunoglobulin-like structures, selectins, and proteoglycans are expressed. These adhesion molecules and receptors are differentially expressed and regulated during differentiation. In MCs integrins are the most studied. Skin, uterine and lung mast cells express a wide range of integrin molecules such as VLA-3/α3,β1 (very late antigen-3), VLA-4/α4,β1, VLA-5/α5,β1, LPAM-1/α4,β7 (lymphocyte Peyer’s patch adhesion molecule-1), VNR/αv,β3 (vitronectin receptor)[62-66].
Adhesion molecules can be regulated by some chemoattractants and chemokines such as SCF which can alter the integrin functions and thereby modulate the adhesion and localisation of MCs [67-70].

Leukocytes are attracted to inflammatory sites and/or sites of infection through the production of chemoattractant mediators. As a result of chemoattractant receptor activation, leukocytes are stimulated to move, adhere and de-adhere, rearrange their cytoskeleton and finally execute their actions [71]. Migration of cells in general can be divided into chemotaxis and chemokinesis. Chemotaxis is a directed migration towards a gradient of chemoattractant. Chemokinesis is an increased undirected, or random, migration. Chemokinesis do not need any chemotactic gradient. Chemotaxis and chemokinesis can be differentiated from each other by means of a checkerboard analysis [72].

Leukocytes navigate through complex chemoattractant arrays, and in doing so, they must migrate from one chemoattractant source to another. For this to occur two interrelated properties must be allowed. First the competing chemoattractant signals must be integrated by the migrating leukocytes. The second property is memory of their recent environment displayed by the cells. The cell’s perception of the relative strength of orienting signals is influenced by their history, so that the cell prioritise newly arising or newly
encountered attractants [73]. This allows combinations of chemoattractants to guide leukocytes in a step-by-step fashion to their destinations within tissues.

The factors that stimulate mast cell migration were until recently not defined. However, the number of known mast cell chemoattractants has recently increased considerably. Examples of human MC chemoattractants are SCF [74], TGF-β [75] (Paper III), platelet activating factor (PAF) [76], serum amyloid A (SAA) [77], C3a and C5a [23, 78], GROα [79], CCL5/RANTES [74, 75, 80](paper IV), CCL11/ eotaxin [80, 81], CCL12/SDF-1α [82, 83], CXCL8/IL-8 [79, 84], and CXCL1/fractalkine [85]. The potency and the efficacy of the different chemoattractants vary significantly, as displayed in table 1.

Table 1. Migratory response and optimal concentration of different known human MC chemoattractants.

<table>
<thead>
<tr>
<th>Chemotaxin</th>
<th>Optimal concentration</th>
<th>Migratory response</th>
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<tbody>
<tr>
<td>SCF</td>
<td>50 ng/ml</td>
<td>175%</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>40 fM</td>
<td>151%</td>
</tr>
<tr>
<td>TGF-β2</td>
<td>40 fM</td>
<td>163%</td>
</tr>
<tr>
<td>TGF-β3</td>
<td>40 fM</td>
<td>185%</td>
</tr>
<tr>
<td>SAA</td>
<td>12.5 µg/ml</td>
<td>157%</td>
</tr>
<tr>
<td>C3a</td>
<td>10 nM</td>
<td>256%</td>
</tr>
<tr>
<td>C5a</td>
<td>1 nM</td>
<td>145%</td>
</tr>
<tr>
<td>PAF</td>
<td>10 nM</td>
<td>NT</td>
</tr>
<tr>
<td>CCL5/RANTES</td>
<td>10 ng/ml</td>
<td>180%</td>
</tr>
<tr>
<td>CCL11/Eotaxin</td>
<td>100 ng/ml</td>
<td>NT</td>
</tr>
<tr>
<td>CXCL1/GROα</td>
<td>10-100 ng/ml</td>
<td>NT</td>
</tr>
<tr>
<td>CXCL8/IL-8</td>
<td>100 ng/ml</td>
<td>NT</td>
</tr>
<tr>
<td>CXCL12/SDF-1α</td>
<td>1 through 3 µg/ml*</td>
<td>App. 250%</td>
</tr>
<tr>
<td>CX3CL1/ Fractalkine</td>
<td>25-125 ng/ml</td>
<td>NT</td>
</tr>
</tbody>
</table>

* The migration reached a plateau level that was maintained at higher concentrations of SDF-1α.
NT = not tested with the leading front technique
ROLE OF MAST CELLS IN INFLAMMATORY DISORDERS – A SHORT BACKGROUND

Two examples of inflammatory diseases where MCs are part of the development and chronicity are rheumatoid arthritis (RA) and asthma. Both of these diseases have been studied in this thesis.

Rheumatoid arthritis

RA is an inflammatory polyarthritis affecting approximately 1% of the population. It occurs worldwide with an increased incidence with age and affect women about 3 times more often than men. The predominant symptoms are pain, stiffness and swelling of peripheral joints. Although the inflammatory process is well characterised, the primary cause of RA is not defined.

It is believed that RA starts with an immune-mediated process leading to inflammation and destruction of the joint. The joint destruction compromises synovial immune complex depositions, neutrophil infiltration, angiogenesis, and T-cell activation. The leukocytes are activated and propagate the cytokine-rich inflammatory environment. The synovial membrane enlarges and become the pannus, which invade the bone and cartilage. Further proliferation of the pannus leads to more profound destruction of cartilage and bone. The role of MCs in this process is not completely clear, but a correlation between MC numbers and the degree of clinical synovitis has been reported [86]. They have been shown to be located in the synovial tissue as well as in the synovial fluid [87], in which increased levels of MC mediators have been detected [88, 89] suggesting that MCs contribute to the initiation and progression of the inflammatory process. A study by Lee et al. show that MCs function as a cellular link between autoantibodies, the complement network and Fc receptors, and other effector cells in the initial development of inflammatory arthritis [1]. MCs has also been detected close
to the cartilage-pannus junction and near metalloproteinase deposits, indicating involvement in matrix remodelling [59, 60]. Despite recent advances in the understanding of MCs role in RA, the involvement and regulatory mechanisms behind it is still poorly understood.

Asthma

Asthma is a chronic airway inflammatory disease characterised by reversible airway obstruction and hyperresponsiveness to external and endogenous stimuli. It is also associated with infiltration of many cell types in the airway in particular mast cells, eosinophils, T-lymphocytes, neutrophils and epithelial cells. Chronic mucosal inflammation plays an important role in the pathogenesis of asthma. Infiltration of activated lymphocytes and mast cells in and around the brochial epithelium, blood vessel dilation, mucosal oedema, and hypertrophy of both submucosal glands and bronchial smooth muscle are some reported histopathological findings associated with asthma [90]. The dominant signal for MC activation in allergic asthma is the allergen-specific cross-linkage of the IgE bound to FcεRI. There are also reports of MC activation by neuropeptides [91], adenosine [92] and by SCF [93]. Stem cell factor, ligand for c-kit, can prime MCs to exhibit a stronger response to other MC activating agents [94]. The activation releases many preformed mediators stored in granules, including histamine, proteases and various cytokines are released. Histamine can activate bronchial epithelial cells, eosinophils, as well as stimulate expression and production of P-selectin, IL-8 and IL-6 in endothelial cells. Histamine also contribute to the bronchospasm, oedema and mucus secretion. The MC protease predominant in BAL is β-tryptase, which can activate matrix metalloproteinases and inactivate fibrinogen and neuropeptides. Leukotriene C4 (LTC4) and LTB4 and prostaglandine D2 (PGD2) are quickly formed and released after activation. LTC4 and PGD2 are powerful bronchoconstrictors and vasodilators increasing the vascular permeability. PGD2 and LTB4 can also activate eosinophils and attract neutrophils. MCs sustain the response by de novo production of numerous, predominantly Th2-type, cytokines, chemokines and growth factors that orchestrate the chronic inflammation and promotion/persistence of a Th2 response. The mediators also contribute to the remodelling of the airway in chronic asthmatics.
A DESCRIPTION OF MAST CELL CHEMOATTRACTANTS: TGF-β, CHEMOKINES, AND T₃H1- AND T₃H2-CYTOKINES

In this thesis I have studied different chemoattractants, i.e. the TGF-β superfamily, chemokines and T₃H1- and T₃H2-cytokines, and if they induce MC migration in vitro.

The TGF-β Superfamily

A few decades ago the multifunctional cytokine TGF-β first captured the attention of scientists. Since then a large family of TGF-β related polypeptides has been discovered. Today nearly thirty members of the TGF-β family has been described in humans. This superfamily is divided into two general branches, the BMP/GDF (Bone Morphogenetic Protein/Growth and Differentiation Factor) and TGF-β/Activin/Nodal branches, whose members have diverse often complementary effects. The TGF-β subfamily members are involved in a wide range of biological responses such as cellular growth inhibition, differentiation, chemotaxis, extracellular matrix formation and wound healing. The TGF-β family members initiate their cellular functions by binding to receptors with intrinsic serine/threonine kinase activity.

TGF-β receptors

The TGF-β superfamily receptors are a heteromeric complex of related transmembrane protein serine/threonine kinases. They are glycoproteins with
a rather short N-glycosylated extracellular region. There are three TGF-β receptors, namely TGF-β receptor-I (TβR-I), TβR-II, and TβR-III, where the former two are directly involved in the signal transduction. TβR-III is structurally related transmembrane proteins with a more indirect role in signalling. TGF-β initially bind to its corresponding type II receptor, after which the type I receptor is recruited to form the signalling complex in a sequential manner. The sequential binding of ligand is characteristic for TGF-β and activin receptors. The two types of receptors have an intrinsic affinity for each other resulting in a more stable complex. Heterodimerisation of TβR-I and TβR-II induced by ligand binding activate a downstream signalling pathway mediated by a certain kind of signalling molecules called Smads.

The intracellular signal transducers for the TGF-β superfamily is the Smad molecules [95]. Smads are divided into three subfamilies, based on their structural and functional properties: receptor-activated Smads (R-Smads), common mediator Smads (Co-Smad), and inhibitory Smads (I-Smads). Heterodimerisation of TβR-I and TβR-II induced by ligand phosphorylate R-Smads (Smad 2/3) leading to homomerization of R-Smads. Co-Smad (Smad 4) then joins the complex forming a heteromeric complex, that translocate to the nucleus where they regulate transcription of target genes, in combination with other transcription factors. There are also inhibitory Smads (Smad 6/7) that interact with the activated TβRI, thereby competing with the R-Smads for receptor association, thus effectively block phosphorylation of R-Smads and the succeeding downstream events.

The Chemokine Superfamily

The chemokines constitute a superfamily of small (8-10kDa) chemoattractant cytokines with 20 to 70 percent homology in the amino acid sequences. Today there are approximately 50 chemokines described. They are distinguished from other cytokines by their usage of G-protein-coupled seven-transmembrane receptors. They have been categorised on the basis of whether the first two cysteins are adjacent to each other or separated by an amino acid. Four classes of chemokines have been described: the CXC (α-chemokines), the CC (β-chemokines), the C (γ-chemokines) and the CX3C (δ-chemokines)(Table 2). The CXC-family has been further subclassified into ELR-motif containing or non-ELR containing chemokines. The ELR-motif is a sequence (glutamic acid-leucin-arginine) near the NH2-terminus.
Another new classification that has come up during recent years is “inflammatory” respectively “homeostatic” chemokines. Here a more physiological approach is used, which include the chemokine production (localisation and conditions) as well as the receptor distribution on different leukocyte subtypes. The inflammatory chemokines are inducible upon proinflammatory stimuli, and are produced by resident and infiltrating cells. The homeostatic group of chemokines, on the other hand, are produced in microenvironments within tissues such as skin and mucosa. These chemokines are constitutively expressed and regulate the positioning and the physiological traffic of cells. A third group of chemokines also exist, where there is no clear distinction on whether they are inflammatory or homeostatic.

Chemokine receptors

Chemokines induce cell migration and activation by binding to specific G-protein-coupled cell-surface receptors. The chemokine receptors are approximately 350 amino acids in length and have a short extracellular N-terminus and a short intracellular C-terminus. There is today ten CC-receptors (CCR), six CXC-receptors, one C-receptor (XCR), and one CX3C-receptors (CX3CR) described in the literature (Table 2) [96, 97].

Chemokines generally signal via active G-protein sensitive phospholipid C (PLC) isoforms, generating inositol 3,4,5-triphosphate and resulting in an increase of intracellular calcium [98, 99]. However, it has been shown that calcium influx is not required to induce chemotaxis [100], suggesting other potential signalling pathways for chemokines. Pathways shown to be activated by chemokines include Janus kinase (JAK) and associated STAT members (signal transducers and activators of transcription) [101, 102]. Also the mitogen/extracellular signal-regulated kinase (MEK)-1 and/or extracellular signal-regulated kinase (ERK)-1/2 can be activated [103, 104].
### Table 2. The chemokines and their corresponding receptors.

<table>
<thead>
<tr>
<th>Systemic name</th>
<th>Common names&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Chemokine receptor(s)</th>
</tr>
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<tr>
<td><strong>C chemokines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XCL1</td>
<td>Lymphotactin/SCM-1α</td>
<td>XCR1</td>
</tr>
<tr>
<td>XCL2</td>
<td>SCM-1β</td>
<td>XCR1</td>
</tr>
<tr>
<td><strong>CC chemokines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCL1</td>
<td>1-309</td>
<td>CCR8</td>
</tr>
<tr>
<td>CCL2</td>
<td>MCP-1</td>
<td>CCR2</td>
</tr>
<tr>
<td>CCL3</td>
<td>MIP-1α/LD78α</td>
<td>CCR1, CCR5</td>
</tr>
<tr>
<td>CCL3L1</td>
<td>LD78β</td>
<td>CCR1, CCR5</td>
</tr>
<tr>
<td>CCL4</td>
<td>MIP-1β</td>
<td>CCR5</td>
</tr>
<tr>
<td>CCL5</td>
<td>RANTES</td>
<td>CCR1, CCR2, CCR5</td>
</tr>
<tr>
<td>CCL7</td>
<td>MCP-3</td>
<td>CCR1, CCR2, CCR3</td>
</tr>
<tr>
<td>CCL8</td>
<td>MCP-2</td>
<td>CCR3, CCR5</td>
</tr>
<tr>
<td>CCL9/10</td>
<td>Unknown&lt;sup&gt;b&lt;/sup&gt;</td>
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</tr>
<tr>
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<td>Eotaxin</td>
<td>CCR3</td>
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<tr>
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<td>CCR2</td>
</tr>
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<td><strong>CXC chemokines</strong></td>
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</tr>
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<td>GROα</td>
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</tr>
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<td>CXCR2</td>
</tr>
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<td>ENA-78</td>
<td>CXCR2</td>
</tr>
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<td>GCP-2</td>
<td>CXCR1, CXCR2</td>
</tr>
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<td>NAP-2</td>
<td>CXCR2</td>
</tr>
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<td>CXCL10</td>
<td>IP-10</td>
<td>CXCR3</td>
</tr>
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<td>I-TAC</td>
<td>CXCR3</td>
</tr>
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<td>SDF-1α/β</td>
<td>CXCR4</td>
</tr>
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<td>BCA-1</td>
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<tr>
<td><strong>CX3C chemokine</strong></td>
<td>Fractalkine</td>
<td>CX3CR1</td>
</tr>
</tbody>
</table>

<sup>a</sup> The common names given in this table are based on human chemokines. Unknown chemokine denotes that no human counterpart has been found.
Chemokines and migration

Chemokines are thought to provide the directional cues for the movement of leukocytes. The trafficking of leukocytes from the circulation into inflammatory tissues requires communication between circulating leukocytes and the vascular endothelial cell wall [105, 106]. The inflammatory lesion releases a series of signals that prime the endothelial wall to become "sticky". Hence, the responding leukocytes begin to roll, slowing their transit through the circulation. The chemokines bound to the heparan sulphate proteoglycans at the endothelial cell wall provide the signals to the leukocyte to convert the low-affinity, selectin-mediated interaction into the higher-affinity, integrin-mediated interaction between leukocytes and the endothelial cell wall. Besides increasing integrin avidity, the leukocyte activation leads to cytoskeletal rearrangement, spreading and cellular polarisation of the leukocyte. Finally the leukocyte migrate through the endothelial cell wall and the basement membrane into the tissue. Well within the tissue the cells migrate toward a gradient of chemokines to the inflammatory site, as shown in Figure 3.

Figure 3. Chemokine regulation of leucocyte migration. The figure was kindly provided by Dr. Juremalm.
The T<sub>H1</sub>- and T<sub>H2</sub>- cytokines in mast cell migration

Antigenic stimulation triggers differentiation of mature naïve T-lymphocytes into effector/memory T-cell populations [107]. The cytokines present at the initiation of the immune response determine the development of functionally distinct subsets of helper T-cells. IL-12 induces differentiation of type 1 T helper (T<sub>H1</sub>) cells, whereas IL-4 promotes T<sub>H2</sub> cell development. The T<sub>H1</sub> cells are characterised by the secretion of TNF-α and IFN-γ, whereas IL-4, IL-5, and IL-13 are typical cytokines secreted by T<sub>H2</sub> cells.

The subpopulation of T-cells and the cytokines secreted can be used to categorise several inflammations into T<sub>H1</sub>- and T<sub>H2</sub>-type. Rheumatoid arthritis, psoriasis and Chron’s disease are examples of T<sub>H1</sub>- mediated inflammations with increased amounts of TNF-α and IFN-γ. A typical T<sub>H2</sub>-mediated inflammation is allergy, where the typical T<sub>H2</sub> cytokines IL-4, IL-5 and IL-13 can be detected in increased amounts. During the last several years it has become evident that MCs are playing a bigger role in the immune responses than previously believed [108-110]. The role of T<sub>H1</sub>- and T<sub>H2</sub>- cytokines capacity to recruit MCs has not yet been characterised.
THE PRESENT INVESTIGATION

In this thesis I provide evidence that BAL fluid and synovial fluid are able to induce MC migration, and that some of the chemotactic activity can be designated to SCF and TGF-β. Among the TGF-β isoforms, which all induce optimal migration at 40 fM, I have found TGF-β3 to be the most effective. The already recognised MC chemotaxin CCL5 is described to act on both CCR1 and CCR4 on MCs. In addition, I show that Th1 and Th2 cytokines are MC chemoattractants, where IL-8, IL-4 and TNF-α play important roles in the attraction of human MCs. These findings contribute to the knowledge of the mechanisms behind the recruitment of human MCs to inflammatory sites.

Aims

The general aim of this work was to investigate MC chemoattractants in vivo and in vitro. The specific goals were to:

- Examine the presence of MC chemoattractants secreted in vivo. For this purpose we investigated MC chemotactic activity in bronchoalveolar lavage (BAL) fluid from patients with allergic asthma, and in synovial fluid from RA patients. (Paper I and II)

- Characterise and compare the in vitro capacity of TGF-β family members to induce migration and growth inhibition in human MC and also to investigate the TGF-β receptor expression (Paper III), and characterise CCL5/RANTES induced MC migration (Paper IV). We also examine the capacity of Th1- and Th2- cytokines to induce MC migration (Paper V).
The chemotaxis assay and the responder cells

In order to study mast cell migration \textit{in vivo} I have used a modified 48-well micro-Boyden chemotaxis chamber and the migration was assessed according to the leading front technique [72, 111, 112]. Briefly, the cells are allowed to migrate toward a gradient of the substance to be tested as shown in Figure 4. The cells migrate into a fibronectin coated nitrocellulose filter for 2.5 hours. The cells are then fixed, stained and the migration distance is measured. The measurement is made according to the leading front technique. The distance is measured between the top layer of nonmigrated cells to the two furthest migrated cells, in a continuous layer of cells. The migration distance of cells against complete medium alone is set to 100% and the response to substances are calculated there after.

\textbf{Figure 4.} The Boyden chemotaxis chamber. Cells migrate into the nitrocellulose filter from the upper well toward a gradient of a chemotactic substance. The chemotactic response is measured according to the leading front technique.

As responder cells we have used a human mast cell line (HMC-1) [113, 114] and \textit{in vitro} developed umbilical cord blood derived mast cells (CBMC) [115].
RESULT AND DISCUSSION

Paper I


Asthma is a very complex disease exhibiting an inflammatory response with infiltration of leukocytes, airway oedema, bronchial hyperresponsiveness and tissue remodelling. During allergic asthma accumulation of MCs have been described in the bronchial epithelium. The mechanism for this infiltration has not been defined.

BAL fluid collected from asthmatic patients before and during pollen season where examined for their capacity to induce MC migration. Before pollen season BAL fluid from eight out of 27 patients were able to induce MC migration, whereas all of the BAL fluid collected during pollen season (27/27) induced a migratory response. This indicates that the allergic inflammation in the airway increases secretion of MC chemotactic factors, and that the chemoattractants present in BAL fluid may be responsible for the accumulation of MCs in the intraepithelial cell layer in allergic asthma. It has been shown that the number of MCs can be correlated to the degree of clinical symptoms [44, 116-118]. Therefore the recruitment of MCs may be of great importance for the pathophysiological mechanisms of asthma.

Studies have shown that SCF and TGF-β can induce MC migration _in vitro_ [74](Paper III), and TGF-β has been shown to be present in BAL fluid [119]. Therefore the presence of SCF and TGF-β in BAL fluid was measured. It was found that TGF-β levels were below the detection limit, whereas SCF could be measured in all BAL fluids. The involvement of the two growth factors in the induction of MC migration in the BAL fluids was assayed by using blocking antibodies against SCF and TGF-β. Only 6 of 14 BAL fluids demonstrated significant decrease in chemotactic activity after SCF-antibody treatment. These results indicate that SCF is a part of the chemoattracting factors present, and that other factors may be of greater importance. SCF however, stimulate many for MCs important functions such as adhesion, activation, survival, growth and differentiation. Hence, SCF may not only play a role in MC recruitment, but also in the perpetuation of the chronic
inflammation. Even though the ELISA could not detect any TGF-β in the BAL fluid, it can be present and function as an MC chemoattractant due to its extreme potency. TGF-β induces migration at concentrations as low as 40fM. The treatment with antibodies against TGF-β resulted in decreased migratory response in 9 of 14 of the BAL fluids collected during season. This suggests that TGF-β, even though below detection in ELISA, can contribute to the MC accumulation in the bronchial epithelium.

To further delineate the different factors present in BAL fluid that can contribute to the MC chemotactic activity, inhibitors blocking different receptor signalling pathways were used. Pertussis toxin (PTx), a G_i protein inhibitor, almost completely blocked the migration of MCs toward BAL fluids. The result indicate that many of the chemotaxins in BAL fluid uses receptors sensitive to PTx, such as C3a, C5a, serum amyloid A and IL-8, of which some have been shown to be present in BAL fluid and also induce MCs migration [23, 77-79, 84, 120-122].

In conclusion this study shows that BAL fluids from asthmatic patients contain mast cell chemoattractants and that most of this activity can be traced to factors mediating their response through G_i-protein coupled receptors. Some of the chemotactic activity can be designated to SCF and TGF-β. We also show that the BAL fluid during season exhibit a significantly higher mast cell chemotactic activity than before season.

**Paper II**


Rheumatoid arthritis is a chronic inflammatory disease characterised by synovial hyperplasia and hypertrophy. Recently, the MCs role as an effector cell in the rheumatoid lesion was proposed [123]. The MC hyperplasia associated with RA is most likely due to directed migration, since it has been reported that MCs in the synovial membrane of patients with arthritis do not proliferate [61]. The synovial fluid from 6 of 7 patients with RA showed MC chemotactic activity.

SCF is a known MC chemotaxin and is expressed in the synovial membrane [61, 74]. We show that MCs are present in the synovial tissue and that SCF is secreted and can be detected in synovial fluids. Thus, the presence of SCF
contributes to the chemotactic activity in synovial fluid. But other chemotaxins are also likely to be involved. SCF may also have other important functions both in activation and survival of synovial MCs as discussed before.

Expression of and probable secretion of TGF-β in the synovium has been reported by Feldmann et al. [124]. All three isoforms of TGF-β are expressed in all specimens in this study and the isoforms have equal potency to induce migration of MCs, with TGF-β3 being the most effective (paper III). The MC hyperplasia in arthritis may therefore partly be due to expression of TGF-β isoforms.

Synovial fluid has a complex composition that may explain why blocking a single cytokine do not completely block the chemotactic activity in the fluid. Many cytokines have capacity to attract MC, and more may remain to be discovered.

Blocking different receptor signalling pathways with inhibitors is one way to sort out the different types of factors involved. Pertussis toxin (PTx) blocks the guanine nucleotide-binding proteins (Gᵢ). Examples of factors mediating their migratory response via Gᵢ-proteins and induce MC chemotaxis are C3a, C5a, serum amyloid A, platelet activating factor (PAF), and IL-8. Some of these are also present in synovial fluid [23, 76, 77, 79, 84, 125-127]. All migration, except for one fluid, was inhibited by PTx, thus suggesting that the fluids contain chemotaxins mediating their response via Gᵢ-protein receptors. A similar response was obtained using genistein, a tyrosine kinase inhibitor. This indicates that several factors are present and acting as MC chemoattractants. Similar complexity have been observed in other body fluids such as nasal lavage from allergic persons and BAL fluid from asthmatic patients (paper I) [128]

Here we provide evidence that SCF and TGF-β are expressed in the synovium, and that soluble SCF is present in the synovial fluid. Both SCF and TGF-β contribute to the mast cell chemotactic activity detected in synovial fluid from patients with rheumatoid arthritis.
TGF-β isoforms induce mast cell chemotaxis in a bell-shaped dose-dependent manner in both CBMC and in HMC-1 with an optimal migration at 40 fM. TGF-β3 induced the strongest migratory response of the three isoforms. TGF-β has previously been shown to induce migration in monocytes, T cells and neutrophils [129-131] at 40 fM. We show that TGF-β3 is the most effective isoform in mast cell chemotaxis, whereas TGF-β2 is the most effective for neutrophils [131]. TGF-β isoforms are the most potent mast cell chemoattractants described so far. In comparison it is approximately 25,000 times more potent than C5a, which is another potent mast cell chemoattractant. A checkerboard analysis revealed that TGF-β was mainly chemotactic, but also possess some chemokinetic properties. However, under certain conditions, no gradient, TGF-β stimulates a chemokinetic movement. Similar responses have been reported for murine mast cells [132]. Activin A and BMP7 did not induce migration at the concentrations tested.

TGF-β1 and TGF-β2 isoforms and to some degree TGF-β3, but not activin A or BMP7, inhibited cell growth in HMC-1 cells in this assay. The same TGF-β preparations where used in both the migration and cell growth inhibition assays, and all three isoforms displayed similar growth inhibition in the positive control, a mink lung epithelial cell line (Mv1Lu). Mv1Lu is known to respond equally well in growth inhibition to all isoforms of TGF-β. These data show that TGF-β3 slightly inhibit mast cell growth, but is still the most potent and effective mast cell chemoattractant. It has previously been described that different isoforms of TGF-β have specific differences in function within the same cell type. An example of this is that TGF-β1 and TGF β2 are involved in cutaneous scarring in rats, whereas TGF-β3 is suggested to be an anti-scarring agent [133].

Affinity cross-linking studies revealed that HMC-1 cells express TGF-β type I and type II receptors. Endoglin, ALK-1 and TGF-β type III receptors could not be detected in this assay. C57, a murine mast cell line, on the other hand has been shown to express TβR-I and TβR-III, but not TβR-II [132]. The lack of responses after stimulation with BMP7 is most likely due to the absence of the BMP receptors on HMC-1 cells. TGF-β2 bound the present receptors with a lower efficiency than TGF-β1 and TGF-β3. This is in
agreement with earlier reports regarding TGF-β2s weak affinity for TβR-II receptors in the absence of TβR-III [134, 135].

TGF-β has been reported to have different biological activities in different cell types [133, 136-138]. In this study we contribute to these differentiated activities of TGF-β isoforms by showing that TGF-β3 does not cause extensive growth inhibition in mast cells, whereas TGF-β1 and TGF-β2 do inhibit cell growth. Taken together, the results provide evidence that TGF-β isoforms are potent chemotaxins for human mast cells and that TGF-β isoforms may be of importance in the recruitment of mast cells to certain inflammatory conditions. The results also show that TGF-β isoforms have differentiated functions in human mast cells.

Paper IV


The underlying mechanisms for the MC accumulation at sites of inflammation are poorly known. Inflammation releases a large amount of chemokines and mediators that may have the capacity to induce chemotaxis of mast cells. CCL5/RANTES, an MC chemoattractant, is an inflammatory chemokine with the ability to bind several CC-chemokine receptors (CCRs), e.g., CCR1, CCR3, CCR4 and CCR5. In this study we found that cord blood derived MCs (CBMCs) express CCR1 and CCR4, but not CCR3 as reported by Romagnani et al. [80]. This difference can be due to the heterogeneity of MCs. CBMC that we use are more mucosal-like (MC\textsubscript{MC}) MCs, whereas Romagnani et al. used connective tissue (MC\textsubscript{CT}) MCs.

The functionality of the expressed CCR1 and CCR4 was analysed by measuring the migratory response of CBMCs to a number of ligands that bind to CCR1 or CCR4. Out of 7 tested chemokines only CCL5 induced migration of CBMCs, and induced migration in a bell-shaped dose-dependent manner. The maximal migratory response was obtained at 10 ng/ml, and the migration was completely inhibited after treatment of the cells with 1.0 μg/ml pertussis toxin. Thus, the migratory response was mediated via G protein-coupled receptors. Specific antibodies against CCR1
or CCR4 could gradually, at increasing concentrations, decrease the migratory response but not completely block it. However, if both antibodies were present at the same time, the migration could be totally inhibited. This suggests that both CCR1 and CCR4 are involved in CCL5-induced MC migration. Other ligands for CCR1, i.e., CCL2, CCL3, CCL7 and CCL14 were unable to induce MC migration in our system. The CCR4 ligands CCL17 and CCL22 where unable to elicit a chemotactic response. Both CCR1 and CCR4 ligands have been shown to mediate a migratory response in other cell systems, so the results were rather unexpected. It has also been shown that CCR4 expressed in mammalian cell lines do not respond to CCL5 [139, 140], thus CCR4 has lately not been considered a CCL5-receptor [141, 142]. Our results show that CCR4 do bind CCL5 in MCs. This indicates that the functionality of the receptors can vary depending on the cell type and the surrounding environment. Recent reports have suggested that chemokine receptors can form homodimers as well as heterodimeres upon ligand binding [143-146]. This indicates that interactions between CCR1 and CCR4 or other chemokine receptors on MCs may affect the cellular response.

In this study we demonstrate that human cord blood derived mast cells express functional CCR1 and CCR4. We also show that CCL5 induce a migratory response in CBMC. The migration is mediated by interaction between CCL5 and the chemokine receptors CCR1 and CCR4. We show that CCL5/RANTES-induced mast cell migration is selectively mediated through interactions with chemokine receptors CCR1 and CCR4.

Paper V

Differential regulation of mast cell migration by T\textsubscript{H}1- and T\textsubscript{H}2- cytokines: Identification of TNF-\alpha and IL-4 as mast cell chemotaxins. Manuscript.

Depending on the subpopulation of T-lymphocytes that are activated and the cytokines secreted, many inflammations can be divided into T\textsubscript{H}1- or T\textsubscript{H}2-type. A T\textsubscript{H}2-type mediated inflammation, like allergy, is associated with increased secretion of IL-4, IL-5, and IL-13. Rheumatoid arthritis, psoriasis and chron’s disease are examples of T\textsubscript{H}1-type of inflammations associated with increased secretion of TNF-\alpha and IFN-\gamma. The role of MCs in the chronic inflammatory process in most inflammatory diseases is not particularly well known. During the last decades MC involvement and roles in immune responses and inflammations have become more clear and show a
greater involvement than previously thought [108-110]. We have earlier showed MC chemotactic activity in Th1 - (paper II), respectively Th2 -type of inflammations (paper I).

To date, no thorough examination on the capacity of typical Th1- and Th2-cytokines to induce MC migration has been performed. To address this issue we used supernatants from established antigen specific Th1- and Th2- clones to investigate MC chemotactic activity released by these clones.

The different clones expressed the typical Th1-, respectively Th2- cytokine profile (IFN-γ, TNF-α and IL-2 and IL-4, IL-5 and IL-13 respectively) in the supernatants. Both Th1- and Th2- clones supernatants where able to induce migration in the responder cell line HMC-1. Clones specific for diphtheria toxin (DTx) exhibited the most effective activity. The cytokine content where further characterised by measuring the levels of different known MC chemoattractants, such as SCF, TGF-β, CCL5 and IL-8, as well as other cytokines. IL-8, GM-CSF and IL-6 could be detected in all clones, whereas CCL5 was predominantly secreted in Th1-clone supernatants and TGF-β predominantly in Th2–clone supernatants.

To further elucidate the MC chemotactic activity found in the different T-cell clones the MCs where treated with pertussis toxin (PTx) to inhibit G_i protein mediated responses. The treatment inhibited the migratory response in all supernatants except from the Th1 tetanus toxin (TTx) specific clone.

To further investigate the MC chemotactic activity in the supernatants, we used antibodies against IL-4, IL-5, IL-8, IL-10, IL-13, IFN-γ and TNF-α. In the Th1 DP and TTx clone supernatants the antibodies against TNF-α decreased the migratory response with 47% and 90% respectively. Also treatment with IL-8 and IFN-γ where able to reduce the migratory response with approximately 25%. In the Th2 DP and DTx clone supernatants migration was inhibited by treatment with antibodies against IL-4 and IL-8. Th2 TTx was however only inhibited by antibodies against IL-8. Surprisingly, anti-IL-4 treatment increased the migration toward Th2 TTx and to some extent also to Th1 TTx. Similarly, anti-IL-5 increased the migration toward Th2 DP and DTx. The reason for this result is not clear.

The results from the antibody inhibition studies show that IL-4 and TNF-a are potential MC chemoattractants. To confirm this we used recombinant cytokines in a migration assay. Both IL-4 and TNF-α induced a bellshaped dose-dependent response curve, with optimal migration at 10 ng/ml for them both.
This work shows that supernatants from different T-cell clones contain chemotactic substances, and determine that the typical T\textsubscript{H1}- and T\textsubscript{H2}-cytokines TNF-\(\alpha\), and IL-4, in addition to IL-8 are involved in the recruitment of MCs to T\textsubscript{H1}- and T\textsubscript{H2}- inflammations. We also show that recombinant IL-4 and TNF-\(\alpha\) are MC chemotaxins.

CONCLUDING REMARKS

In this work we provide evidence that different body fluids and recombinant cytokines have the capacity to induce migration in human MCs. We demonstrate that BAL fluid from asthmatic patients contains MC chemotactic factors, and that BAL fluids collected during pollen season induce a significantly higher chemotactic response than those collected before season. This indicates that the cells involved in the asthmatic reaction in the lung produce and secret MC chemoattractants. The growth factors SCF and TGF-\(\beta\) are partly responsible for the MC chemotactic activity. Similarly, synovial fluid from rheumatoid arthritis also induces MC migration and also in these fluids we found that SCF and TGF-\(\beta\) contribute to the chemotactic activity. Furthermore, investigation of T\textsubscript{H1}- and T\textsubscript{H2}-clone supernatants revealed TNF-\(\alpha\) and IL-4 as potential MC chemoattractants. Using recombinant IL-4 and TNF-\(\alpha\) we confirmed the results. We also show that human MCs express CCR1 and CCR4 and that CCL5/RANTES selectively induce migration through these receptors, whereas other ligands for the same receptors where unable to induce migration. In addition we demonstrate that TGF-\(\beta\) isoforms, with TGF-\(\beta\)3 being the most effective, are MC chemotaxins. TGF-\(\beta\) isoforms are, by far, the most potent MC chemotaxins known, inducing MC chemotaxis at 40 fM.

The results of this thesis increase our understanding of the complex events leading to accumulation of MCs at sites of inflammation. We believe that TGF-\(\beta\), CCL5, TNF-\(\alpha\) and IL-4, secreted by epithelial- or inflammatory-cells, are involved in the redistribution of mature human MCs within the tissue. The MC hyperplasia is of pivotal importance for the onset/progression of the inflammatory response. By targeting the recruitment of MCs it would be possible to reduce the severity of the inflammation. There are complicating factors in the understanding of the control of cell migration, such as how different chemotaxins interact with each other. They
could be additive or synergistic or even counteract each other. The complexity makes it difficult to find an antibody or inhibitor that can inhibit the accumulation, but rather a cocktail of different inhibitors/antibodies would be needed. The knowledge about MC chemoattractants and how they interact has increased considerably during the last decade, but there is still many more clues to be found to solve the complete enigma of mast cell migration.
Avhandlingen handlar om vad som gör att, den så kallade mastcellen, kan förflytta sig från blod till vävnad och ansamlas i stort antal vid en inflammation. Jag har undersökt hur olika kroppsvätskor och olika enstaka ämnen förmår att locka till sig mastceller.

Avhandlingen visar att vätska från lungsköljningar av astmatiker och ledvätska från patienter med ledgångsreumatism kan locka till sig mastcellerna. Även så kallade T-celler (förknippade med olika inflammationer) har förmågan att locka till sig cellerna. Jag har också identifierat vissa specifika ämnen som kan locka till sig mastceller, nämligen CCL5/RANTES, TGF-β, TNF-α och IL-4.

Vad är då mastceller?
Mastceller är en av många olika celltyper som utvecklas från benmärgen. Härifrån kommer de flesta celler som ingår i vårt immunförsvar mot mikroorganismer. Genom att ursprungscellerna, stamcellerna, kommer i kontakt med olika tillväxtfaktorer i benmärgen utvecklas cellerna till olika celltyper. Trots att mastcellen är involverad i många viktiga funktioner för att hålla oss hela och friska, som att läka sår, nybilda blodkärl, försvara oss mot parasit- och bakterieinfektioner, samt reglera immunförsvar, så är mastcellen mest känt för att spela huvudrollen i allergiska reaktioner.

Mastceller är enormt potenta celler som när den binder ett främmande ämne kan frisläppa en mängd inflammatoriska substanser. Dessa substanser, till exempel histamin, orsakar reaktioner som är typiska för de allergiska symtommen.

Som omogna förstadicellern lämnar mastceller benmärgen för blodomloppet. Därifrån vandrar de in i vävnaden, där de utvecklas till mogna celler. Mastceller finns utspredda i de flesta av kroppens vävnader och vid en inflammation samlas de snabbt vid inflammationshärden. Som som lockar dit mastcellerna är hittills dåligt känt. De arbeten som ingår i denna avhandling bidrar till att höja kunskapsnivån om vad som styr mastcellernas förflyttnings


Även ledvätska från patienter med ledgångsreumatism har förmåga att attrahera mastceller, vilket vi visar i det andra arbetet. Här visar vi att SCF och TGF-β finns uttryckt i både ledvätskan och i synovium. Både SCF och TGF-β bidrar till ledvätskans attraherande förmåga.

Vi har i arbete fem undersökt om så kallade T-celler kan frisläppa ämnen som attraherar mastceller. T-celler är en viktig cell i inflammationen och kan delas in i två grupper T_{\text{H}1}- (T-hjälpar celler typ 1) och T_{\text{H}2}-celler. Både T_{\text{H}1}- och T_{\text{H}2}-celler utsöndrade ämnen som attraherade mastceller. Vi visar att TNF-α och IL-8 är involverade i attraktionen av mastceller till inflammationer av T_{\text{H}1} typ samt IL-4 och IL-8 för inflammationer av T_{\text{H}2} typ.

Sammanfattningsvis har detta arbete identifierat sex ämnen som attraherar mastceller. Vidare visar arbetet att kroppsvätskor kopplade till inflammatoriska sjukdomar innehåller ämnen som attraherar mastceller. Arbetet har bidragit till en ökad förståelse av mastcellers rörelse. Detta i sin tur ger ledrådar i arbetet att finna nya läkemedel mot till exempel astma och ledgångsreumatism.
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