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MAST CELL RESPONSES TO DANGER SIGNALS

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A problem solved is a problem caused

- Karl Pilkington

ABSTRACT

Detecting and responding to danger is a paramount function of the immune system. Compounds heralding danger can be divided into two groups: exogenous and endogenous danger signals. The former group consists of conserved microbial structures such as lipopolysaccharide (LPS), while the latter consists of host compounds released or exposed by dead or dying cells as a consequence of trauma, stress or infection. Mast cells are long-lived immune cells present in almost all tissues, and are especially numerous at sites facing the external environment, making them ideal responders to danger signals. The aim of the work presented in this thesis was to investigate mast cell responses to danger signals of exogenous and endogenous origin.

In **Paper I**, we investigated mast cell responses to the exogenous danger signal M-TriDAP, a bacterial peptidoglycan degradation product. We found that cord blood-derived mast cells (CBMCs) express NOD1, the receptor for M-TriDAP. Furthermore, M-TriDAP-treatment of CBMCs resulted in degranulation-independent release of cytokines and chemokines such as TNF, IL-8/CXCL8, MIP-1α/CCL3 and MIP-1β/CCL4. Importantly, we observed an augmented response when M-TriDAP was combined with the TLR4 agonist LPS, indicating cooperation between intracellular and extracellular pattern recognition receptors.

In **Paper II**, we investigated mast cell responses to cell injury by subjecting murine mast cells to the supernatant of fibroblasts rendered necrotic by freeze-thawing. We found that mast cells respond to cell injury in this model by initiating a pro-inflammatory response, characterized by degranulation-independent release of cytokines and leukotrienes. By using genetically modified mice and molecular inhibitors, we found that the recognition of cell injury was MyD88-, T1/ST2- and p38-dependent. Finally, by using RNA-interference, we could pinpoint IL-33 as the necrotic cell compound that was responsible for the mast cell activation.

In **Paper III**, we investigated responses to IL-33 administration *in vivo*. Here we found that wild-type C57BL/6 mice respond to intraperitoneal IL-33 administration with neutrophil infiltration. This response was not observed in mast cell-deficient mice but could be restored upon mast cell reconstitution, thus demonstrating a mast cell dependent mechanism.

In **Paper IV**, we investigated the hypothesis that mast cells might function as sensors of damaged epithelia by responding to IL-33 during chronic inflammations of the airways, for instance in asthma. We found that IL-33 is released from necrotic airway epithelial cells and that CBMCs respond to the necrotic supernatant of these cells by secreting IL-5, IL-8/CXCL8, TNF and GM-CSF. However, no release of histamine, LTB_4 or PGD_2 could be detected. Interestingly, the exact same mediator release pattern was observed when CBMCs were treated with recombinant IL-33, suggesting that IL-33 might be an important factor released by injured airway epithelial cells that activates mast cells.

In conclusion, the work presented in this thesis provides further evidence for important roles of mast cells in innate immune responses. The function of mast cells as sensors of cell injury is highlighted; a role that potentially can be either beneficial or detrimental. Finally, novel evidence is provided for the notion that IL-33 is an important danger signal capable of mast cell activation.

LIST OF PUBLICATIONS

I. Enoksson M, Ejendal KFK, McAlpine S, Nilsson G, Lunderius-Andersson C
Human cord blood-derived mast cells are activated by the Nod1 agonist M-TriDAP to release pro-inflammatory cytokines and chemokines
J Innate Immun. 2011;3:142-149

II. Enoksson M, Lyberg K, Möller-Westerberg C, Fallon PG, Nilsson G, Lunderius-Andersson C
Mast cells as sensors of cell injury through IL-33 recognition
J Immunol. 2011 Feb 15;186(4):2523-8

III. Enoksson M, Fallon PG, Lunderius-Andersson C, Nilsson G
Intraperitoneal influx of neutrophils in response to IL-33 is mast cell dependent
Submitted

IV. Enoksson M, Andersson C, Heinz G, Chatterjee M, Saluja R, Erjefält J, Nilsson G
Human mast cell responses to injured airway epithelial cells – implications for chronic airway inflammation
Manuscript

Publications not included in this thesis

Lunderius-Andersson C, Enoksson M, Nilsson G
Mast cells respond to cell injury through the recognition of IL-33
Front Immunol. 2012, In press. Review

McAlpine SM, Enoksson M, Lunderius-Andersson C, Nilsson G
The effect of bacterial, viral and fungal infection on mast cell reactivity in the allergic setting
J Innate Immun. 2011;3:120-130. Review

CONTENTS

LIST OF ABBREVIATIONS

AHR	Airway hyper-responsiveness
AIM	Absent in melanoma
APC	Antigen-presenting cell
ATP	Adenosine triphosphate
BMMC	Bone marrow-derived mast cell
CARD	Caspase activation and recruitment domain
CBMC	Cord blood-derived mast cell
CIA	Collagen-induced arthritis
CLR	C-type lectin receptor
DAMP	Danger-associated molecular pattern
DC	Dendritic cell
ELISA	Enzyme linked immunosorbent assay
GM-CSF	Granulocyte macrophage colony-stimulating factor
HMGB1	High-mobility group box 1 protein
HSP	Heat shock protein
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IL-1RAcP	IL-1R accessory protein
IPAF	ICE-protease-activating factor
IRF	Interferon regulatory factor
LGP2	Laboratory of genetics and physiology 2
LPS	Lipopolysaccharide
LRR	Leucine-rich repeat
LT	Leukotriene
MCP	Monocyte chemoattractant protein
MDA5	Melanoma differentiation associated factor 5
MDP	Muramyl dipeptide
MEF	Mouse embryonal fibroblast
MIP	Macrophage inflammatory protein
NALP	NACHT, LRR and PYD-containing protein
NBD	Nucleotide-binding domain
NK	Natural killer
NLR	NOD-like receptor
NLRP	NACHT, LRR and PYD-containing protein
NOD	Nucleotide-binding oligomerization domain
PAMP	Pathogen-associated molecular pattern
PBS	Phosphate-buffered saline
PG	Prostaglandin
PGN	Peptidoglycan
PNEC	Primary nasal epithelial cell
PRR	Pattern recognition receptor
PYD	Pyrin domain
qPCR	Quantitative real time PCR

RA	Rheumatoid arthritis
RAGE	Receptor for advanced glycation endproducts
RIG-I	Retinoic acid-inducible gene I
RLR	Retinoic acid-inducible gene (RIG)-I-like receptor
SCF	Stem-cell factor
siRNA	Small interfering RNA
TGF	Transforming growth factor
Th	T helper
TIR	Toll/IL-1R homology
TLR	Toll-like receptor
TNF	Tumor necrosis factor

1 INTRODUCTION

The immune system has evolved to combat infections, a function that represents a prerequisite for life. Therefore, strict control and regulation of the immune system is paramount, as both excessive and insufficient activation can be fatal, leading to autoimmunity and persistent infections, respectively. Consequently, context-appropriate activation is vital [1], i.e. the nature, magnitude and duration of the immune response need to be appropriate for a given situation and location. In essence, the immune system can be activated by two means: *i*) by recognizing non-self molecules of infectious agents such as bacteria, virus, parasites and fungi (pathogen-associated molecular patterns; PAMPs) [2] or *ii*) by recognizing self molecules released by damaged or dying cells (danger-associated molecular patterns; DAMPs) [3].

To face the ever present threat of detrimental pathogens, the immune system has evolved into a highly organized network of organs, cells and molecules that together can be regarded as the barriers, first line-defenders and the heavy artillery of the immune system. Traditionally, the immune system is described to be composed of two branches: the innate and the adaptive branch. While the former is unspecific but fast in onset, the latter is slow but extremely specific. The first obstacles faced by invading pathogens are physical and chemical barriers, which can be considered part of the innate immune system. Such barriers include for example the skin, mucosal surfaces and low gastric pH. Should pathogens circumvent these barriers, the cellular part of the innate immune system will be activated. The cellular part is composed of a variety of cell types including macrophages, monocytes, dendritic cells (DCs), eosinophils, basophils, natural killer cells (NK cells), neutrophils and mast cells. Together, these cells possess the abilities to directly kill pathogens, produce compounds that enhance pathogen elimination and to activate the adaptive branch of the immune system. Most pathogen infestations can be handled by the innate immune cells, but sometimes a more forceful response is needed, and this is where the adaptive branch of the immune system is introduced.

Antigen-presenting cells (APCs) of the innate immune system, such as DCs, are specialized in carrying antigens through the lymphatic system to local lymph nodes, where activation of the adaptive immune system takes place. The adaptive immune system is composed of T and B lymphocytes, cells that carry receptors specific for a vast amount of antigens. When activated, these cells expand and participate in pathogen killing by producing mediators and antibodies that enhance pathogen elimination. The adaptive immune system also possesses memory, which facilitates a fast response to re-invading pathogens.

In order to fully understand the immune system, and eventually translate this understanding into therapeutical use, detailed knowledge of the various components of the immune system is of great importance. This thesis deals with the activation of such a component; the mast cell. More specifically, activation of mast cells by exogenous and endogenous danger signals is investigated.

1.1 MAST CELLS

The mast cell was discovered in 1878 by the German scientist Paul Ehrlich [4]. Mast cells are most well-known for their role in allergic inflammation, where they act as effector cells which release compounds causing classical allergic symptoms. For many years, mast cells were mostly discussed in the context of allergy, but it is becoming increasingly clear that mast cells have many other functions. We now know that mast cells possess a "Dr Jekyll and Mr Hyde"-nature, where mast cell responses sometimes are beneficial and sometimes detrimental [5]. Thus, a more complex role for mast cells is emerging, where mast cells are more and more appreciated as important sentinel cells possessing regulatory functions. Key characteristics of mast cells include their granulation, their wide tissue distribution, their long life span, and their ability to selectively release mediators upon activation. In addition, mast cells are endowed with several types of receptors, enabling them to react to a multitude of activators. In this chapter, important aspects of mast cell biology are highlighted.

1.1.1 Mast cell distribution and heterogeneity

Mast cells are evolutionary conserved inflammatory cells; early mast cells are thought to have appeared approximately 450-500 million years ago [6]. Murine mast cells originate from a pluripotent, hematopoietic stem cell population in the bone marrow and human mast cells derive from a corresponding $CD34^+/CD117^+/CD13^+$ bone marrow population [7-9]. Unlike other cells of the hematopoietic lineage, mast cells exit the bone marrow as immature precursors, circulate in the bloodstream and eventually migrate into peripheral tissues where they mature [7, 9]. Mast cells are commonly found near blood vessels and nerves in vascularized tissues throughout the body, and are especially numerous at sites facing the external environment, such as the skin, the airways and in the gastrointestinal tract [7, 10, 11]. As a result of this tissue distribution, mast cells are among the first cell types to encounter invading pathogens, and are therefore often referred to as gate-keepers, or sentinels, of the immune system. This important function of mast cells will be discussed in more detail in chapter 1.1.3.

Mast cells represent a heterogeneous cell population, where the tissue microenvironment and growth factor milieu dictates the characteristics of the mature mast cell. Mast cell heterogeneity was originally described by Enerbäck in the 1960s [12, 13]. In this work, mast cells in the intestinal mucosa and submucosa were called "mucosal mast cells" and "connective-tissue mast cells", respectively, depending on their dye-binding properties. In humans, corresponding mast cell populations are referred to as MC_T (tryptase containing) and MC_{TC} (tryptase and chymase containing) [9]. However, this division probably represents an oversimplification, as it is more likely that there is a wide spectrum of mast cell variants, depending on localization and local microenvironment. These types of mast cells might also change during inflammation or infection [11]. Interestingly, it has even been suggested that mast cell phenotypes can be reversible under certain conditions [9, 14]. Stem-cell factor (SCF) is the most important growth factor for mast cells. However, other mediators such as interleukin-3 (IL-3), IL-4, IL-5, IL-6, IL-9 and transforming growth factor-β1 (TGF-β1) can also influence mast cell proliferation and survival [7, 11, 15-17]. The importance of SCF or its receptor c-Kit, which is highly expressed by mast cells, is

demonstrated in mice deficient in c-Kit or SCF, as mast cells are almost completely absent in these mice [18, 19].

1.1.2 Mast cell activation and mediator release

Mast cells are best known for their role in allergic reactions. Therefore, the most extensively studied mast cell reaction is immunoglobulin E (IgE)-dependent mast cell activation [20, 21]. This kind of activation is what underlies an allergic reaction, eventually resulting in classical allergic symptoms, such as sneezing and a runny nose. IgE-dependent mast cell activation is schematically depicted in figure 1. When susceptible individuals come into contact with for example birch pollen, their B cells produce birch-specific IgE antibodies (a process that also requires T cell help). These antibodies will then attach to the surface of mast cells by binding to high affinity IgE-receptors (FcεRI). Upon a second exposure to birch pollen, IgE-primed mast cells are activated through FcεRI-crosslinking, causing the mast cells to degranulate and thereby release mediators, such as histamine, that cause allergic symptoms.

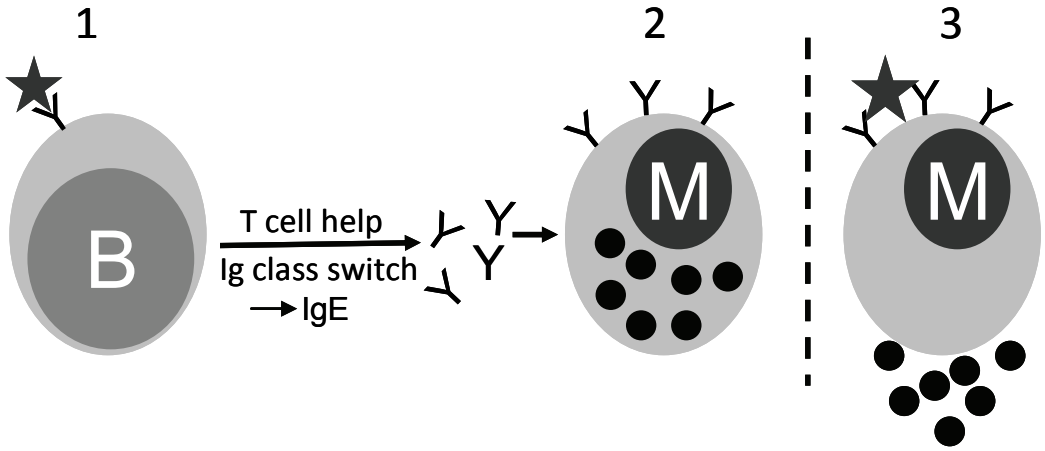

Figure 1. IgE-dependent mast cell activation. In this example, birch allergen (black star) induces the production of IgE antibodies from B cells (1). These birch-specific IgE-antibodies attach to mast cells by interacting with the FcεRI receptor (2). When the same birch allergen is encountered a second time, IgE-primed mast cells become activated and degranulate (3), thereby releasing mediators that elicit allergic inflammation.

Aside from IgE-dependent mast cell activation, mast cells can become activated through many other means. Mast cell activators derive from a wide variety of sources, including viruses, parasites, cytokines, chemokines, chemicals, venoms, endogenous peptides, complement [21], neuropeptides such as substance P [22], IgG [23, 24] fungal components such as zymosan [25], and CD30/CD30L interactions [26]. Studying "alternative" or IgE-independent routes of mast cell activation is of great importance in order to better understand the different roles mast cells have in innate immune responses. While considering that there are many different types of signals capable of mast cell activation, it is important to remember that mast cells do not aimlessly release all their content when activated. Instead, mast cells release different mediator profiles depending on the triggering factor [27]. This means that a certain stimuli might cause degranulation, while another might induce release of cytokines or chemokines without triggering degranulation. For example, many pathogen products induce release of

cytokines and chemokines from mast cells, but do not induce degranulation [11]. The ability of mast cells to selectively produce and release mediators means that they can tailor responses as required for a certain situation. This is of particular importance for their role as sentinel cells, as it gives them the opportunity to fine tune mediator release depending on the invading pathogen.

In the same way as mast cells can be activated by many different triggers, they also produce and release many different mediators. To simplify, mast cell mediators can be divided into two groups; *i*) mediators that are preformed and stored in granules and *ii*) mediators that are synthesized upon activation [21]. Mediators belonging to the first group are released immediately during degranulation, while mediators of the second group can be released minutes to hours following mast cell activation. Mast cell mediators are summarized in figure 2.

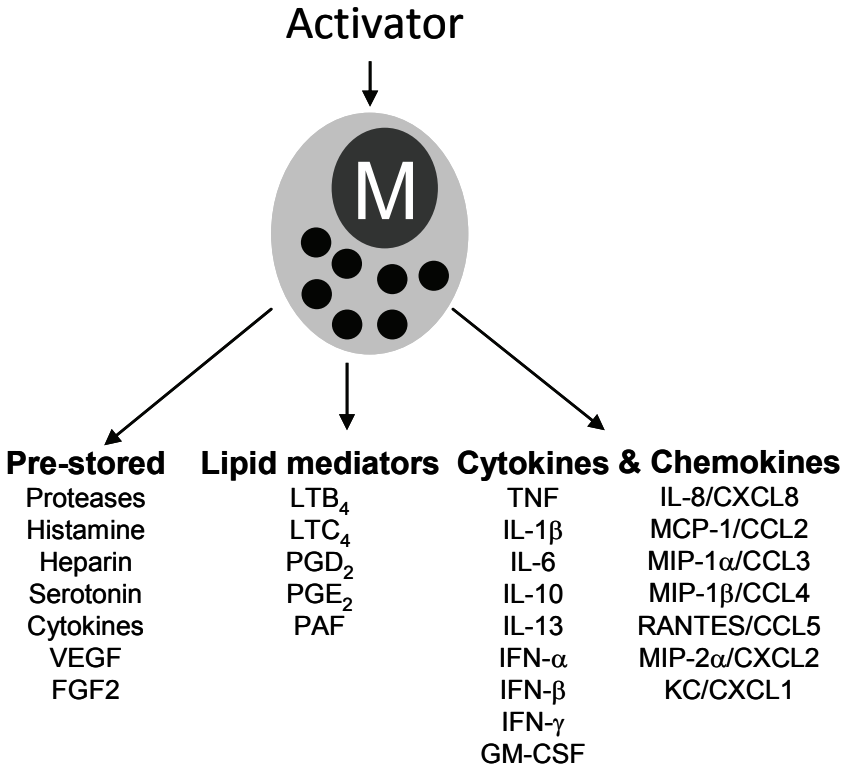

Figure 2. Examples of mast cell mediators.

Of the pre-stored mediators, different proteases (including tryptases, chymases and carboxypeptidases) constitute the largest group. Other pre-stored mediators include histamine, serotonin, heparin and tumor necrosis factor (TNF) [11, 21, 27]. Mediators synthesized upon activation include lipid mediators such as leukotriene B_4 (LTB_4), LTC_4, prostaglandin D_2 (PGD_2) and PGE_2 [11] and many different cytokines and chemokines, including TNF, IL-1β, IL-3, IL-4, IL-5, IL-6 IL-8/CXCL8, IL-9, IL-10, IL-13, IL-16, GM-CSF, MCP-1/CCL2, MIP-1α/CCL3, IFN-α, IFN-β and IFN-γ [11, 27]. In addition, mast cells can also produce and release antimicrobial peptides such as LL-37 [28].

The ability to selectively release different mediators depending on the activating stimuli underlies the fact that mast cells participate in, and help orchestrate, many different

responses. These can be beneficial or detrimental, depending on the circumstances. The impact of various mast cell responses are discussed in chapter 1.1.3.

1.1.3 Mast cell function in health and disease

In 2007, Mitch Leslie published a *News* article in *Science* entitled *"Mast cells show their might"*, in which Leslie stated: "Once dismissed as "allergy cells," mast cells have proven crucial for immunity. But they've also shown a dark side" [5]. Today, it is clear that mast cells can be beneficial, detrimental, or even fatal, depending on the current setting that they are present in, a fact that renders them particularly interesting targets of research. An overview of mast cell roles in health and disease is depicted in figure 3.

In order to understand mast cell function in health and disease, the use of mast cell-deficient mice has been an invaluable tool. The two most commonly used strains are referred to as WBB6F1-Kit$^{W/W-v}$ [29] and C57BL/6-Kit$^{W-sh/W-sh}$ [30]. These mice almost completely lack mast cells due to mutations in the *c-kit* gene, which encodes the receptor for SCF. Comparing results in wild-type mice, mast cell-deficient mice and mast cell knock-in mice has provided important knowledge concerning the role of mast cells in different diseases [31, 32]. In addition, reconstituting mast cell-deficient mice with mast cells deficient in a certain mediator facilitates studies on the role of mast cell-derived mediators, for instance TNF [9, 33]. In addition to Kit-mutant mice, two novel mouse models were recently generated in which mast cells are selectively targeted and eradicated using genetic engineering [34, 35], allowing for more precise future studies of mast cell roles in the immune system.

When considering the mast cell's characteristics, it is easy to understand why they are now recognized as important sentinel cells. By being pre-positioned in tissues, long-lived and able to selectively release inflammatory mediators, mast cells are well equipped for being first hand-responders to different pathogens [36]. The importance of mast cells in bacterial defense has been extensively studied. For instance, mast cells have been shown to be of vital importance in acute bacterial peritonitis models [37], as well as in bacterial clearance after intraperitoneal challenge with enterobacteria [38]. In addition, mast cells protect against other bacteria, such as *Helicobacter* [39], *Pseudomonas* [40] and *Mycoplasma* [41]. Mast cells also seem to have important roles in the defence against viruses and fungi. For instance, mast cells are activated by poly(I:C) (a dsRNA analogue), reovirus [42] and dengue virus [43]. In addition, fungal products such as zymosan and fungal extract (from *Malassezia sympodialis*) also activate mast cells [25, 44, 45]. Supporting a role for mast cells in the recognition of virus and fungi, mast cells express receptors important for the recognition of such pathogens, including toll-like receptor-(TLR)3, TLR7, TLR9 [46], Dectin-1 [25, 44, 45] and Mincle [44]. Pattern recognition receptors (PRRs) such as these are discussed in more detail in chapter 1.2.

Even though mast cells have been shown to be able to phagocytose bacteria [38, 47], release antimicrobial peptides [28, 48], form extracellular traps [49] and even act as antigen-presenting cells [11, 50-52], one of their most important functions in host defense might be to promote inflammation. This is achieved for instance by facilitating the recruitment of effector cells such as neutrophils by releasing pro-inflammatory

cytokines, chemokines and lipid mediators. Examples of when mast cell-derived mediators are important for inflammation are numerous. For instance, TNF derived from mast cells is important for neutrophil infiltration in peritonitis [53], and mast cell-derived lipid mediators such as LTB_4 and LTC_4 contribute to early neutrophil influx during bacterial infection [54]. In addition, chemokines secreted by activated mast cells, such as KC/CXCL1, play important roles in mediating neutrophil recruitment during skin inflammation [55].

Due to the same characteristics that renders mast cells efficient in recognizing and responding to pathogens, mast cells are also ideal contributors to wound healing. Indeed, mast cells have been suggested to participate in various stages of wound healing [56]. Aside from their already mentioned ability to initiate inflammation, mast cells are involved in other important aspects of wound healing as well, such as angiogenesis [57] and matrix remodeling [58]. Furthermore, early wound closure is delayed in mast cell deficient-mice, while wound closure, extravasation and neutrophil recruitment are restored upon mast cell reconstitution in these mice [59]. Mast cells are not only important in facilitating processes required for tissue repair; they are also important in actually sensing cell injury. The function of mast cells as sensors of cell injury was suggested already in 1958 by G.B. West [60], but has surprisingly not yet been completely explored. The role of mast cells as sensors of cell injury is discussed in Papers II and IV of this thesis.

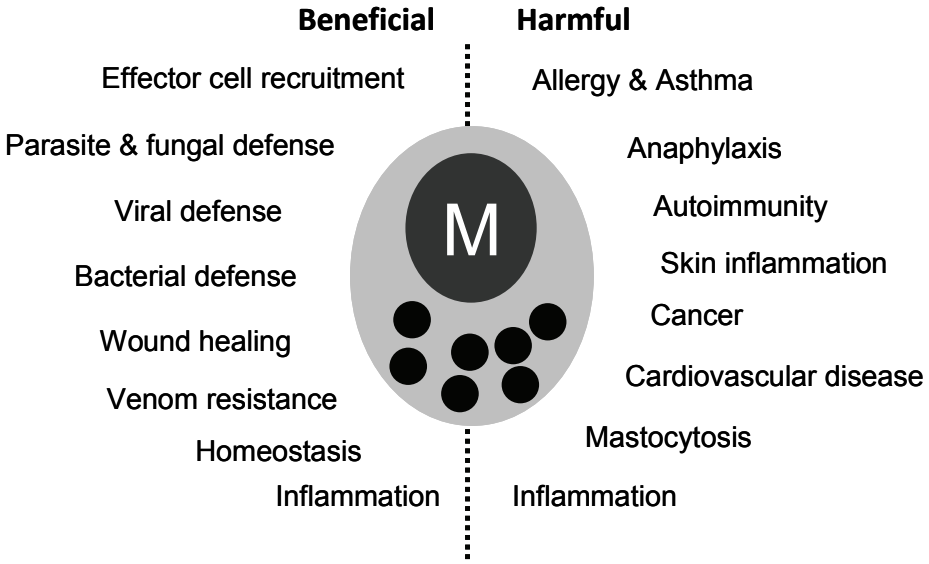

Figure 3. Mast cells can be helpful or detrimental, depending on localization, environment, triggering stimuli, infections and inflammation and other factors. Listed here are some processes where mast cells are involved.

Even though mast cell responses are beneficial in many instances, there is also a darker side to mast cell biology, most commonly linked to their involvement in allergies, anaphylactic reactions and asthma, but mast cells are also believed to be involved in diseases such as multiple sclerosis and rheumatoid arthritis (RA) [61]. In addition, mast cells are involved in the development of heart disease and cancer by, for instance, promoting atherosclerosis [62-64] and tumor growth [61], respectively. Evidence has also been presented that connects mast cells to the development of diabetes and diet-induced obesity [65].

After allergen-specific IgE-binding and subsequent cross-linking of FcεRI-receptors, mast cells are activated and release their content. This causes an immediate or early phase reaction, which has an impact on epithelial, endothelial and smooth muscle cells due to the actions of released histamine, proteoglycans, lipid mediators, tryptase and other mediators. The release of such mediators cause increased vascular permeability, mucus production and smooth muscle contraction [66]. Sometimes, this leads to an anaphylactic shock, which can have fatal outcomes. The immediate phase is followed by late-phase reactions, which are, partly, caused by mediators *de novo* synthesized and subsequently released by mast cells, such as leukotrienes, prostaglandins and cytokines such as IL-5 and IL-13 [9, 61, 66]. Mast cells also play a fundamental role in asthma, which is a chronic inflammatory disease of the airways characterized by bronchoconstriction, mucus secretion, edema and tissue remodeling. Mast cells contribute to these symptoms by releasing mediators that initiate and sustain inflammation in the airways [67].

Conclusively, mast cells are heterogeneous cells shaped and adapted by their local milieu. They can be activated by a multitude of triggers, and produce selective responses tailored to a certain stimuli. Mast cell activation can sometimes have detrimental effects, but they also function as important pathogen sensors, and are among the first cells to encounter invading pathogens. Aside from their longevity, their ability to rapidly produce pro-inflammatory mediators and their favorable tissue distribution, mast cells are also endowed with a multitude of receptors for sensing pathogens. Such receptors are collectively referred to as PRRs, and constitute an essential part of the innate immune system.

1.2 PATTERN RECOGNITION

Germline-encoded PRRs constitute an essential part of the innate immune system, as they allow for the detection of microorganisms. This is achieved by recognizing conserved microbial structures, referred to as PAMPs [2, 68]. The discovery that PAMP recognition by PRRs is essential for the induction of immune responses, made by Charles Janeway [68], represents a key finding in immunology research. There is also accumulating evidence that PRRs are involved in the recognition of endogenous molecules released by damaged cells, so called DAMPs. Up to date, four classes of PRRs have been identified [69]. These include the TLRs, C-type lectin receptors (CLRs), NOD-like receptors (NLRs) and Retinoic acid-inducible gene (RIG)-I-like receptors (RLRs). As an undisputable testament to the importance of PRRs, the discovery of TLRs by Bruce A. Beutler and Jules A. Hoffman was awarded the Nobel Prize in Physiology or Medicine in 2011 (The prize was shared with Ralph M. Steinman for his discovery of DCs).

1.2.1 Toll-like receptors

TLRs were the first PRRs to be discovered and are thus most extensively studied. So far, 10 TLRs have been identified in humans and 12 in mice, each recognizing PAMPs derived from a wide variety of sources, including bacteria, virus, fungi and parasites [70]. TLRs are expressed both extracellularly (TLR1, TLR2, TLR4, TLR5 and TLR6) and intracellularly in endosomes and lysosomes (TLR3, TLR7, TLR8 and TLR9) [69,

71]. The TLRs that are expressed extracellularly are involved in the recognition of bacterial membrane components such as lipopolysaccharide (LPS) (TLR4), peptidoglycan (PGN) (TLR2) and flagellin (TLR5). TLR2 also forms heterodimers with TLR1 or TLR6, where TLR2/TLR1 and TLR2/TLR6 recognize triacyl and diacyl lipoproteins, respectively [69]. Intracellular TLRs are implicated in the recognition of nucleic acids such as dsRNA (TLR3), ssRNA (TLR7 and TLR8) and DNA (TLR9). This is of particular importance for the initiation of antiviral responses, as the triggering of intracellular TLRs often result in the production of type I interferons (IFNs).

TLRs are composed of N-terminal leucine-rich repeats (LRRs), followed by a transmembrane region and a cytoplasmic Toll/IL-1R homology (TIR) domain [69]. Upon recognition of a certain PAMP, TLRs initiate signaling events starting with the recruitment of a TIR-domain-containing adaptor molecule such as MyD88, TRIF, TRAM or TIRAP. All TLRs signal through MyD88, except TLR3 which utilizes TRIF, to eventually activate transcription factors such as nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) and interferon regulatory factor-(IRF)3 to induce production of pro-inflammatory cytokines and IFNs, respectively [70, 72].

TLRs have been mostly studied in APCs such as dendritic cells, but it is becoming increasingly clear that TLRs play important roles in a wide range of cell types, including B cells and mast cells. For instance, it has been demonstrated that mast cell-deficient mice reconstituted with TLR-mutated bone marrow-derived mast cells (BMMCs) display higher mortality than mice reconstituted with wild-type BMMCs after cecal-ligation and puncture-induced peritonitis [73]. Furthermore, the TLR4 ligand LPS has been shown to induce production of several cytokines in mast cells, such as IL-1β, TNF-α, IL-6, IL-10 and IL-13 [73-75], while treatment of mast cells with the TLR2 ligand PGN induces TNF, IL-4, IL-5 and IL-13, but not IL-1β [75]. These types of cytokine responses have also been shown to be synergistically enhanced during IgE cross-linking [74]. It has also been shown in one study that TLR2- but not TLR4-dependent mast cell activation results in degranulation [75]. This further exemplifies that mast cell responses can be fine-tuned depending on stimuli type [75]. While many studies on TLRs and mast cells have been conducted in a murine system, it has also been described that human cord blood-derived mast cells (CBMCs) express mRNA for TLR1, TLR2 and TLR6, but not TLR4 [76]. CBMCs do not respond to LPS [76] unless primed by IL-4 [77], and this also requires the presence of serum components such as CD14.

1.2.2 Nod-like receptors

The NLRs are cytosolic sensors of PAMPs and DAMPs, and they can be divided into three subgroups: NODs (nucleotide-binding oligomerization domain), NLRPs/NALPs (NACHT, LRR and PYD-containing domain) and IPAFs (ICE-protease-activating factor) [78]. Some NLRs form large cytoplasmatic complexes termed inflammasomes, and are involved in proteolytic activation of the inflammatory cytokines IL-1β and IL-18. Proteins of the NLR family utilize LRRs (similarly to TLRs), as well as nucleotide-binding domains (NBD) which are thought to be involved in PAMP recognition [79]. Other domains common to NLR proteins include the caspase activation and recruitment domains (CARDs) and pyrin domains (PYDs). Two of the

first NLRs to be described, and thus most widely studied, are NOD1 and NOD2 [78-81]. NOD1 and NOD2 function as intracellular PRRs and recognize PGN, a component of bacterial cell walls. NOD1 recognizes muropeptides (iE-DAPs), found mainly in PGN of Gram-negative bacteria, while NOD2 recognizes muramyl dipeptide (MDP), found in both Gram-positive and Gram-negative bacteria [78]. Interestingly, it was recently demonstrated that ssRNA activates NOD2 [82], indicating that new NOD ligands from different sources are likely to be found in the future. Both NOD1 and NOD2 recruit the kinase RIP2 upon activation, which subsequently activates NFκB, and eventually results in production of pro-inflammatory cytokines [79].

As stated above, several members of the NLR family form cytosolic multiprotein complexes called inflammasomes [83, 84]. Examples of inflammasomes are NACHT, LRR and PYD-containing protein-(NLRP)1, NLRP3 and Absent in melanoma-(AIM)2, which function as sensors of endogenous or exogenous PAMPs and DAMPs. In general, inflammasomes are though to be activated by a wide range of compounds, including DAMPs (uric acid crystals, adenosine triphosphate (ATP)), PAMPs (bacteria, virus, parasites) and environmental agents (silica, asbestos, alum) [78]. It is not yet completely understood how inflammasomes are activated *in vivo*, but inflammasome activation is thought to be achieved in two steps [79, 85], and is best studied in the NLRP3 inflammasome (which belongs to the NALP subgroup of NLRs). The first step is though to be provided by TLR and/or NLR signaling, which initiates expression of NLRP3, as well as transcription of important components such as pro-caspase-1, proIL-1β and proIL-18 [79]. The second step, which is not yet fully understood, involves interactions between the activator and the inflammasome, and result in inflammasome assembly which eventually drives the release of active IL-1β and IL-18.

Very little is known about the role for NLRs in mast cells responses, but it has been demonstrated that mouse mast cells express inflammasome components: *Casp1* and *Asc* are constitutively expressed in BMMCs, while *Nlrp3* and *Il1b* are inducible by LPS treatment [86]. In humans, it has been shown that the numbers of intestinal $NOD2^+$ mast cells are significantly increased in patients with Crohn's disease [87]. The role of NOD1 in human CBMCs is discussed in Paper I in this thesis. Given the roles of mast cells in innate immunity, it is clear that in time, much will be learned about the role of NLRs in mast cells.

1.2.3 C-type lectin receptors

The term C-type lectin was originally used to separate Ca^{2+}-dependent and independent carbohydrate binding lectins. CLRs initiate responses to pathogens by recognizing mannose, fucose and glucan carbohydrate structures. Thus, CLRs can recognize several types of pathogens including virus, fungi, mycobacteria and helminths [88]. For instance, mannose-structures are present in virus, fungi and bacteria, fucose structures in helminths (and some bacteria), and glucans are present in cell walls of fungi, plants and mycobacteria. Since all pathogens express different sets of PAMPs, these PAMP profiles elicit different immune responses. This is reflected also in CLR signaling, where activation can lead to direct gene expression through NFκB activation, or to modulation of for instance TLR signaling mechanisms [88-91]. Pathogen recognition

through CLRs leads to pathogen internalization, followed by degradation and subsequent antigen presentation. Therefore, CLRs have so far mainly been studied in DCs.

The mannose receptor [92] and Langerin [93] recognize high-mannose and fucose, and these were among the first CLRs to be shown to detect pathogens [91]. Since then, many different CLRs have been described, including Mincle, dectin-1, dectin-2 and DC-SIGN. Dectin-1 recognizes β-glucans in fungal cell walls, while dectin-2 binds high-mannose and α-mannans, for instance in *C. albicans, M. tuberculosis* and *S. cerevisiae*. DC-SIGN is expressed by myeloid DCs and can recognize many different pathogens such as mycobacteria, *C. albicans* and *Leishmania* spp. through mannose and fucose recognition. SIGNR3 is the closest mouse homologue of human DC-SIGN. Mincle is a CLR mainly expressed by macrophages, and recognizes α-mannans which allows for recognition of for instance *C. albicans* and *Malasezzia* spp [91]. Interestingly, Mincle is a PRR capable of responding also to endogenous danger signals. For instance, it has been demonstrated that Mincle-expressing cells respond to SAP130, a small nuclear ribonucleoprotein released by dead cells [94]. In addition, DNGR-1 (also known as CLEC9A) is a CLR with no known PAMP ligand, but binds a yet uncharacterized endogenous ligand released upon necrosis [91, 95].

Currently, the knowledge about CLRs in mast cells is very limited. However, human mast cells have been shown to express Mincle [44], and the mRNA levels were up-regulated upon treatment with *M. sympodialis* extract. Likewise, human mast cells express dectin-1 [25, 44], and respond to treatment with zymosan and *A. fumigatus* hyphae with leukotriene production and IgE-independent degranulation [96], respectively.

1.2.4 Retinoic acid-inducible gene (RIG)-I-like receptors

RLRs are cytoplasmatic PRRs implicated in antiviral immunity as important sensors of viral RNA, but represent the least characterized class of the PRRs. RLR detection of viral components leads to signaling mechanisms eventually resulting in IFN production required for controlling virus infections. To date, only three RLRs have been identified: retinoic acid-inducible gene I (RIG-I), melanoma differentiation associated factor 5 (MDA5) and laboratory of genetics and physiology 2 (LGP2) [97]. RIG-I and MDA5 are expressed in many tissues and share several structural domains, including CARD, a RNA helicase domain, and a C-terminal repressor domain. LGP2 lacks CARD, and is thought to function as a regulator of RIG-I and MDA5 [97, 98].

RIG-I detects many different viruses, including members of Paramyxoviridae, Orthomyxoviridae, Coronaviridae and Caliciviridae, and also DNA viruses such as Epstein-Barr virus, while MDA5 responds to Picornaviridae. There are also some viruses that are detected by both receptors, including West Nile virus, reovirus and dengue virus [97]. RLR signaling is to date not fully understood, but both RIG-I and MDA5 form CARD-CARD interactions with IPS-1 (an adaptor protein) upon recognition of viral PAMPs. IPS-1 forms a signalosome complex with IRF3 and IRF7, which together with NFκB mediate production of type I IFNs [97].

As is the case with mast cells and CLRs, the functions of RLRs in mast cells are to date largely unknown. However, some studies have emerged in which this issue is addressed. For instance, mast cells have been shown to be activated by dengue virus [43, 99]. In a recent study by St John *et al.*, it was shown that both RIG-I and MDA5 were activated in mast cells treated with dengue virus and that this led to mast cell degranulation and *de novo* synthesis of TNF and IFN [99]. In addition, mast cell-deficient mice infected sub-cutaneously with dengue virus displayed increased viral burdens in lymph nodes compared to mast cell-sufficient mice, suggesting mast cells to be important in fighting the viral infection.

PRR-mediated recognition of PAMPs represents a cornerstone in immunology, as it is a prerequisite for innate responses to pathogens and also represents an important step in the induction of adaptive immune responses. Importantly, it is becoming increasingly clear that mast cells participate in pathogen recognition by utilizing PRRs such as those described in this chapter. While individual functions of the four classes of PRRs are becoming better and better characterized, much remains to be learned concerning their cross-talk, and how these receptors modulate and regulate each other. When a pathogen is encountered, many different PAMPs are detected by different sets of PRRs. Therefore, it is now clear that cross-talk between different PRRs is essential in order to mount proper immune responses to a given pathogen.

1.3 DAMAGE RECOGNITION

While pathogen recognition through PRRs initiates inflammatory responses, pathogens are not the only cause of inflammation; damage, or trauma, is another one. Blunt trauma is a good example, where inflammation is rapidly initiated without pathogens having entered the body. The question is how the immune system can respond to injured cells, thus initiating repair mechanisms and eventually restoring tissue homeostasis?

Polly Matzinger, a pioneer in the field of immunology in general, and DAMPs in particular, once stated that the immune system needs to concern itself with two questions when faced with a threat; i) to respond or not, and ii) if the answer is yes, what kind of response should be initiated [100]? During the years, different models have been proposed to answer such questions [3]. One important model was the "Infectious-nonself model", proposed by Janeway in 1989 [68]. This model stated that APCs, such as DCs, are not constantly active, but are resting until activated by PAMP recognition utilizing different PPRs. Thus, this model described how APCs could discriminate between infectious-nonself and noninfectious-self [3, 101]. However, as pointed out by Matzinger, this model does not answer questions concerning why transplants are rejected, why alum works as an adjuvant (since it is non-microbial), or what induces autoimmunity. In an attempt to provide a model capable of answering such questions, Matzinger, in 1994, proposed the "Danger model" [102]. This model suggested that the immune system is more concerned about recognizing damage-causing substances rather than only foreign substances. Importantly, it was proposed that APCs can be activated not only by PAMPs, but also by danger/alarm signals released by cells undergoing for instance mechanical injury or injury caused by pathogen exposure [3].

In 2000, Shi *et al*. found that injecting dead/dying cells provided a strong adjuvant activity, and that the adjuvant activity was present in the cytosol, releasable for instance by mechanical rupture [103, 104]. This finding emphasizes the concept underlying the Danger model, and indeed, during the last decade, many different endogenous danger signals (or DAMPs) have been characterized, providing additional validity to the danger model. Different DAMPs are discussed in chapter 1.3.1.

1.3.1 Endogenous danger signals

A paramount function of the immune system is to monitor the health status of different cells. In this context, cell death (other than apoptotic death) is heralding trouble; a sign of alarm that needs to be recognized and responded to. Responses to injured or killed cells, aiming at repair and a return to tissue homeostasis, can be initiated by the recognition of intracellular molecules exposed as a result of cell injury or death. Thus, an important characteristic of endogenous danger signals is that they should not be released by healthy cells.

Of all DAMPs characterized so far, high-mobility group box 1 protein (HMGB1) is perhaps most extensively studied. HMGB1 functions as a chromatin-binding nuclear factor, but can also be secreted and initiate inflammatory responses [105]. Importantly, release of HMGB1 by necrotic cells has been demonstrated to generate an inflammatory response [106]. Here, bone marrow cells were challenged with dead wild-type or $HMGB1^{-/-}$ fibroblasts. Interestingly, while treatment with dead wild-type fibroblasts triggered a TNF response, this was not observed using $HMGB1^{-/-}$ fibroblasts, or apoptotic wild-type fibroblasts, clearly demonstrating that HMGB1 released from necrotic cells initiates inflammation. In parallel, another group demonstrated similar findings, where also HMGB1-blocking reduced activation induced by necrotic cells [107].

As mentioned in chapter 1.3, Shi and colleagues demonstrated that dead cells possessed adjuvant activity of unknown identity [103]. In a later study by the same group, the cytosol of irradiated 3T3 fibroblasts was fractionated and individual fractions were tested for adjuvant activity [108]. One single fraction was responsible for most of the activity, and identified as uric acid by using mass spectrometry. In line with this finding, the adjuvant activity of necrotic fibroblasts could be decreased if the cells were pre-treated with allopurinol (an inhibitor of uric acid formation) or uricase [108]. Thus, the authors of this study identified uric acid as an important endogenous danger signal released by necrotic cells.

Aside from HMGB1 and uric acid, several additional compounds of different origin have been described as endogenous danger signals. For instance, necrotic cells have been shown to release heat shock proteins (HSPs) such as HSP70 [109], and SAP130 (a ribonucloprotein recognized by Mincle) [94]. Also DNA [110], RNA [111] and ATP [112] can act as DAMPs.

It is interesting to note that several endogenous danger signals are detected by receptors previously thought to react only to foreign molecules (PAMPs). For example, HMGB1 signals through TLR2, TLR4, TLR9 and RAGE (receptor for advanced glycation endproducts) [113], self RNA-complexes can be detected by TLR7 and TLR8 [111], SAP130 by Mincle [94], hyaluronan fragments by TLR2 [114] and Granulysin by TLR4 [115]. Thus, several PPRs have "over-lapping" functions and are capable of eliciting inflammatory responses both in response to pathogens, but also in response to endogenous compounds released by necrotic cells.

IL-1α is another endogenous danger signal released upon necrosis, and has important functions in subsequent neutrophil recruitment [116] and initiation of inflammation [117]. Interestingly, another cytokine of the IL-1-family, IL-33, has been suggested to function as an alarmin [118, 119]. IL-33 biology, its role in the immune system, and its role as a danger signal is discussed in chapter 1.4. Furthermore, IL-33-mediated mast cell responses are discussed in Papers II-IV.

To summarize this chapter, endogenous danger signals are compounds exposed or released by damaged or dead cells as a consequence of infection or trauma. The release of such danger signals functions as a warning signal to the immune system, and allows for damage recognition and actions aiming at containing the damage, and eventually repairing the damaged tissue. Due to their ability to instigate innate responses as well as present antigens to T-cells and thus initiate adaptive responses, recognition and responses to danger signals have largely been studied in DCs. However, a key hypothesis in this thesis is that mast cells also are important sensors of cell injury, based on mast cell characteristics such as a pre-positioning in tissues, their ability to selectively secrete mediators upon activation and their longevity.

1.4 IL-33

IL-33 is a recently discovered member of the IL-1 family of cytokines, and signals through the T1/ST2 receptor [120]. Originally, IL-33 was thought mainly to be involved in induction of T helper 2 (Th2) responses, but many additional functions for this cytokine have been discovered. IL-33 is a potent activator of several immune cells (including mast cells), and is involved in the pathogenesis of several diseases, but it does also mediate protective functions. Interestingly, IL-33 has been shown to function as an endogenous danger signal.

1.4.1 IL-33 expression and signaling

In 2005, IL-33 was characterized by Schmitz *et al*. [120] as a member of the IL-1 family of cytokines, together with IL-1, IL-18 and IL-1RA. Two years prior to this study, Baekkevold *et al.* characterized a nuclear factor expressed in high endothelial venules, termed NF-HEV [121], which was later shown to be identical to IL-33 [122]. IL-33 is expressed in many different tissues and organs, especially by structural cell types such as epithelial cells [123], endothelial cells, fibroblasts, keratinocytes [118], airway smooth muscle cells [124] and astrocytes [125]. IL-33 is also expressed in osteoblasts [126, 127], adipocytes [126, 128], monocytes [129] and pancreatic stellate cells [130]. In some cell types, pro-inflammatory stimuli such as LPS can up-regulate IL-33 mRNA and protein levels [129].

The nuclear role of IL-33 is still incompletely understood. However, it has been demonstrated that IL-33 can associate with heterochromatin in the nucleus, where it exerts transcriptional repression [122]. In addition, IL-33 has been shown to interact with the p65 subunit of NFκB, thus reducing NFκB-triggered gene expression [131]. The recent generation of IL-33$^{-/-}$ mice [132] will definitively be an important tool in future studies seeking to further investigate nuclear roles of IL-33.

The IL-33 receptor consists of two subunits; IL-1R accessory protein (IL-1RAcP) and T1/ST2 [133]. While IL-1RAcP is utilized also by IL-1RI [134], T1/ST2 is specific for IL-33, and was for a long time an orphan receptor until Schmitz *et al.* identified IL-33 as its ligand [120]. T1/ST2 was originally described as a stable surface marker expressed by Th2 but not Th1 cells, and readily used to distinguish between these cell types [135]. Besides Th2 cells, mast cells were early on shown to strongly express T1/ST2 [136]. Complex formation between T1/ST2 and IL-1RAcP is essential for functional IL-33 signalling [133]. For instance, IL-1RAcP$^{-/-}$ mast cells secrete reduced cytokine levels compared to wild-type mast cells after IL-33 treatment [137], and the use of neutralizing antibodies targeting IL-1RAcP abrogates mast cell responses to IL-33 [138]. The IL-33/ST2 signalling pathway involves recruitment of MyD88, IRAK, IRAK4 and TRAF6, eventually resulting in NFκB activation [120]. TRAF6 has been shown to be a crucial component in this signalling pathway, as activation of NFκB, p38 and JNK was absent when TRAF6$^{-/-}$ fibroblasts were stimulated with IL-33 [139]. Additionally, JAK2$^{-/-}$ fibroblasts fail to induce IκBα degradation and NFκB activation upon IL-33 stimulation, suggesting this tyrosine kinase to be of importance in the signalling mechanism of IL-33 [140].

While IL-1β and IL-18, two other members of the IL-1 family of cytokines, both require proteolytical processing by caspase-1 in order to be rendered biologically active [134], this does not seem to be the case for IL-33. Instead, full-length IL-33 (IL-33$_{1\text{-}270}$, ~30kDa) has been reported by several studies to be biologically active [141-144], thus clearly distinguishing IL-33 from IL-1β and IL-18. This characteristic provides one explanation for the proposed role of IL-33 as an endogenous danger signal, a role discussed further in chapter 1.4.3. Initially, caspase-1-processing was also thought to be required for IL-33 [120], until several studies demonstrated that IL-33 was a poor substrate for caspase-1 [141, 143, 144]. Consequently, proteolytical processing of IL-33 has been a much debated subject. For instance, one study reported calpain to be mediating IL-33 processing *in vivo* [145], while another study observed IL-33 release in macrophages treated with inhibitors of both calpain and caspase-8, as well as in caspase-1$^{-/-}$ cells [146]. Furthermore, IL-33 has even been shown to be inactivated by caspase-1 [142] as well as by the apoptotic caspases caspase-3 and caspase-7 [143]. Many published studies have utilized an artificially truncated form of IL-33 (IL-33$_{112\text{-}270}$, ~18kDa). While this shorter form of IL-33 can activate T1/ST2 similarly to full-length IL-33 [143], it remains unclear whether the shorter form is produced naturally.

1.4.2 IL-33 activity

Since early results demonstrated that T1/ST2 is expressed on Th2 but not Th1 cells, IL-33 activity was first studied in T cells. In this experiment, Schmitz *et al.* observed that Th2 cells stimulated with anti-CD3 and anti-CD28 together with IL-33 responded with production of IL-5 and IL-13 [120]. Further evidence for the importance of IL-33 in generating Th2 responses was demonstrated in T1/ST2$^{-/-}$ mice, which were shown to produce reduced levels of Th2 cytokines in response to immunological challenges normally characterized by Th2 responses in wild-type mice [147]. IL-33 is also a potent regulator of mast cell activity (figure 4). Several studies have demonstrated that IL-33 induces release of cytokines and chemokines in mouse mast cells, including IL-1β, IL-6, IL-13, TNF, macrophage inflammatory protein 1α (MIP-1α)/CCL3, monocyte chemoattractant protein 1 (MCP-1)/CCL2, MCP-3/CCL7 and granulocyte macrophage colony-stimulating factor (GM-CSF) [138, 148-151]. Similar results have been reported in human mast cells, where stimulation with IL-33 induce release of IL-5, IL-6, IL-8/CXCL8, IL-10, IL-13, TNF, GM-CSF and CCL1 [152, 153]. IL-33 has also been shown to promote maturation [152], survival, adhesion [153] and release of lipid mediators in human mast cells [154]. Furthermore, IL-33 was recently shown to modulate tryptase expression in mast cells [155]. The fact that IL-33 induces pro-inflammatory responses in mast cells implies a role for IL-33 and mast cells in inflammatory diseases, as discussed more in chapter 1.4.2.

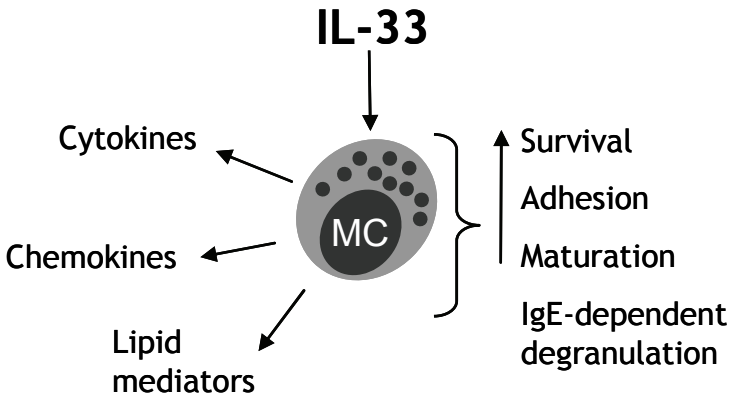

Figure 4. IL-33 induces cytokine, chemokine and lipid mediator release by mast cells. In addition, IL-33 increases mast cell survival, adhesion and maturation. While IL-33 by itself does not induce mast cell degranulation, IgE-primed mast cells do degranulate in response to IL-33.

Aside from Th2 cells and mast cells, IL-33 activates several additional cell types (figure 5). For instance, IL-33 induces IL-4 and IL-13 release from mouse and human basophils [156-159], and promotes survival and adhesion in eosinophils [159, 160]. In macrophages, IL-33 enhances responses to LPS by increasing expression of MD2, TLR4, CD14 and MyD88 [161]. In addition, IL-33 induces release of pro-inflammatory cytokines in DCs [162], and importantly, DCs treated with IL-33 have been shown to prime naïve T-cells to produce IL-5 and IL-13 [162, 163]. Furthermore, IL-33 can activate B1 cells to release IgM, IL-5 and IL-13 through an IL-5 dependent mechanism [164]. Interestingly, it was recently discovered that nuocytes, a population of innate Th2 effector leukocytes, expand *in vivo* in response to IL-33, resulting in IL-13 production [165]. While IL-33 often has been associated with Th2-skewed responses, it has been reported that iNKT cells produce both IFN-γ and IL-4 upon stimulation with

IL-33 and α-galactosylceramide [166]. Regarding effects of IL-33 on cells of the central nervous system, not much is currently known. However, one study has reported that IL-33 induces proliferation and production of IL-1β, TNF and IL-10 in microglia [167].

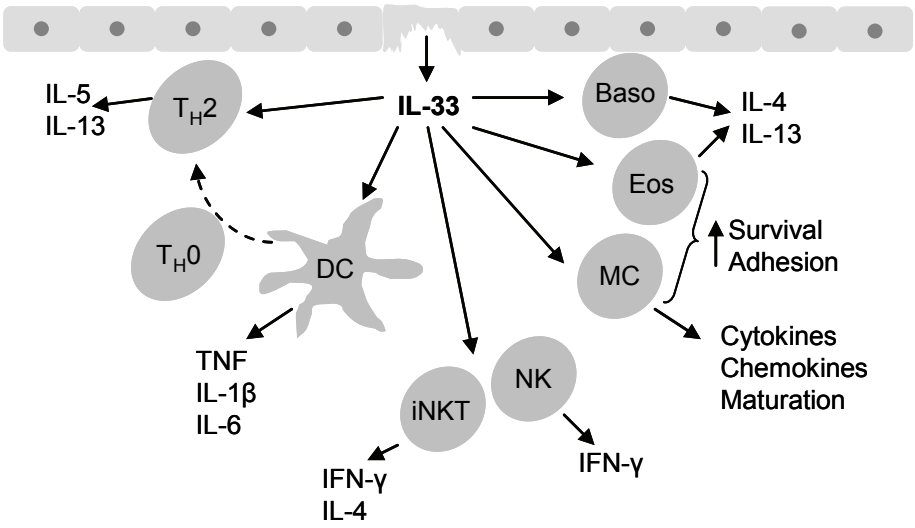

Figure 5. Effects of IL-33 on immune cells. Aside from regulating mast cell (MC) activity, IL-33 activates many other cell types. As depicted here, IL-33 released from necrotic cells induces release of IL-4 and IL-13 from basophils and eosinophils and, together with appropriate co-stimulation, IFN-γ from iNKT cells and NK cells. DCs treated with IL-33 can prime naïve T cells to produce Th2 cytokines.

1.4.3 IL-33 in health and disease

Like all other cytokines, IL-33 is part of our immune system, and thus contributes to different immune mechanisms. Due to the rather recent discovery of IL-33 however, a bona fide role for IL-33 in our immune system remains to be completely established. Due to the capability of IL-33 to activate several cell types and initiate pro-inflammatory responses, IL-33 has been implicated in the pathogenesis of several diseases. Less is known about protective immune mechanisms involving IL-33, even though some recent studies have highlighted such roles. Beneficial and detrimental consequences of IL-33 actions are discussed below.

An interesting protective effect of IL-33 was recently demonstrated in a study by Alves-Filho *et al.* [168], in which mice were subjected to cecal ligation and puncture with or without preceding IL-33 administration. In this experimental model of sepsis, mice pre-treated with IL-33 displayed a reduced mortality rate due to increased recruitment of neutrophils to the peritoneum compared to phosphate-buffered saline (PBS)-treated control mice. (IL-33-mediated neutrophil recruitment in the context of mast cell activation is discussed in Paper III.) Further supporting a protective role for IL-33 in sepsis, the authors could detect elevated serum concentrations of soluble ST2 in patients who died from sepsis, compared to patients who survived. In a parasite infection model in mice, Humphreys *et al.* demonstrated that infected mice could expulse the parasite if treated with IL-33 [169]. This was achieved through a mechanism where IL-33-treatment prevented a Th1-polarized response, and instead promoted IL-4, IL-9 and IL-13. Thus, the authors provided functional evidence for the

notion that IL-33 is a Th2-driving cytokine. In a model of atherosclerosis, IL-33-treated mice displayed reduced lesion development compared to PBS-treated mice; this was also accompanied by an increase in Th2 cytokines such as IL-4, IL-5 and IL-13, and reduced levels of IFN-γ [170]. Furthermore, several studies suggest IL-33 to have cardio-protective functions [171, 172], by prolonging cardiac allograft survival [173, 174], and improving survival after experimental myocardial infarction [175].

In addition to the protective effects of IL-33 discovered so far, IL-33 has been connected to several inflammatory diseases, including ulcerative colitis and RA. IL-33 mRNA is increased in patients with ulcerative colitis [176-178], and several studies have reported elevated levels of IL-33 in serum and synovial fluid from patients with RA [179-181]. In addition, IL-33 has been shown to be expressed in synovial fibroblasts from RA patients [151]. This increase in IL-33 levels is partially decreased after anti-TNF treatment [180], and the mechanism for this has been suggested to involve downregulation of T1/ST2 on neutrophils [182]. Several studies of animal models of arthritis have implicated mast cells as important players in disease exacerbations. For instance, Xu *et al.* demonstrated that T1/ST2$^{-/-}$ mice develop attenuated collagen-induced arthritis (CIA) and a reduced induction of IL-17, TNF and IFN-γ [151]. Here, IL-33-treatment exacerbated CIA in wild-type mice, but not in T1/ST2$^{-/-}$ mice. Moreover, CIA was exacerbated also in T1/ST2$^{-/-}$ mice reconstituted with wild-type mast cells, indicating an important role for mast cells in the disease mechanism. Similarly, another study by Xu *et al.* demonstrated that IL-33-treatment failed to induce autoantibody-induced arthritis in mast cell-deficient and T1/ST2$^{-/-}$ mice [183]. In addition, administration of a blocking T1/ST2 antibody at disease onset has been shown to attenuate disease severity in a CIA model [184].

Injection of IL-33 in the skin of mice induces an increased expression of IL-13 mRNA and development of cutaneous fibrosis [185]. A recent study also reported that T1/ST2$^{-/-}$ mice developed reduced cutaneous inflammation in a phorbol ester-induced model of skin inflammation, [186]. The observed inflammatory response in this study was shown to be partly mast cell dependent, as delayed inflammatory responses were observed in mast cell-deficient mice.

More and more studies are suggesting that IL-33 has important roles in allergic inflammation and asthma. For instance, recent genome-wide association studies have implicated both *IL-33* and *IL1RL1* (T1/ST2) as important candidate genes for asthma [187, 188]. Endobronchial biopsies from asthmatic patients display higher IL-33 levels compared to healthy controls [124], and epithelial cells from patients with severe bronchial asthma display increased IL-33 expression [123]. In addition, patients with moderate asthma also have elevated IL-33 levels in their bronchoalveolar lavage fluid [123]. The involvement of the IL-33/ST2-axis in allergic airway inflammation and asthma has also been studied in several mouse models. For instance, intraperitoneal IL-33-administration induces airway hyper-responsiveness (AHR) to methacoline in mouse lungs, and production of IL-4, IL-5 and IL-13 [189]. In addition, intranasal IL-33-administration rapidly triggers allergic airway responses in mice [190]. Correspondingly, AHR resolution has been suggested to be dependent upon disruption of the IL-33/ST2-axis [191]. IL-33 and mast cells in the context of chronic airway inflammation is discussed in Paper IV.

1.4.4 IL-33 as a danger signal

It was originally thought that the main role of IL-33 was to drive the production of Th2-associated cytokines [120], but later studies demonstrated that IL-33 also has an intracellular role as a heterochromatin-associated nuclear factor [122]. In the wake of this latter finding, the connection between IL-33 and a possible role as a danger signal was made relatively quickly [119]. This connection was based on similarities between IL-33 and well-known danger signals such as HMGB1 and IL-1α. Both HMGB1 and IL-1α are dual-function proteins that have intracellular roles, but also act extracellularly as cytokines [192]. Importantly, both these danger signals are released upon necrosis, and are known to initiate inflammatory responses [106, 116, 117].

Further evidence for the notion that IL-33 is a danger signal was provided when questions regarding the secretion and processing of IL-33 were being asked. As stated in chapter 1.4.1, IL-33 was at first thought to require proteolytical processing in order to be biologically active, similarly to IL-1β and IL-18, and subsequently, caspase-1 was reported to mediate this cleavage [120]. However, several studies proved this notion to be wrong [141, 143, 144]. For instance, Lüthi and colleagues demonstrated that while caspase-1 could convert proIL-1β into active IL-1β, similar treatment failed to process human as well as mouse IL-33 [143]. Another study published at the same time reported that caspase-1-processing of IL-33 was not required for biological activity [144], and a third study reported that IL-33 was inactivated by caspase-1-processing [142]. Together, these three studies independently showed that full-length IL-33 is biologically active, a characteristic well-suited to a danger signal. In addition, Lüthi *et al.* could observe that while IL-33 was inactivated during apoptotic cell death, it was released upon necrosis induced by hydrogen peroxide or sodium azide [143]. Similarly, Cayrol *et al.* noted that mechanical injury of endothelial cells resulted in the release of biologically active full-length IL-33 [142]. Thus, another similarity between IL-33 and HMGB1 and IL-1α was revealed. Importantly, Moussion *et al.* have demonstrated that IL-33 is constitutively expressed in normal human tissues and that endothelial and epithelial cells constitute the major sources of IL-33 [118]. Epithelial cells are present at sites exposed to the local environment and are thus exposed to pathogens and other causes of tissue damage (toxins, UV-light, mechanical trauma etc.). In keratinocytes, UV-light exposure leads to IL-33 mRNA up-regulation [193, 194], something that has also been reported in human skin subjected to tape-stripping [195].

Hypothetically, should cells suffer injury, one important step towards restoring tissue functions might be the detection of IL-33 released from necrotic injured cells, and the subsequent inflammatory responses generated upon this recognition. The role of IL-33 as an endogenous danger signal, and its recognition by mast cells, is discussed in Papers II and IV.

2 THE PRESENT STUDY

2.1 AIM

The overall aim of the work presented in this thesis was to investigate mast cell responses to danger signals of endogenous and exogenous origin.

The specific aims for papers I-IV were as follows:

Paper I: To investigate how mast cells respond to treatment with the NOD1 specific agonist M-TriDAP.

Paper II: To investigate mast cells responses to treatment with necrotic cells.

Paper III: To investigate the role of mast cells *in vivo* in response to IL-33 injections.

Paper IV: To investigate whether human mast cells have a role in recognizing and responding to injured airway epithelial cells.

2.2 METHODOLOGY

The methods used during the work of this thesis are described here. More detailed method descriptions are found in the "material and methods" sections of Papers I-IV.

Enzyme linked immunosorbent assay (ELISA) (Papers I-IV)
Cytokines in cell culture supernatants were quantified using ELISA.

Flow cytometry (Papers I, III, IV)
To detect surface expression or intracellular expression of various markers, cells were stained with fluorochrome-conjugated antibodies and analyzed on a FACSCalibur. Data was analyzed using CellQuestPro and FlowJo.

Histology (Paper IV)
Airway epithelial cell lines were stained with a fluorochrome-conjugated antibody targeting human IL-33 and analyzed by confocal microscopy.

Intraperitoneal injections (Paper III)
Recombinant IL-33 or PBS was injected intraperitoneally in wild-type or mast cell-deficient W^{sh}/W^{sh} mice. The mice were sacrificed at different time points, and peritoneal cells were harvested by flushing the peritoneum with ice-cold PBS. Recovered cells were analyzed by flow cytometry.

In vitro *mast cell cultures (Papers I-IV)*
To generated human CBMCs, $CD34^{+}$ cells were purified from umbilical cord blood and maintained in medium supplemented with SCF and IL-6. CBMC purity was assured by tryptase staining. BMMCs were generated by retrieving bone marrow cells from femur and tibia of mice. These cells were subsequently maintained in IL-3-containing medium for four weeks. BMMC purity was assessed by toluidine blue staining and by flow cytometry. PCMCs were obtained by flushing the peritoneum of mice with ice-cold PBS. Peritoneal cells were cultured for four weeks in SCF-containing medium. The purity of mast cells used in experiments exceeded 95%.

In vitro *stimulation of mast cells (Papers I-IV)*
Human or mouse mast cells were washed and seeded in plates before being incubated with different stimuli for different time periods at 37°C. After this, cell free supernatants were collected and stored at -20°C until further analysis.

Mice (Paper II-III)
C57BL/6 wild-type mice and mast cell-deficient W^{sh}/W^{sh} mice were used in experiments. BMMCs were generated from C57BL/6 wild-type mice, $TLR1^{-/-}$, $TLR2^{-/-}$, $TLR4^{-/-}$, $TLR5^{-/-}$, $TLR6^{-/-}$, $TLR7^{-/-}$, $TLR8^{-/-}$, $TLR9^{-/-}$, $MyD88^{-/-}$, $T1/ST2^{-/-}$, $A_{2A}R^{-/-}$ and $A_3AR^{-/-}$ mice. All experiments involving animals were approved by the Swedish local ethics committee for animal welfare.

Mast cell reconstitution in W^{sh}/W^{sh} mice (Paper III)
In order to locally reconstitute mast cell-deficient W^{sh}/W^{sh} mice with mast cells, BMMCs ($2.5x10^6$/mouse) were injected intraperitoneally. Four weeks later, reconstituted mice were used in experiments.

Multiplex assays (Papers I-IV)
Cytokines and chemokines in cell culture supernatants were measured using multiplex assays such as Luminex. In multiplex assays, multiple analytes are measured simultaneously.

Necrotic supernatant generation (Papers II, IV)
Different cell types were rendered necrotic by being subjected to repeated freeze-thaw cycles. The cells were then centrifuged in order to remove debris, and the cell supernatant was collected and stored at -80°C until use.

siRNA-mediated gene silencing (Paper II)
siRNA targeting IL-33 was transfected into mouse embryonal fibroblasts (MEFs) using electroporation in order to transiently knock down expression of the IL-33 gene.

Statistical analyses (Papers I-IV)
The data was analyzed using the Mann Whitney U-test, the Wilcoxon matched pairs test, the Kruskal-Wallis test with Dunn's post test or Students T-test. $p<0.05$ was considered significant.

Western blot (Papers II, IV)
Proteins in total cell lysates or in necrotic cell supernatants were analyzed using Western blotting. Briefly, proteins were separated by electrophoresis, transferred to a protein-binding membrane and eventually detected using HRP-conjugated antibodies.

Quantitative real time PCR (qPCR) (Paper II)
To validate siRNA-mediated knock-down of IL-33 in MEFs, total RNA was extracted from siRNA-treated MEFs and synthesized to cDNA by reverse transcription. cDNA was then amplified using specific primer pairs, and results analyzed in relation to the expression of a house-keeping gene.

2.3 RESULTS AND DISCUSSION

2.3.1 Human cord blood-derived mast cells are activated by the NOD1 agonist M-TriDAP to release pro-inflammatory cytokines and chemokines (Paper I)

The immune system relies on PRRs to recognize and respond to invading pathogens. Among the different PRRs, NLRs such as NOD1 and NOD2 are important intracellular sensors of bacteria, recognizing peptidoglycan fragments from bacterial cell walls. While mast cells are appreciated as important cells of the innate immune system lining the border between host and external environment, the role of intracellular PRRs such as NOD1 in mast cells is currently not completely investigated. Therefore, we here investigated responses of human mast cells to the NOD1 agonist M-TriDAP.

We started by investigating whether NOD1 and NOD2 are expressed intracellularly in human mast cells derived from cord blood (CBMCs). While CBMCs from all tested donors expressed NOD1 intracellularly, only one donor out of four expressed NOD2, at low levels (figure 6A). This is in contrast to earlier studies, where NOD2 expression was reported in the mast cell line HMC-1.1 [196] and also in primary human mast cells from patients with the inflammatory disorder Crohn's disease [87]. Even though it has previously been reported that NOD2 expression can be up-regulated [87, 197], we were unable to up-regulate NOD2 expression in CBMCs using either LPS, IFN-γ or vitamin D3. As a consequence, we continued by investigating CBMC responses to M-TriDAP, a peptidoglycan degradation product specifically recognized by NOD1 [198].

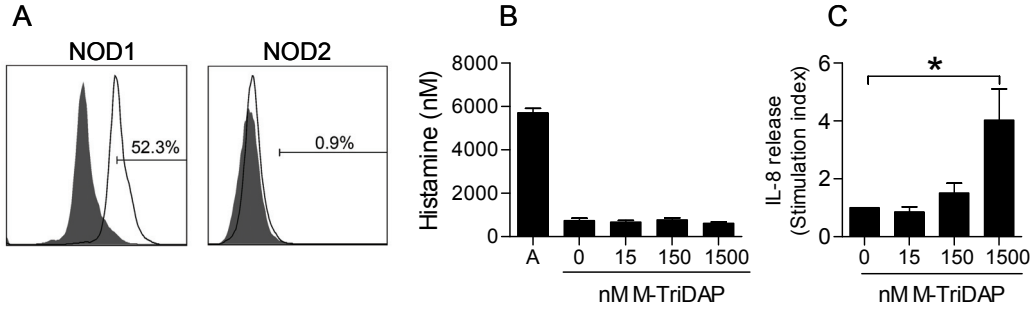

Figure 6. Human mast cells express the NOD1 receptor, and respond to a NOD1 agonist by degranulation-independent cytokine release. (**A**) CBMCs express NOD1 but not NOD2 intracellularly. Representative data from one donor is shown. (**B**) Histamine release in CBMCs exposed to various concentrations of the NOD1 agonist M-TriDAP for 30 minutes. (**C**) IL-8/CXCL8 release in CBMCs treated with M-TriDAP for 24 hours. Values are presented as mean ± SEM (n=4-5). *p<0.05. A= A23187 ionophore used in (B) as positive control

Since mast cell activation often is characterized by rapid degranulation, we first investigated whether histamine and/or leukotrienes would be secreted from M-TriDAP-stimulated mast cells. However, we did not detect any release of neither histamine (figure 6B) nor leukotrienes. As mast cell mediator release is selective and highly dependent on the triggering factor [27], and pathogens often induce *de novo* synthesis of cytokines/chemokines unaccompanied by degranulation [11], we continued by investigating cytokine and chemokine release. After 24 hours of treatment with M-TriDAP, we found that CBMCs from all investigated donors secreted IL-8/CXCL8, an important pro-inflammatory chemokine (figure 6C). The IL-8/CXCL8 release was

partly p38-dependent, as cells pretreated with the p38 inhibitor SB203580 released reduced IL-8/CXCL8 levels. In agreement with this observation, this inhibitor has previously been demonstrated to inhibit RIP2 [199], an important molecule in NOD1 signaling [200].

To further characterize the mediator release pattern of CBMCs in response to M-TriDAP, we assessed the release of other pro-inflammatory mediators. Here, we could observe a dose-dependent release of MIP-1α/CCL3, MIP-1β/CCL4 and TNF, while no release of IL-1 β, MCP-1/CCL2 or GM-CSF was observed. Taken together, these results demonstrate that human mast cells are able to mount an inflammatory response to a NOD1 agonist, thus participating in the defense against invading bacteria.

NOD1 activation has been shown to synergistically enhance responses to TLR ligands in mononuclear cells [201], and Pam(3)CSK(4)-mediated IL-6 production in mast cells has been demonstrated to be increased by simultaneous treatment with NLR agonists [202]. Since mast cells are activated by TLR2 and TLR4 ligands [76, 77], we next investigated whether M-TriDAP together with low concentrations of the TLR2 and TLR4 agonists zymosan and LPS would elicit augmented IL-8/CXCL8 release. Here, we found that the release of IL-8/CXCL8 was increased when M-TriDAP was combined with LPS (figure 7A), suggesting co-operation between extra- and intracellular means of pathogen detection. On the other hand, no augmented effect was observed when M-TriDAP was combined with zymosan (figure 7B).

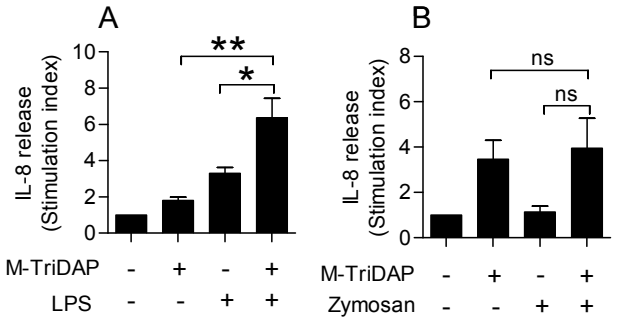

Figure 7. M-TriDAP-induced IL-8/CXCL8 secretion is augmented if M-TriDAP is combined with LPS. CBMCs were treated with 1500 nM M-TriDAP either alone, or combined with (**A**) 0.1 µg/ml LPS or (**B**) 1 ng/ml zymosan, and IL-8/CXCL8 secretion was determined after 24 hours. Values are shown as stimulation index (treated/untreated). The values shown are mean ± SEM (n = 7–8). *p<0.05, **p<0.01 in comparison to single treated cells.

Taken together, this paper demonstrates that human mast cells express the NOD1 receptor intracellularly. CBMCs are activated by the NOD1 agonist M-TriDAP to secrete pro-inflammatory cytokines and chemokines, independently of mast cell degranulation, suggesting selective mediator release. Cross-talk between different classes of PRRs such as NLRs and TLRs is becoming increasingly appreciated, and we here show that mast cells responses are augmented if the mast cells are treated with NLR and TLR agonists simultaneously. Most importantly however, this study is one of the first specifically targeting NOD1 activation in human mast cells, and provides additional evidence for the fact that mast cells are important sentinel cells.

2.3.2 Mast cells as sensors of cell injury through IL-33 recognition (Paper II)

Recognition and response to cell injury requires the initiation of several inflammatory processes to restore and maintain tissue function [203]. Detecting cell injury is a prerequisite for proper subsequent responses, but the mechanisms underlying cell injury responses are not fully understood. In this paper, we hypothesized that mast cells are important sensors of cell injury due to characteristics such as their positioning at sites facing the external environment [11, 204], their longevity, and their ability to selectively produce mediators upon activation [205]. Remarkably, while the notion of mast cells as responders to cell injury was suggested over 50 years ago [60], underlying mechanisms remain to be deciphered.

In order to study whether mast cells play a role in cell injury recognition, we generated an *in vitro* model of cell injury by rendering mouse embryonal fibroblasts (MEFs) necrotic by repeated cycles of freeze-thawing. Next, we incubated BMMCs with cell-free necrotic supernatant from MEFs and monitored mast cell responses. While the mast cells did not release histamine in response to necrotic supernatant, we observed secretion of cysteinyl leukotrienes, IL-6 and TNF (figure 8A-D), demonstrating the capability of mast cells to recognize and respond to components released by necrotic cells. Initially, we suspected well-known endogenous danger signals such as HMGB1 [106] or uric acid [108] to be responsible for the observed effect, and therefore generated necrotic supernatant from HMGB1$^{-/-}$ MEFs [206], and from MEFs pre-treated with allopurinol, an inhibitor of uric acid production. However, we could not observe decreased mast cell IL-6 production in neither case, thus suggesting other danger signals than HMGB1 or uric acid to be involved.

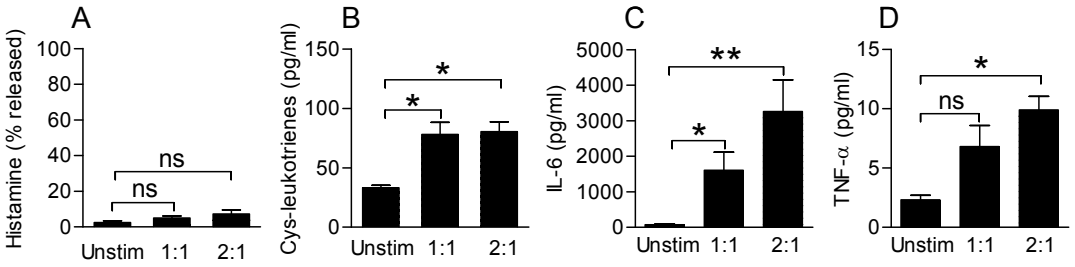

Figure 8. Mast cells respond to necrotic supernatant by generating a pro-inflammatory response. BMMCs treated with supernatant from fibroblasts rendered necrotic through cycles of freeze-thawing (**A**) do not release histamine, but secrete (**B**) cys-leukotrienes, (**C**) IL-6 and (**D**) TNF. Values are presented as mean ± SEM (n=5). *p<0.05, **p<0.01 in comparison to unstimulated BMMCs.

Since several exogenous danger signals are recognized by TLRs utilizing MyD88 as adaptor protein [207], we next investigated responses to necrotic supernatants in MyD88$^{-/-}$ BMMCs. Here, the IL-6 response to necrotic supernatant was completely absent (figure 9A), which prompted us to further pinpoint the upstream receptor by generating BMMCs from mice lacking various TLRs. However, BMMCs from all tested TLR knock-out mice generated comparable IL-6 responses to necrotic supernatant. T1/ST2 [208] is a receptor of the IL-1R family which also utilizes MyD88 as adaptor protein. When treating T1/ST2$^{-/-}$ BMMCs with necrotic supernatant, the IL-6

response was completely abolished (figure 9B), leading us to suspect the T1/ST2 ligand IL-33 to be important for mast cell-mediated cell injury recognition. This suspicion was strengthened when we could detect IL-33 in the necrotic supernatant by both Western blot and ELISA. In addition, we could also demonstrate that BMMC responses to necrotic supernatant are p38-dependent (figure 9C), which is in agreement with what is known regarding IL-33 signaling. Definite evidence for the importance of IL-33 in this system was provided when we generated necrotic supernatant from MEFs treated with small interfering-(si)RNA targeting IL-33, and found impaired IL-6 secretion in comparison to BMMCs subjected to necrotic supernatant from MEFs treated with non-targeting siRNA (figure 9D).

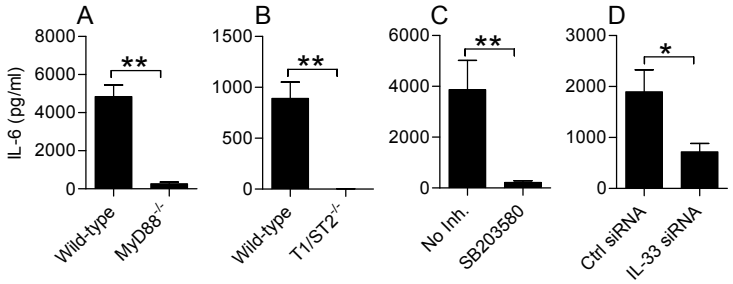

Figure 9. IL-33 in the necrotic supernatant activates mast cells through a MyD88-, T1/ST2- and p38-dependent pathway. (**A-B**) IL-6 release was determined in wild-type, MyD88$^{-/-}$ or T1/ST2$^{-/-}$ BMMCs after treatment with necrotic supernatant for 24 hours. (**C**) IL-6 release in BMMCs pre-treated or not with SB203580 before addition of necrotic supernatant. (**D**) IL-6 release in wild-type BMMCs stimulated with necrotic supernatant from fibroblasts treated with IL-33 siRNA or non-targeting control siRNA. Values are presented as mean ± SEM (n=5). *p<0.05, **p<0.01

We also investigated whether necrotic supernatant from other cell types than MEFs could elicit similar responses in BMMCs, and found that necrotic supernatants from many structural cell types, including keratinocytes, smooth muscle cells and neuronal cells induced IL-6 secretion by BMMCs. Correspondingly, these cell types also contained the highest IL-33 levels, leading us to the conclusion that IL-33 preferentially seems to be released from necrotic structural cells.

This paper describes a plausible mechanism by which mast cells might function as sensors of cell injury. Rather remarkably, responses to necrotic supernatant from MEFs were solely dependent upon IL-33 recognition. Interestingly, mast cells have been shown to be required for normal wound healing in mice, as wound closure is delayed in mast cell-deficient mice, but restored upon mast cell reconstitution [59]. Keeping this in mind, it is tempting to speculate that this impairment could be attributed to the lack of mast cells recognizing IL-33 released upon injury.

Taken together, we here provide evidence for an important role of mast cells in early responses to cell injury, and also provide additional evidence for the notion that IL-33 is an important endogenous danger signal.

2.3.3 Intraperitoneal influx of neutrophils in response to IL-33 is mast cell dependent (Paper III)

IL-33 is a recently described cytokine that induces Th2-responses and also functions as an endogenous danger signal. Several cell types, including mast cells, eosinophils and basophils are activated by IL-33, and IL-33 has been associated with the pathogenesis of several diseases [209]. Despite many studies investigating IL-33, much remains to be revealed. For instance, possible roles of IL-33 *in vivo* remain to be fully understood. Therefore, we here investigated responses to intraperitoneal injections of IL-33 in mice, with emphasis on mast cell involvement, as IL-33 is a potent regulator of mast cell activity.

Initially, we investigated peritoneal cells in untreated C57BL/6 mice, and found that approximately 1 % of the peritoneal cells were mast cells. All peritoneal mast cells were shown to be T1/ST2$^+$, and we could show that mast cells constitute about 75 % of the peritoneal T1/ST2$^+$ population, indicating mast cells to be well positioned and equipped to respond to IL-33. We next cultured peritoneal mast cells *in vitro* and monitored responses to IL-33 treatment. Here, we observed dose-dependent secretion of several cytokines and chemokines, including IL-6, TNF, MIP-2/CXCL2 and KC/CXCL1.

We next investigated cellular responses to intraperitoneal injections of IL-33 in C57BL/6 mice. Interestingly, we found that mice treated with IL-33 displayed remarkable peritoneal neutrophil infiltration compared to PBS-treated animals (figure 10A-B), while the amount of other cell types such as B-cells and macrophages remained unchanged after IL-33-treatment. In addition, we found neutrophil influx already 1 hour after IL-33 injection, suggesting an innate function for IL-33 in early neutrophil recruitment *in vivo*.

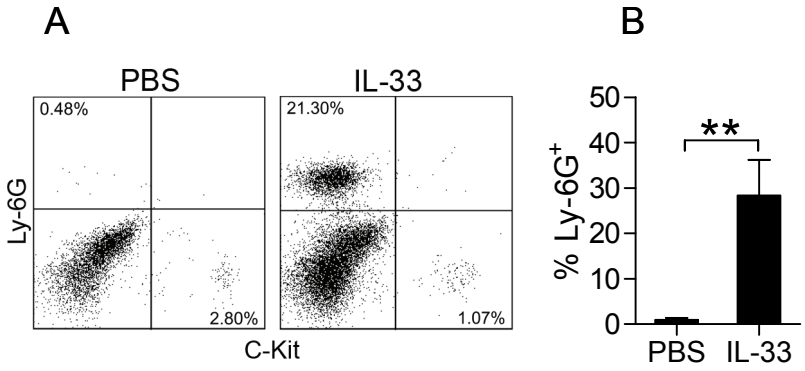

Figure 10. Intraperitoneal IL-33-administration results in neutrophil influx in C57BL/6 mice. Wild-type C57BL/6 mice were injected intraperitoneally with 0.1 µg IL-33. After 6 hours, peritoneal cells were retrieved and the cells were analyzed by flow cytometry. (**A**) Representative FACS plot, neutrophils are represented by a Ly-6G$^+$ population and mast cells by a C-Kit$^+$ population. (**B**) Quantification of neutrophils in the peritoneum. Results are presented as percentage of total peritoneal cells in peritoneal lavage obtained from injected mice. Values are presented as mean ± SEM (n=4-5) **p<0.01.

Given our earlier observation that IL-33 induces release of several pro-inflammatory mediators in peritoneal mast cells, we next investigated whether mast cells were involved in the observed neutrophil recruitment. To investigate this, we injected mast cell-deficient W^{sh}/W^{sh}-mice with IL-33, and found that these mice were unable to recruit neutrophils, indicating a possible role for mast cells in this recruitment mechanism. To strengthen this hypothesis, we observed that when W^{sh}/W^{sh}-mice were reconstituted with wild-type BMMCs, but not with T1/ST2$^{-/-}$ BMMCs, the neutrophil influx response was rescued, thus demonstrating the importance of T1/ST2-expressing mast cells in this system (figure 11A-B).

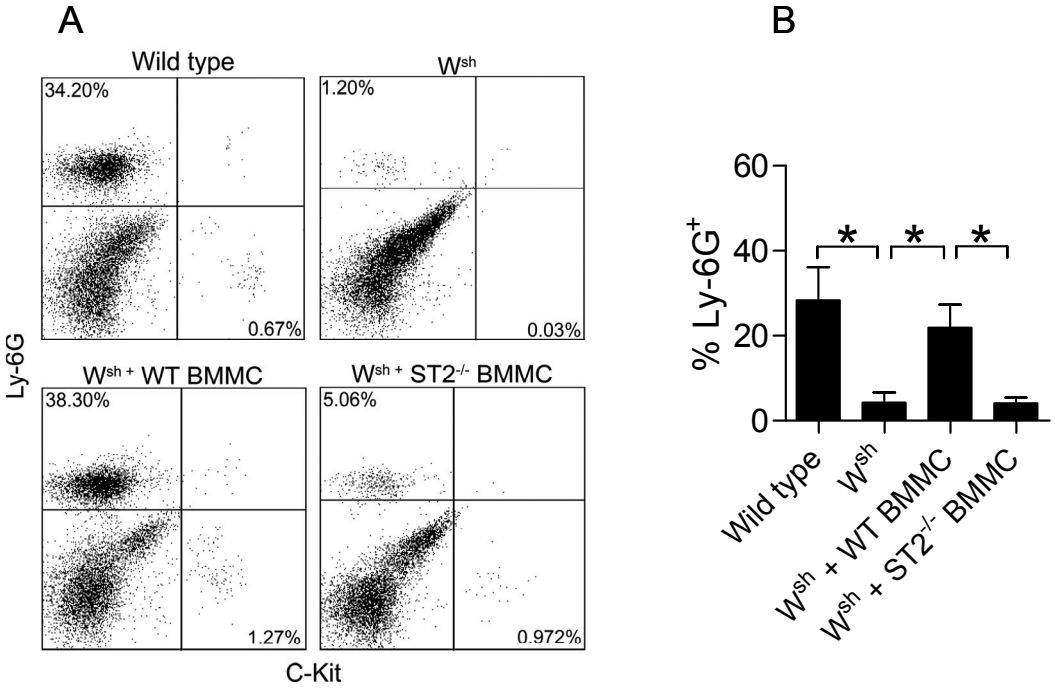

Figure 11. IL-33-induced neutrophil recruitment is mast cell dependent. (**A**) Flow cytometry plots of C-Kit^{+} mast cells and Ly-6G^{+} neutrophils from different groups of mice injected intraperitoneally with 0.1 µg IL-33. Representative plots are shown. (**B**) Quantification of (A). Values are presented as mean ± SEM (n=4-5). *p<0.05.

In this paper, we demonstrate that IL-33-induced neutrophil recruitment into the peritoneum is mast cell dependent. Recently, it was reported that mice injected with IL-33 prior to cecal ligation and puncture display more efficient bacterial clearance, increased neutrophil influx and improved survival compared to mice treated with PBS [168]. This suggests IL-33 to have a therapeutical role in conditions such as sepsis, and it is likely that IL-33 actions on mast cells contribute to detrimental as well as beneficial mechanisms in various diseases. In summary, our work in this paper provides further insights into IL-33 biology, and demonstrates that mast cells are important regulators of IL-33-induced innate immune responses.

2.3.4 Human mast cell responses to injured airway epithelial cells – implications for chronic airway inflammation (Paper IV)

Asthma is a chronic inflammatory disease of the airways characterized by Th2-skewed responses, airway hyper-reactivity and airway remodeling [124, 187]. Another characteristic feature of asthma is airway epithelial damage [210], which has been reported both in adults [211, 212] and children [213] with asthma. Here, the airway epithelial cells are more susceptible to damage, and fail to recover after injury [214], which results in a chronic state of wound healing responses [215]. Which cell type that responds to damaged airway epithelial cells, and the nature of such a response, is unclear however. Since we demonstrated in Paper II that mast cells can function as sensors of cell injury by recognizing IL-33 released by necrotic cells, we here explore the hypothesis that human mast cells can respond to damaged airway epithelial cells.

IL-33 expression has previously been reported in airway epithelial cells [216], and also in airway smooth muscle cells [124]. In line with this, we could detect IL-33 in human primary nasal epithelial cells (PNECs) and in the human airway epithelial cell lines A549 (lung epithelia), 9HTEo (tracheal epithelia) and 16HBE (bronchial epithelia) by immunohistochemistry. To investigate whether IL-33 would be released from necrotic airway epithelial cells, we rendered A549 and 9HTEo cells necrotic through cycles of freeze-thawing. As expected, we could detect IL-33 by ELISA and Western blot in the supernatant of the necrotic cells. In order to be able to respond to IL-33, mast cells need to express the IL-33 receptor, T1/ST2. Therefore, we next investigated T1/ST2 expression (surface and intracellular expression), in primary CBMCs, and also in the human mast cell lines HMC-1.1, HMC-1.2 and LAD2. In accordance with earlier studies [152, 153], we could detect only meager T1/ST2 expression on the surface of the investigated mast cells, while T1/ST2 expression was more pronounced intracellularly. We were unable to up-regulate surface T1/ST2 expression in CBMCs by using pro-inflammatory stimuli such as LPS, TNF or IL-1β, but we could observe a slightly increased T1/ST2 surface expression in all mast cell types if the cells were first incubated 24 hours with necrotic supernatant from A549 cells (figure 12), indicating that human mast cells respond to this stimulus.

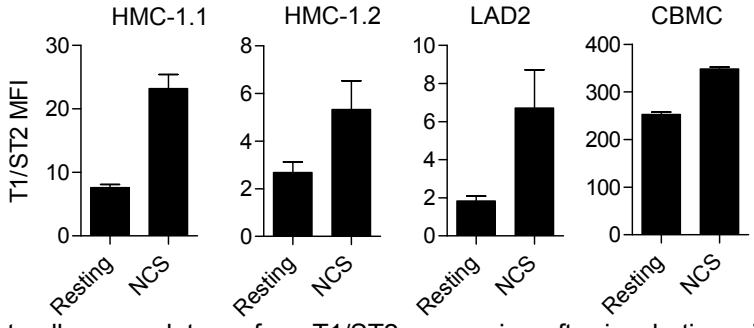

Figure 12. Mast cells upregulate surface T1/ST2 expression after incubation with necrotic cell supernatant. T1/ST2 surface expression was investigated in resting mast cells, or in mast cells treated with necrotic cell supernatant (NCS) for 24 hours. Values are shown as mean ± SEM (n=3). MFI= mean fluorescence intensity.

To further characterize this response, we incubated mast cells with A549 necrotic supernatant. In response to this, HMC-1.1, HMC-1.2 and LAD2 cells secreted IL-8/CXCL8, which we could also demonstrate in CBMCs. When we investigated CBMC responses to the necrotic supernatant more closely, we saw that the cells did not

release histamine, LTB_4 or PGD_2 upon stimulation. This suggests a selective mediator release in response to necrotic cell supernatant, as opposed to a full-scale mast cell activation as achieved for instance by FcεRI cross-linking. Interestingly, the same mediator release pattern was observed in CBMCs treated with either recombinant IL-33 or full length IL-33. Likewise, CBMCs treated with either necrotic supernatant or with IL-33 released IL-5, GM-CSF and TNF (figure 13), while no release of IL-1β, MCP-1/CCL2 or MIP-1α/CCL3 could be detected.

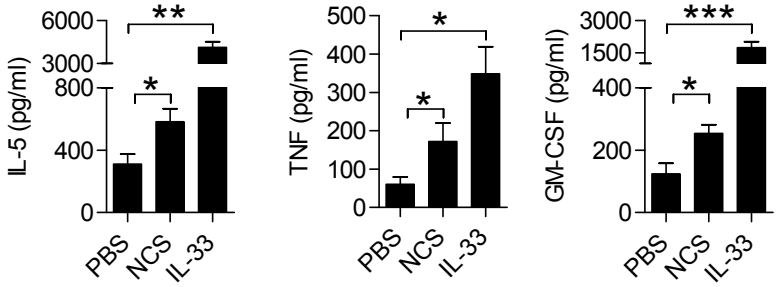

Figure 13. Human CBMCs treated with necrotic supernatant or IL-33 share mediator secretion patterns. CBMCs were treated with necrotic supernatant (NCS) or 10 ng/ml IL-33 for 24 hours and cytokine release was measured by Luminex. IL-5, GM-CSF and TNF was secreted in response to either NCS or IL-33, while no release of IL-1β, MCP-1/CCL2 or MIP-1α/CCL3 could be detected (data not shown). Values are shown as mean ± SEM (n=6-9). *p<0.05, **p<0.01, ***p<0.001 in comparison to PBS-treated cells.

In Paper II, we show that mast cell responses to cell injury are largely dependent on IL-33 released from necrotic structural cells. This was achieved by generating mast cells from various knock-out mice. Lacking this possibility in human cells, we were here unable to provide definite evidence for the involvement of IL-33 in this experimental setting. We attempted to block IL-33 activity in necrotic supernatant from A549 cells using either neutralizing antibodies or soluble ST2, but supernatant treated in these ways activated mast cells equally well as did necrotic supernatant treated with relevant controls. We did observe, however, that mast cells up-regulate T1/ST2 upon exposure to necrotic supernatant from airway epithelial cells, and that mast cells treated with necrotic supernatant or IL-33 share mediator release profiles. Consequently, future studies seeking to further investigate the roles of IL-33 as a danger signal in the context of damaged airway epithelia should aim at providing additional evidence supporting a role for IL-33 in this setting.

Taken together, our results support the hypothesis that mast cells can respond to injured airway epithelial cells, as we show that mast cells respond to necrotic supernatant from airway epithelial cells with cytokine and chemokine release, unaccompanied by histamine or eicosanoid release. When exposed to necrotic supernatant or IL-33, CBMCs respond by secreting IL-5, GM-CSF and TNF. Both IL-5 and GM-CSF are important in airway inflammation [217], where they for instance prolong eosinophil survival [210], and mast cell-derived TNF contributes to the production of Th2 cytokines in models of airway inflammation [218]. Such responses might be beneficial in some instances in order to promote tissue repair, but may on the other hand be detrimental in patients with chronic airway inflammations such as asthma. Here, a possible scenario would be that injured airway epithelia chronically releases factors such as IL-33, which mast cells react to and respond by secreting Th2 cytokines, thus participating in the maintenance of the chronic inflammation.

3 FUTURE PERSPECTIVES

The work presented in this thesis presents four main findings, which together have contributed to broaden the understanding of mast cell responses to danger signals. In Paper I, we show that human mast cells express NOD1 and are activated to induce pro-inflammatory responses if treated with NOD1 agonists. This demonstrates that mast cells contribute to innate immune responses also through NLRs. In Paper II, we show that mast cells can function as sensors of cell injury, here demonstrated by their ability to recognize IL-33 released from necrotic structural cells. This study highlights the role of mast cells in cell damage responses, and provides additional evidence for the importance of IL-33 as a danger signal. In Paper III, we found that intraperitoneal injections of IL-33 in mice results in rapid neutrophil recruitment through a mast cell dependent mechanism, demonstrating an important role for mast cells in early innate immune responses to IL-33. Finally, in Paper IV, we show that human mast cells play an important role in recognizing injured airway epithelial cells, and speculate that this might have important implications for chronic inflammation in conditions such as asthma. Taken together, the papers presented in this thesis provide additional knowledge regarding mast cells as important contributors to innate immune responses, further insights in the actions of IL-33 on mast cells, and additional evidence for the notion that IL-33 acts as an alarmin. In this section, I speculate on future perspectives for the research field addressed in this thesis.

The mast cell is unquestionably an interesting cell type to study. While for long considered only as an effector cell in allergic responses, the mast cell is now well established as an important sentinel cell of the innate immune system. However, there is much left to discover about the mast cell and its role in the immune system. In my opinion, one very important way to further advance our knowledge on mast cell actions is to develop better models of mast cell deficiency. For instance, if we could find ways to selectively turn on/off mast cells in a certain tissue/organ, we could pinpoint site-specific mast cell involvement in different immunological processes in greater detail. The most commonly used mouse models for mast cell deficiency (WBB6F1-Kit$^{W/Wv}$ and C57BL/6-Kit$^{W\text{-}sh/Wsh}$ mice are the most commonly used models) have undoubtedly helped tremendously in understanding mast cell functions. One major issue with these models, however, is that the mast cell deficiency depends on lack of Kit expression, which is expressed by several cell types, for instance hematopoietic stem cells [35]. This leads to alterations that may be unrelated to the lack of mast cells. For example; WBB6F1-Kit$^{W/Wv}$ mice display basopenia, while C57BL/6-Kit$^{W\text{-}sh/Wsh}$ mice instead display basophilia [219]. Recently, two novel Kit-independent mouse models of mast cell deficiency were published [34, 35]. Here, Dudeck *et al*. generated a mouse completely lacking connective tissue mast cells, and Feyerabend *et al*. generated mast cell-deficient mice by using targeted expression of Cre recombinase from the *Cpa3* locus. (It should be pointed out here that the latter of these mast cell-deficient mice also display reduced basophil counts.) It will be very interesting to see how such new models will be put to use in the future, and whether it will be necessary to re-evaluate results obtained in Kit-mutant mice. I would like to point out two cases where results from the new Kit-independent mast cell-deficient mouse models imply that re-evaluation of the role of mast cells in disease models is needed. It has been shown

previously, by reconstituting mast cell-deficient mice, that mast cells limit pathology in contact dermatitis [220]. In accordance with this, an enhanced contact hypersensitivity response was confirmed in WBB6F1-Kit$^{W/Wv}$ and C57BL/6-Kit$^{W\text{-}sh/Wsh}$ mice by Dudeck *et al*. [35]. However, the authors also found that contact hypersensitivity responses were drastically decreased in their Kit-independent model of mast cell deficiency, resulting in the conclusion that enhanced contact hypersensitivity responses in Kit-mutated mice is related to Kit deficiency, and not to mast cell deficiency. Secondly, Cpa3$^{Cre/+}$ mice and corresponding controls are equally susceptible to arthritis in a K/BxN serum transfer model, while WBB6F1-Kit$^{W/Wv}$ were more or less protected [34], thus challenging the impact of mast cells in this type of model. Similarly, it is also worth noting that WBB6F1-Kit$^{W/Wv}$ have been shown to be protected in a model of antibody-mediated arthritis, while C57BL/6-Kit$^{W\text{-}sh/Wsh}$ develop full arthritis [221].

One mast cell function I find particularly intriguing is the ability to recognize pathogens. In essence, mast cells are too well equipped and too strategically positioned not to play a major part in (many of) our defense systems, be it against bacteria, virus, fungi, parasites or trauma. So far, we know, thanks to revolutionary studies for instance by Echtenacher *et al.* [37] and Malaviya *et al.* [38], that mast cells are crucial for the defense against bacteria, and we also know that various TLR ligands can activate mast cells. We do not know equally much regarding the roles of NLRs in mast cells, however. For instance, there are very few studies investigating the inflammasome in mast cells, even through one study has demonstrated the expression of inflammasome components in response to pro-inflammatory stimuli [86]. Considering this lack of knowledge on NLRs and mast cells, it is interesting that we demonstrate in Paper I that human mast cells express NOD1, and that they can be activated to release pro-inflammatory mediators in response to NOD1 agonists. We found mRNA for NOD2 in CBMCs (unpublished observation), but to our surprise, the receptor was not expressed. Therefore, we could not detect any responses in CBMCs treated with the NOD2 agonist MDP. Similar results have been reported in another study [202]. One possibility that would be interesting to investigate is that NOD2 signaling induced by MDP-stimulation might require a so far uncharacterized co-factor, such as ATP, in order to assemble the receptor complex and mediate signaling. Also, NOD2^{+} mast cells seem to be over-represented in patients with Crohn's disease [87], possibly suggesting that the NOD2 receptor has a more pronounced role in patients with chronic diseases than in healthy people.

In addition to further exploring the role of NLRs in mast cells, many interesting future studies will describe how mast cells participate in defense mechanisms through CLR and RLR signaling. An especially interesting field is interactions between mast cells and viruses, of which we have just scratched the surface. For instance, it was only recently demonstrated that mast cell responses to virus involve intracellular antiviral pathways involving MDA5 and RIG-I [99]. In addition, mast cells are activated during dengue infection to secrete TNF, which was shown to trigger the activation of endothelial cells [43]. Infections with respiratory viruses (such as influenza) represent a major global health hazard today. Given that aerosols containing viruses are inhaled, mast cells, which are numerous in the airways, may well play important roles in virus recognition. On the other hand, I believe that mast cells could also be detrimental in this

setting. For instance, when virus-infected airway epithelial cells eventually die, released IL-33 recognized by mast cells might trigger responses contributing to maintained airway inflammation.

At present, many studies focus on *one* of the four classes of PRRs that are known today. I think, however, that to better understand PRRs in the future, studies should be focused on PRR cross-talk. This is because treating cells in culture with single, purified agonists of a given PRR poorly represents reality, in which cells constantly encounters a mixture of different triggers, thus rendering reality immensely more complex. Therefore, advances in the area of PRR cross-talk will be very important, and it will be interesting to see just how big impact mast cells have in this system. In Paper I, we made an attempt to investigate such a response, and could see that low doses of LPS could augment IL-8/CXCL8 secretion by M-TriDAP-treated CBMCs. In future studies seeking to shed light upon the role of mast cells in PRR cross-talk, it will be very important to remember that mast cells represent a very heterogenous population, and that mast cell responses in this regard likely will be context-dependent, and might thus vary greatly between tissues.

Even though many coming studies will teach us more about how mast cells function in the innate immune system, I think that the next decades of mast cell research will shift focus towards studying mast cell actions in, and fine-tuning of, adaptive immune responses. In 2005, Galli *et al.* named mast cells "tunable effectors" and "immuno-regulatory cells" [205], and I believe that this view will gain more ground in the coming years. To exemplify this, it has for instance been shown that mast cells are crucial for allograft tolerance, and that T_{regs} produce IL-9 to recruit mast cells to mediate immune suppression [222]. In addition, a recent study demonstrated that IL-2-producing mast cells expanded in the spleen after induction of chronic dermatitis, and that this suppressed dermatitis development by regulating the activated to regulatory T cell ratio in a model of oxazolone-induced allergic contact hypersensitivity [223].

Future studies investigating various danger signals will be as interesting as those targeting PRRs. Indeed, it is my belief that we will find endogenous ligands for many receptors that are today believed to only detect pathogen components, and vice versa. I find it very likely that many additional danger signals of both exogenous and endogenous origin will be discovered, and that we will learn more about those already discovered. For example, uric acid was discovered in 2003 as a danger signal heralding cell death [108], and was found in 2011 to be released in airways of allergen-challenged patients and necessary for Th2 immunity [224]. Likewise, it will be interesting to follow discoveries of novel danger signal sensors. For instance, since 2007, two new intracellular sensors of DNA have been discovered; DAI (DLM-1/ZBP1) [225] and DDX41 [226].

The effects of danger signals such as HMGB1, uric acid, IL-1α and heat-shock proteins on mast cells are surprisingly uninvestigated. While this is a field in which we have a lot to learn, this thesis does provide some information regarding mast cell responses towards exogenous danger signals (M-TriDAP, Paper I) as well as endogenous ones (IL-33, Papers II-IV). Regarding IL-33, is has been very exiting to follow the development of this "new" member of the IL-1 family of cytokines. Since IL-33 was

characterized by Schmitz *et al.* [120] in 2005, we have learned a lot about IL-33 biology, and now know that IL-33 truly represents one of immunology's (in)famous double-edged swords. IL-33 acts on the inside of cells as a nuclear factor, outside of cells as an alarmin, exacerbates inflammation and plays protective roles for instance in sepsis or during helminth infection. A PubMed search in January 2012 on IL-33 generates just over 300 hits. This means that, on average, roughly one new study on IL-33 has been published each week since Schmitz *et al.* characterized IL-33 in November 2005; a testament to the great interest and rapid development of this field. It has been very interesting to not only follow but also contribute to the development of this field, and I am looking forward to follow its further advances. However, while many new answers will be generated, I also think that we will need to revise some of the current beliefs regarding IL-33. For example, we already know that IL-33 is not activated through caspase-1-mediated proteolysis as was originally described [120]. Instead, full-length IL-33 is biologically active [142-144], and IL-33 is even inactivated by caspase-1 [142]. In addition, many studies have used an artificially truncated from of IL-33, and these studies might, potentially, have had other outcomes if full-length IL-33 had been used. The belief that IL-33 is (only) a pro-Th2 cytokine will probably also be challenged in future studies. Already now, it has been shown that IL-33 induced IFN-γ release in iNKT cells through an IL-12-dependent mechanism [227]. In addition, T1/ST2 was recently demonstrated to be expressed by type 1 cytotoxic T cells, which displayed enhanced T-cell receptor-triggered IFN-γ production upon IL-33 treatment [228]. Furthermore, IL-33 is so far the only described ligand for the T1/ST2-receptor. It will be interesting to see whether additional ligands will be discovered, and also whether IL-33 might signal through other receptors.

IL-33 processing has been, and still is, a matter of some controversy, and it is also unclear how IL-33 is secreted from cells. So far, a majority of studies have suggested necrosis to be the only way in which IL-33 is liberated from cells. However, one recent study has demonstrated IL-33 release from airway epithelial cells treated with common environmental aeroallergens [216]. This is especially interesting when keeping our results from Paper IV in mind, in which we suggest that mast cells responding to IL-33 released from injured airway epithelial cells might participate in the maintenance of chronic airway inflammation.

Rather surprisingly, it took some 3 years before IL-33 knock-out mice were generated [132]. This will be an invaluable tool for future studies seeking to investigate possible nuclear roles of IL-33, and it will also help to determine the role of IL-33 in various disease models. Since we suggest mast cells to be important sensors of cell injury though the recognition of IL-33 released from damaged structural cells (Paper II), it would also be interesting to study injury responses in IL-33$^{-/-}$ mice. Are responses to cell injury impaired if IL-33 is completely missing, or will other danger signals compensate for this deficiency?

To summarize my thoughts on IL-33, I believe that IL-33 is beneficial in small amounts, for instance in injury recognition (Paper II) and early innate immune responses such as neutrophil recruitment (Paper III), while chronic release of IL-33 might exacerbate various inflammatory disorders (for instance asthma, as suggested in Paper IV). Therefore, future therapeutics involving IL-33 will likely aim at sometimes

promoting and sometimes suppressing IL-33. If allowed to speculate, one possibility where it would be beneficial to promote IL-33, or administer it, is during helminth infections. In this scenario, IL-33 could promote Th2-responses and thus promote parasite expulsion. Another situation could be in cardiac care, where IL-33 administration possibly could promote the survival of transplanted hearts. A third possibility would be to administer IL-33 immediately after brain trauma to facilitate injury responses, or to give IL-33 to patients suffering from sepsis. On the other hand, several future drugs will probably attempt to counteract IL-33 actions. This might be beneficial in diseases characterized by chronic inflammation, such as asthma, RA and atopic dermatitis.

Lastly, it is with great interest that I will follow the field of IL-33 biology and see whether the accumulated knowledge on this cytokine will result in any therapeutical applications in the years to come. As one of the main cellular targets of IL-33, I believe that mast cells will be attributed pronounced roles in such future discoveries.

4 POPULÄRVETENSKAPLIG SAMMANFATTNING

Den här avhandlingen består av fyra delarbeten, i vilka jag och mina kollegor vid Karolinska Institutet studerat en celltyp som kallas för mastceller. Mastceller är en del av kroppens immunförsvar och finns utspridda över hela kroppen. De är extra många på ställen som kommer i kontakt med omgivningen, som till exempel huden och lungorna.

För många människor ställer mastcellerna till en mängd bekymmer eftersom de kan sätta igång allergiska reaktioner och astma. Både astma och allergi är vanligt förekommande i Sverige. Enligt Astma- och Allergiförbundet har ungefär 10 % av Sveriges vuxna befolkning astma, och ungefär var femte vuxen har någon form av allergiska besvär. När en person med tendenser att till exempel utveckla pollenallergi kommer i kontakt med pollen för första gången, bildar kroppen så kallade IgE-antikroppar mot pollenet. Dessa antikroppar fastnar sedan på ytan av mastceller i olika delar av kroppen, till exempel i luftvägarna. Nästa gång personen i fråga kommer i kontakt med björkpollen känner mastcellerna igen det, eftersom de nu har antikroppar mot pollen på sin yta. Mastcellerna reagerar nu på björkpollenet med att släppa ut en mängd substanser i kroppen. Dessa substanser orsakar tillsammans allergiska symptom, som rinnande näsa och ögon, klåda och andningssvårigheter. I vissa fall kan den allergiska reaktionen till och med bli så pass kraftig att den drabbade avlider. Jordnötsallergi är ett exempel på en allergi som kan ge en sådan kraftig reaktion.

Man kan undra varför vi egentligen har mastceller i kroppen? Intressant nog så finns det faktiskt många fördelar med mastceller, något som forskningsvärlden har börjat inse under de senaste femton åren. En studie som gjordes 1996 av en tysk forskargrupp visade att möss som helt saknar mastceller är mycket känsligare mot bakterieinfektioner än möss med normalt antal mastceller. Detta var det första beviset på att mastcellerna spelar en viktig roll för vår förmåga att försvara oss mot bakterier. På senare år har ett flertal andra studier visat att mastcellerna inte bara är viktiga för kroppens förmåga att försvara sig mot bakterier, utan även mot till exempel virus- och svampinfektioner. Eftersom mastcellerna finns på ställen i kroppen där bakterier och andra inkräktare kan ta sig in, till exempel i huden och i olika slemhinnor, är mastcellerna ofta den första celltypen i kroppen som kommer i kontakt med olika inkräktare. Man kan säga att mastcellerna fungerar som vaktposter, vilka snabbt kan sätta igång processer som leder till att bakterieinfektioner kan upptäckas och stoppas.

För att bättre förstå hur mastcellerna bidrar till vårt immunförsvar, måste man veta hur de gör för att känna igen (och reagera på) bakterier och andra inkräktare. Syftet med den här avhandlingen är därför att ta reda på mer om hur mastceller gör just detta. Mer specifikt handlar den om ifall mastceller kan bli aktiverade av så kallade varningssignaler. Det finns två olika typer av varningssignaler: varningssignaler som kommer från omvärlden, till exempel delar av bakterier och virus, och varningssignaler som kommer från vår egen kropp. Om en cell i kroppen blir skadad kan det hända att den dör. Det som händer då är att den döda cellens innehåll läcker ut och kommer i kontakt med de friska cellerna. De friska cellerna kan i vissa fall reagera på att en skada har inträffat genom att känna igen varningssignaler som kommer från den döda cellen.

Detta är ett första steg i att reparera skadan eftersom det gör att kroppen kan lokalisera skadan och starta olika typer av läkningsprocesser.

I det första delarbetet undersökte jag och mina kollegor om mastceller kan upptäcka bakterieinfektioner genom att använda en sorts sensor som heter NOD1. När immunförsvarets celler upptäcker bakterier och andra mikroorganismer använder de sig ofta av sensorer som sitter på ytan av cellen, eller i vissa fall finns på cellens insida. NOD1 tillhör en ganska nyligen upptäckt grupp av sensorer som finns på cellers insida och man vet inte så mycket om den ännu. Därför började vi med grundläggande frågeställningar som huruvida NOD1 finns i mastceller. När vi bekräftat att mastceller har NOD1 var nästa steg att se vilken funktion NOD1 har i mastceller. Vi behandlade mastceller med en liten bakteriedel som heter M-TriDAP, vilken är känd för att identifieras av NOD1. När vi gjorde detta kunde vi se att mastcellerna reagerade med att utsöndra flera olika ämnen som orsakar inflammation. Vi såg även att man kunde få mastcellerna att utsöndra högre halter av inflammationsämnen om vi behandlade med M-TriDAP och andra bakteriedelar samtidigt. Den här studien tillför ytterligare bevis för att mastceller är viktiga för att känna igen bakterieinfektioner.

I den andra studien undersökte vi om mastceller kan bli aktiverade av (det vill säga reagera på) innehåll som läcker ut från döende celler, och på det viset känna igen en cellskada. Till att börja med kunde vi se att mastcellerna aktiverades, när de behandlades med en blandning av komponenter från döda celler. För att ta reda på vilken komponent i innehållet från de döda cellerna som aktiverar mastceller, använde vi oss av olika genetiskt modifierade möss, som saknar olika typer av sensorer. När vi tog mastceller från en sådan typ av mus som saknar sensorn T1/ST2, såg vi att komponenter från döda celler inte längre kunde aktivera mastcellerna. T1/ST2 känner normalt sett igen en molekyl som heter IL-33, och vi kunde nu påvisa att döende celler släpper ut IL-33 vilket aktiverar mastceller. Mastcellerna släpper då ut substanser som är viktiga för att kroppen ska kunna starta en inflammation, och på så sätt påbörja en läkningsprocess. Den här studien beskriver en delvis ny roll för mastcellen, nämligen att känna igen en cellskada. Studien visar även att IL-33 är en viktig molekyl som frisätts av döende celler, och därmed fungerar som en varningssignal.

I det tredje delarbetet fortsatte vi att studera IL-33. Detta är en ganska nyligen upptäckt molekyl, och man vet ännu inte så mycket om hur den fungerar inne i kroppen. För att ta reda på mer om detta undersökte vi vad som händer när man injicerar IL-33 i bukhålan på möss. Redan någon timme efter själva injektionen kunde vi se att neutrofiler, en typ av vita blodkroppar, ansamlades i bukhålan, ”ditlockade” av IL-33. (En ansamling av neutrofiler är vanligtvis ett tecken på att en inflammation startats i kroppen.) Detta experiment utfördes även på möss som helt saknar mastceller; då såg vi ingen ansamlig av neutrofiler. Detta tyder på att det är mastceller, vilka känner igen och reagerar på IL-33, som är ansvariga för att locka till sig neutrofiler, och därmed starta en inflammation. Detta delarbete visar därmed att mastceller även känner igen IL-33 inne i kroppen, och att de är mycket viktiga rekryterare av neutrofiler vid inflammation.

I det fjärde delarbetet studerade vi en karakteristisk egenskap för sjukdomen astma, nämligen att celler i luftvägarna skadas under den långvariga inflammation som astma innebär. I delarbete II visade vi att skadade celler släpper ut IL-33, vilket aktiverar

mastceller. Många tidigare studier har visat att IL-33 finns inne i celler längs luftvägarna. Vi började därför med att döda olika typer av luftvägsepitelceller, och kunde då se att de mycket riktigt släppte ut IL-33. När vi sedan behandlade olika varianter av humana mastceller med komponenter utsläppta av döende epitelceller kunde vi se att de aktiverades av detta, och släppte ut flera olika komponenter som kan starta en inflammation. Intressant nog släppte mastcellerna ut samma typer av komponenter om de enbart behandlades med IL-33, vilket tyder på att IL-33 som släpps ut från döende celler även kan ha en viktig funktion som varningssignal i människor. Dessa upptäckter kan vara av speciellt stor vikt för att i framtiden bättre kunna behandla astma. Astma karakteriseras av en kronisk inflammation i luftvägarna, vilket leder till bland annat skada på epitelcellerna. Dessa celler skulle då, hypotetiskt sett, släppa ut IL-33 vilket i sin tur skulle leda till att mastceller i luftvägarna släpper ut komponenter som upprätthåller inflammationen i luftvägarna.

Sammanfattningsvis bidrar studierna i den här avhandlingen till en bättre förståelse för hur mastceller deltar i kroppens försvarssystem, och bidrar också med information om hur IL-33 kan fungera som en varningssignal som släpps ut av skadade celler.

I delarbete II och III har djurförsök använts. De experiment som innefattat djurförsök har genomgått etisk granskning av Stockholms norra djurförsöksetiska nämnd, och godkänts.

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