INTRODUCTION

Chapter 1

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The immune system protects the body against pathogens by recognizing dangerous invasive organisms and subsequently eliminating them. Different parts of the immune system cooperate to achieve this, including the innate immune system, which is able to recognize pathogens based on repetitive signals associated with danger, and the adaptive immune system, which is able to improve recognition over time and to memorize this recognition (*1*). Mast cells and basophils are immune cells mainly considered as part of the innate immune system, although recent evidence has suggested they might be at the basis of certain adaptive immune responses as well.

MAST CELL DISCOVERY

Mast cells were discovered more than a century ago, by Paul Erlich, who based his discoveries on immunohistochemical studies of "Mastzellen", cells in connective tissue containing granules which stained with basic aniline dyes, and supposedly had a feeding or nutritional function. He used similar techniques to describe granulocytes in blood, and discovered basophils based on their staining with basophilic dyes. In the 1950's, these granules were found to contain histamine, and thus, mast cells and basophils were hypothesized to participate in allergic reactions by releasing their granular contents (*2*).

Mast cell granules contain several mediators besides histamine, including the enzymes tryptase and chymase, discovered in the 1980's. In both human and mouse, these enzymes have a heterogeneous expression pattern, allowing for the identification of two main mast cell subsets in different tissues. In humans, mucosal mast cells express only tryptase (MC_T), whereas skin and submucosal tissue mast cell granules contain both tryptase and chymase (MC_{TC}).

As mast cell granules contain several mediators that could cause wheal-and-flare reactions when administered locally or anaphylactic shock when administered systemically, mast cells were long considered in the context of these allergic reactions. However, the physiological role of mast cells was not clear, and it was questioned why these cells with potent detrimental effects to the host existed without obvious benefits in terms of physiological function. It was not until the last two decades, that our perception of mast cell and basophil function has been broadened to other immune responses as well, and now, these cell types are thought to play a role in various immune responses, ranging from acute to chronic and from innate to adaptive immune responses.

MAST CELL MEDIATORS

Besides releasing their granule content, mast cells can release several other mediators, including membrane-derived lipid mediators, and cytokines and chemokines which are produced *de novo* (Figure 1).

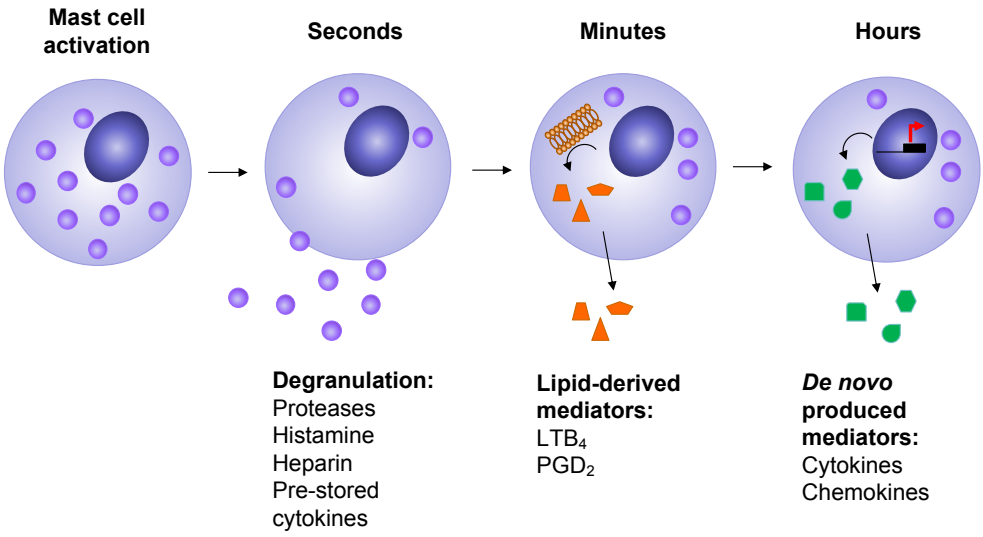

Figure 1: Mediator release by mast cells. Upon activation, mast cells have the ability to degranulate within seconds, thereby releasing pre-formed molecules residing in their granules. Lipid mediators, derived from fatty acids in membranes through arachidonic acid, are synthesized through enzymatic reactions inside the cell and can be released within minutes after activation. De novo transcription and translation leads to secretion of cytokines and chemokines within hours after activation.

GRANULE MEDIATORS

Mast cell granules are structures within the cell which contain inflammatory mediators, which can be released rapidly (within seconds) upon activation of the cell. The granule structure serves mainly two functions: First of all, the granules contents contain very high concentrations of mediators allowing their rapid release into extracellular space. Second, these mediators are often destructive to the intracellular environment (such as proteolytic enzymes), and therefore need to be contained in a separate structure. They are initially produced as inactive precursor proteins (pre-pro-enzymes), after which they are processed through the ER-golgi pathway. Once they enter the granule, they are cleaved into the active protein. This is important, as it allows for immediate proteolytic activity in the affected tissue, and makes the mast cell response so potent.

The granule-resident mediators can be divided into different functional groups. As mentioned, histamine is an important mast cell mediator associated with allergic symptoms. Four different histamine receptors exist, which each lead to different downstream effects, including increased vascular permeability and chemotaxis, each enhancing influx of immune cells into the affected tissue.

Next, proteases such as tryptase, chymase (both serine proteases) and carboxypeptidase (zinc-containing metalloprotease) are the most abundantly expressed proteins in mast cells, constituting 25-50% of the total proteins (*3, 4*). Release of these proteases can lead to pro- and anti-inflammatory effects, depending on the extracellular substrates that are being cleaved. For, example, these proteases can cleave pro-inflammatory cytokines into active forms, or in contrast, degrade pro-inflammatory cytokines or other molecules (*5*). Furthermore, they can degrade toxic substance, such as derived from snake and bee venoms or endogenous toxins released upon injury (see below).

Proteoglycans are another major constituent of granules. These are mainly serglycin proteoglycans that contain heparin and/or chondroitin sulfate chains. They form complexes with mast cell proteases in the granules, which is essential for correct storage and release of these proteases (*6*). The heparin which is released upon degranulation also serves as anti-coagulant.

A fourth type of mediator which has been suggested to be present in granules are cytokines, such as TNF-α (*7*). However, it is not clearly established whether human mast cell granules also contain cytokines, in particular TNF-α.

LIPID-DERIVED MEDIATORS

The second wave of mediators released by mast cells consists of eicosanoids, or arachidonic acid metabolites, which are released within minutes after activation. Arachidonic acid is released from membrane phospholipids by phospholipase A2, and subsequently converted to leukotrienes and prostaglandins by arachidonate 5-lipoxygenase (5-LO) and cyclooxygenases (COX) enzymes respectively. Leukotrienes induce bronchoconstriction, increased vascular permeability and neutrophil chemotaxis, and can induce anaphylactic shock when present in excess. Prostaglandins, and in particular PGD_2 released by mast cells, leads to recruitment of Th2 cells, eosinophils, and basophils, as well as vasodilation and bronchoconstriction.

Together, these early mast cell-derived molecules induce an early inflammatory reaction, allowing other immune cells to enter the tissue.

CYTOKINES AND CHEMOKINES

Upon activation, transcription factors induce *de novo* transcription and production of several molecules, including secretion of cytokines and chemokines. The production and release of these molecules takes several hours, and this response is therefore sometimes referred to as the late phase response, especially in the context of allergic inflammation. Although the release of pre-formed and lipid derived mediators leads to general inflammation, the release of cytokines and chemokines can lead to more versatile functions, as different mast cell triggers can induce the differential production of cytokines, probably through inducing different transcription factors.

Mast cells can produce cytokines affecting many different cell types. For example, several cytokines are most known for their effects on stromal or parenchymal cells. Likewise, the effect of mast cell-derived IL-13 on non-immune cells can lead to parasite expulsion through increased mucous production, epithelial cell turnover, and contraction by intestinal muscle. Mast cell-derived cytokines can also affect various immune cells, both from innate and adaptive immune system, as discussed below. These include IL-3, which can activate T cells, IL-5, which can recruit and activate eosinophils, and GM-CSF, which can affect neutrophil and monocyte expansion and function.

Chemokines produced by mast cells lead to recruitment of different cell types, with neutrophils and other granulocytes as main responder cell types being attracted to the site of injury by mast cell-derived chemokines, such as IL-5, IL-8, MIP1α (CCL3), TNF-α. However, these and other chemokines, such as CCL2/MCP-1, can also attract T cells, monocytes and natural killer cells. This suggests that the mast cell chemokine response can lead to potent influx of a variety of other immune cells.

MAST CELL MEDIATORS AS IMMUNOSUPPRESSORS

In contrast to the pro-inflammatory role that mast cells play during injury and infection, mast cell derived cytokines and granule-derived mediators can also induce regulatory responses. Examples of such cytokines are IL-10 and TGFβ. In particular mast cell-derived IL-10 has been shown to be involved in immune regulation (*8-10*).

In addition, mast cell proteases can cleave proinflammatory cytokines, and thereby limit inflammation. Many cytokines can be cleaved by chymase (at least in the mouse), sometimes leading to inactivation of the cytokines and alarmins, such as IL-3, IL-6, SCF, HSP70 and IL-33 (*5, 11-13*). Therefore, the response of mast cells can modulate the immune system towards anti-inflammatory responses as well.

MAST CELL ACTIVATION

The most well-known receptor for activation of mast cells and basophils is the high affinity receptor for IgE, FcεRI. Due to its high affinity, IgE from the circulation is constantly bound to the cell surface, and upon antigen recognition, this receptor is crosslinked causing the full-blown mast cell response characterized by degranulation and production of lipid derived mediators, cytokines and chemokines. As the IgE is already bound to the receptor, minute amounts of antigen are needed for activation, and activation can occur within seconds after antigen recognition. Therefore, this is a very potent mast cell response.

Several other pathways for mast cell activation have been described. These include Fcγ receptors, complement receptors and innate receptors, such as Toll like receptors. In addition, mast cells can be activated by various cytokines or growth factors, which often influence their proliferation as well.

APPROACHES TO STUDY MAST CELL FUNCTION

Human

Mast cells are not present in blood, and are therefore a difficult cell type to obtain for functional studies. Several mast cell-lines exist, such as LAD-2 and HMC-1, however, each of these have several molecular abnormalities compared to tissue mast cells, including low expression of FcεRI, dysfunction of ckit (receptor for stem cell factor; SCF), and lack of granules. These features constitute a limitation of studies employing these cell lines. Therefore, most studies of mast cell function in the human rely on in vitro expansion of stem cells or tissue mast cells, such as using hematopoietic stem cells from peripheral or cord blood. Although some discrepancies have been described, these have been shown to closely resemble tissue mast cells in most characteristics, such as granule constituents, expression of FcεRI and degranulation.

In vivo approaches in humans include immunohistochemistry or measurement of mast cell specific mediators in serum or other body fluids. Although useful, these studies can merely be used for descriptive studies. Due to the low frequency of mast cells in most tissues, characterization of tissue mast cells in humans is still difficult, but new technologies, such as next generation sequencing, are likely to give us more insight into the exact function of mast cells in different tissues.

Mouse

In the mouse, several in vivo models for mast cell function are being used. The most frequently used is the so-called mast cell knockin model, using the $kit^{W/Wv}$ or $kit^{Wsh/sh}$ mice which display profound mast cell-deficiency. However, the mutation in kit in these mice

affects some other cell types as well. This includes neutropenia observed in $kit^{W/Wv}$ mice and neutrophilia in $kit^{Wsh/sh}$ mice (*14, 15*). Therefore, mast cell reconstitution using mast cells derived from bone marrow of wildtype mice is usually required to confirm that the phenotype is mast cell-specific.

In the last years, several novel mouse strains have been developed which target mast cell molecules, including carboxypeptidase A3 (cpa3), and mast cell proteases (such as Mcpt5) (*16-19*). Several findings using kit mutant mice with knockin mast cells, have been recently challenged using the more specific mast cell deficient mice (*15, 17, 20*). Therefore, these new mast cell deficient mouse models might change the paradigms on the contribution of mast cells to various immune responses.

Differences between mouse and human mast cells

It is important to note that mouse mast cells and human mast cells differ considerably. Their constitution of granules is different, as in humans there is only one chymase gene, whereas in mice, several different chymases can be expressed at the same time (*21*). Their origin and development also differs considerable: in humans the proliferation and differentiation is thought to mainly depend on SCF, whereas in mice, IL-3 alone can induce differentiation and proliferation of mast cells from stem cells (*22*). Also, the expression of activating receptors such as TLR and FcγR might differ between these species.

Importantly, several cytokines which have been shown to be crucial for certain mast cell functions in the mouse (TNF-α and IL-4) were not found in human mast cells (*22, 23*). So, although mouse studies are very important to obtain understanding of mast cell function in vivo, caution needs to be applied for extrapolation of these results to human conditions. As it is difficult and expensive to obtain functional human mast cells, there is a lack of translation of the findings in mice to human disease, and this is therefore an important area of research.

PHYSIOLOGICAL FUNCTIONS OF MAST CELLS

The presence and homology of mast cells between different species suggests that they are essential to our survival. In support of this notion, mast cell-deficient persons have not been identified. Mast cells are located in strategic locations where they can encounter pathogens upon entry of the body. The following paragraphs describe the protective immune responses in which mast cells play an important role.

THE ROLE OF MAST CELLS IN INNATE IMMUNE RESPONSES

Venoms

A physiological role for mast cell degranulation has recently emerged, hypothesizing that such an immune response has evolved as a protective mechanism against venoms which need to be eradicated in a quick manner. Besides direct toxicity of venom contents, venoms can induce toxic endogenous (neuro-) peptides, such as endothelin-1, neurotensin and vasoactive intestinal polypeptide (VIP) (*24-26*). Granule-derived mast cell enzymes have been shown to detoxify or degrade several venom-derived toxins as well as endogenous peptides produced in response to venoms (*26-28*), giving protection to the host (Figure 2).

Venom-induced mast cell degranulation is usually triggered by innate recognition, but their receptors are not known. Nonetheless, as venoms resemble neuropeptides, they might act on mast cells through similar G protein coupled receptors. Besides activation of mast cells via innate receptors, a recent study has demonstrated that IgE memory can also contribute to venom-induced mast cell activation (*29*). In this mouse model, IgE was produced after sensitization with bee venom, which later mediated resistance to a lethal dose of the same venom in a mast cell-dependent manner.

These mast cell responses to venoms are a good example on how immune responses need to be tightly balanced. As described above, both innate and IgE-mediated responses can contribute to protection against these dangerous venoms through release of mast cell proteases. However, uncontrolled mast cell degranulation in allergic individuals can lead to anaphylaxis such as during severe reactions to bee and wasp stings in sensitized individuals.

Parasites

Another type of innate immune response in which mast cells have been shown to play a role is the protection against various parasites, in particular intestinal helminths. These are multicellular pathogens, and can therefore not be controlled or eliminated by traditional immune responses such as phagocytosis or cytotoxicity. Helminth parasites are well-known for their induction of Th2 immune responses (*30*), but they also have several immune evasion strategies, including upregulation of Tregs and anti-inflammatory cytokines such as IL-10 (*31, 32*).

Parasites trigger TLRs and other pattern recognition receptors, generally leading to Th2 responses (*33-37*). Due to their Th2-skewing properties, parasitic infection often promote production of both total and parasite-specific IgE.(*38-40*) In addition, the helminth parasite Schistosoma mansoni has been shown to directly crosslink non-specific IgE/FcεRI in an antigen-independent manner (*41*). The contribution of IgE antibodies or FcεRI to protective

immunity is not completely clear, and seems to depend on the type of parasites and the site of infection (*42-44*).

Mast cells are also thought to contribute to immunity during primary helminth infections in mice (*45, 46*). The mast cell effector molecules during protective responses against parasites are IL-4, IL-13, TNF-α, histamine and mast cell proteases, such as mouse MCP-1, -2 and -6 (Figure 2) (*47-50*). These cytokines play different roles; IL-13 specifically has been suggested to induce goblet cell hyperplasia, causing increased mucus production, leading to trapping of parasites in the mucus as well as enhancing expulsion of the parasites (*51*). Mast cell proteases can enhance intestinal permeability, thereby directly contributing to parasite expulsion (*46, 52*). The other cytokines and proteases are mainly thought to contribute to intestinal inflammation, for example through recruitment of eosinophils (*49*). Although most of these cytokines are also produced by Th2 cells, IL-4 and IL-13 derived from innate cells was shown to be most important for worm clearance during infection with N Brasiliensis (*51*).

Recent data suggest a non-redundant role for basophils in the context of secondary parasite infection. Basophils were shown to be important in the secondary immune response against diverse intestinal helminthes (N. brasiliensis, H. polygyrus, T. muris) and ticks, probably by activation through parasite-specific IgE (*44, 53, 54*).

Together, these observations suggests that whereas mast cells are potent effector cells to prevent parasites from entering the body in a primary infection, basophils may be more potent during IgE-dependent secondary responses against parasites.

Bacteria

The first study to show an important role for mast cells in protection against bacteria originated in 1996, when mast cell-derived TNF-α was shown to confer protection against *E. Coli* (*55*). Several studies have since then shown a protective role of mast cells against various bacteria, including *K. pneumoniae*, *M. pneumoniae*, and *C difficile* (*56-59*). In most of these models, the crucial mechanism of mast cell-mediated protection is recruitment of immune effector cells, mainly neutrophils (Figure 2). Due to their ability to rapidly degranulate, mast cells are thought to be the first cell to initiate an inflammatory response upon bacterial invasion of a tissue. Such an acute response is characterized by increased vascular permeability mediated by histamine and proteases, and recruitment of effector cells by release of lipid-derived mediators (LTB4) and cytokines or chemokines present in granules (TNF) (*7, 60*). This early response is important, as mast cell-deficient mice have a wider spread of bacterial infection, suggesting that mast cells can contain infections to a local tissue, such as lung or skin (*55, 56, 61*).

In addition to their role in acute infection, mast cell may initiate adaptive immune responses to bacteria as well. Mast cell derived TNF was shown to regulate recruitment of T cells to draining lymph nodes during intradermal infection with *E. Coli* (*62*). Furthermore, DCs were recruited to the infected tissue by mast cells, followed by their migration to draining lymph nodes (*63, 64*). Therefore, mast cells may orchestrate T cell activation during bacterial infection by regulating the migration and activation of both T cells and antigen presenting cells.

Different receptors have been shown to contribute to mast cell activation during bacterial infections, including complement receptors (CR1, CR2) and TLR4 (*65-67*). In addition, bacterial toxins may directly activate mast cells through yet unknown receptors (*68*). The exact receptors inducing mast cell degranulation upon bacterial infection are not known, but can consist of a variety of bacterial products and endogenous ligands released upon invasion of the body.

Mast cells are not crucial for protection against all bacteria strains. However, a recent study suggested that this may originate from evasion strategies employed by bacteria to prevent mast cell activation. At least two bacterial strains, Salmonella Typhimurium and Yersinia Pestis, were capable of such mast cell inhibition (*69*).

These studies show that mast cell play a non-redundant role in protection against a variety of bacteria, although some bacteria have developed immune evasion strategies.

Other pathogens

Although fewer studies have been performed evaluating the role of mast cells to viruses and fungi, some studies suggest they can also contribute to protective immunity against these pathogens. For example, mast cells were shown to induce migration and activation of $CD8^+$ T cells and NK cells in the context of viral infections (*70-75*).

Not much is known about the role of mast cells in fungal infections, but the overlapping mechanisms in protection against bacteria, parasites and fungi (such as TLR recognition), suggest that mast cells potentially play a role against fungi as well (*76*).

THE ROLE OF MAST CELLS IN TISSUE HOMEOSTASIS

Mast cells are usually associated with acute responses due to their potent degranulation mechanism; however, they may be an important effector cell in the context of tissue homeostasis. Several findings implicate a role for mast cells in tissue remodeling. Mast cell numbers are often associated with tissue remodeling processes, such as during scarring and fibrosis and pathologic conditions such as bullous pemphigus and scleroderma (*77-79*).

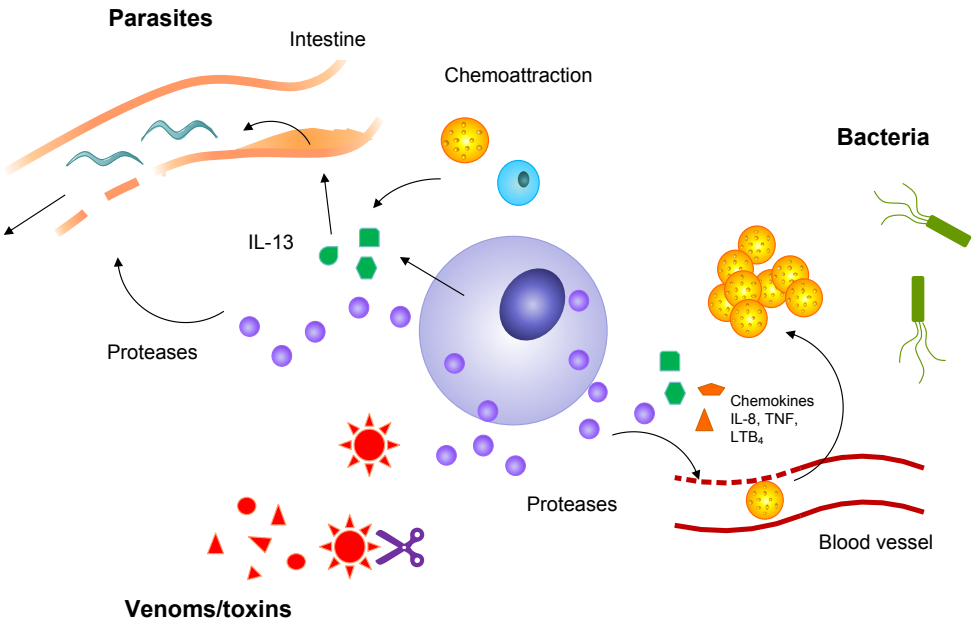

Figure 2: The role of mast cells in protective immunity. Mast cells have been shown to contribute to protection against a variety of pathogens. During parasitic infection, mast cell-derived proteases can lead to intestinal permeability. Cytokines contribute to recruitment of eosinophils and other granulocytes to the site of parasitic infection, and IL-13 specifically can induce the production of mucous; together these processes contribute to expulsion of intestinal parasites. Upon exposure to venoms or toxins, mast cell-derived proteases can degrade or detoxify these molecules, thereby protecting the host. Upon bacterial infection, tissue-resident mast cells are one of the first cell types to respond by releasing proteases, lipid mediators and chemokines. These mast cell-derived molecules increase vascular permeability and recruitment of immune effector cells, including neutrophils.

In addition, mast cells can produce a variety of mediators involved in tissue remodeling, including proteases which can activate several matrix metalloproteinases (MMPs), thereby contributing to breakdown of extracellular matrix proteins (*80, 81*). Mast cells can activate fibroblasts through various growth factors and cytokines and induce TGFβ-dependent collagen production by fibroblasts through a variety of mechanisms (*82-85*). These studies indicate that mast cells can contribute to extracellular matrix turnover by both production and breakdown of extracellular matrix constituents.

Mast cells were also thought to contribute to wound healing and fibrosis in the skin. Mast cells accumulate at the site of skin injury, and some studies using kit-mutant mice showed a functional role for mast cells in fibrosis (*78, 86*). However, some studies using kit-independent mast cell-deficient mice have recently challenged the role of mast cells

in wound healing and fibrosis in the skin (*87*), indicating that more research is needed to understand the role of mast cells in tissue remodeling (*87-90*). Furthermore, most studies have only addressed the role of mast cells during acute wound healing responses, whereas tissue remodeling during chronic inflammatory responses has only been sparsely studied. As mast cell-mediated tissue remodeling could be detrimental in the context of chronic inflammation, this is an important area of research.

THE ROLE OF MAST CELLS IN PATHOGENIC PROCESSES

Although the physiological role of mast cells and basophils is being more acknowledged in recent years, and as it is now generally accepted that these cells play important roles in the first line of defense against a number of pathogens, the route that mediates the recognition of pathogens, is not firmly established. For example, there is a lack of knowledge concerning expression of Toll-like receptors and the specific response that is generated by ligands for these receptors, especially in human mast cells and basophils. Activation through such receptors is important, not only for their role in protective immunity, but also during pathogenic processes, as described below.

MAST CELLS IN ALLERGIC REACTIONS

Mast cells are classically associated with allergic responses, in particular type I hypersensitivity responses, related to IgE. Allergic reactions consist of two phases, the early and late reaction, of which the exact symptoms depend on the location where the body comes in contact with the allergen. The early reaction in the lungs is characterized by bronchoconstriction, edema and mucus production, whereas in the skin it leads to redness, edema and itching. The late phase reaction in all tissues is associated with influx of eosinophils, other granulocytes and lymphocytes. Basophils and mast cells are the main cell types expressing FcεRI, but because mast cells are already present in tissues where allergens are first encountered, and basophils need to be recruited from the blood, mast cells are usually considered as the main cell type in the early phase reaction. Their mediators histamine, proteases, leukotrienes and prostaglandins can directly induce the early allergic symptoms (Figure 3) (*91*). Through their release of cytokines, such as IL-5 and IL-13, mast cells can also contribute to the late phase reaction, in particular by recruiting and activating eosinophils (through IL-5), and inducing mucus production (through IL-13) (*92-95*).

Besides the early and late responses to allergen exposure in sensitized individuals, allergy often leads to chronic inflammation, associated with tissue remodeling. The most well-known example is chronic atopic asthma, which is thought to be driven at least partially

by repeated FcεRI triggering. During chronic asthma, several tissue remodeling processes occur, including fibrosis, matrix deposition, vascular remodeling and mucus secretion. Mast cells are thought to contribute to these processes through their release of proteases (tryptase, chymase), angiogenic factors (VEGF, IL-8), and cytokines (IL-13). However, the role of mast cells in such chronic IgE-mediated responses is not clearly understood (*96*).

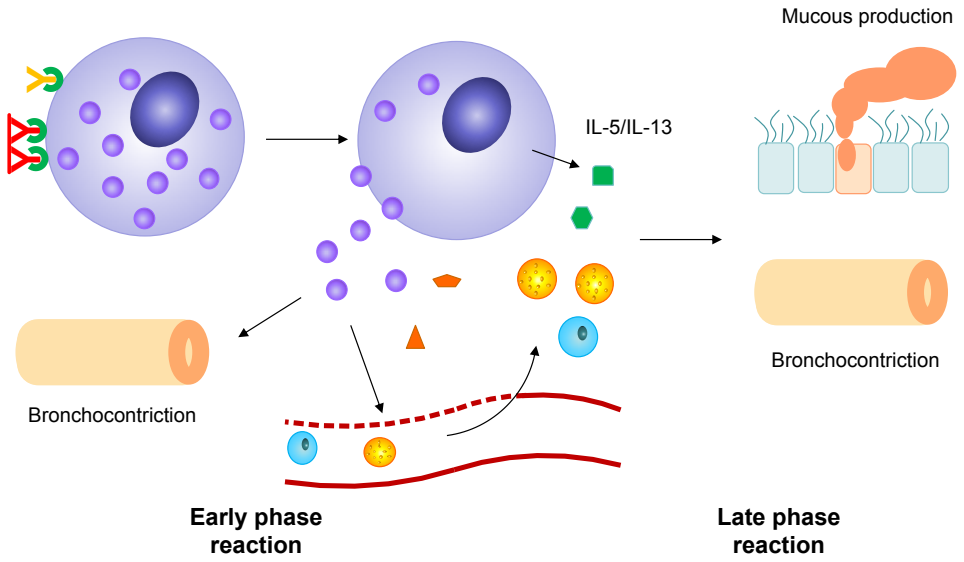

Figure 3: Role of mast cells in allergy. Once specific IgE recognizing allergens (red Y) has been formed, and mast cells have been sensitized, re-exposure to the allergen leads to activation of mast cells, characterized by degranulation, release of lipid mediators and production of cytokines, including type 2 cytokines, IL-5 and IL-13. Degranulation of mast cells during acute allergic reactions in the lung can lead to bronchoconstriction and increased vascular permeability. Production of leukotrienes and chemokines then leads to recruitment of granulocytes and T cells, which further increase allergic symptoms. During the late phase reaction, IL-13 and other mast cell products can increase mucous production and bronchoconstriction in the lung, thereby contributing to further narrowing of the bronchial tubes.

MAST CELLS IN AUTOIMMUNE DISEASE

Mast cells have been correlated to several autoimmune diseases, including T cell-dependent type IV hypersensitivity, and antibody-dependent type II and type III hypersensitivities (*97*). However, in most cases, this association is mainly correlative, depending on the findings of increased mast cell numbers or evidence of mast cell activation in the affected tissues in human disease. For example, increased mast cell numbers and levels of their mediators have been found in the synovium of rheumatoid arthritis patients, blisters of patients with bullous pemphigoid, spinal fluid of multiple

sclerosis patients, salivary glands of Sjögren's syndrome, as well as the skin of scleroderma patients (*98-102*).

Although this provides some suggestion for mast cell involvement, mast cell activation in these tissues is sometimes regarded as merely a consequence of tissue inflammation, rather than playing an important role in the pathogenesis of autoimmune disease. The functional contribution of mast cells to autoimmune disease is difficult to verify in humans, as currently, no specific mast cell inhibitors are available for clinical studies. Therefore, such studies rely on the use of animal models, which in most autoimmune diseases do not fully reflect human disease, but rather, just one component of the disease (*103*). For example, immunization models for arthritis are used to study the initiation of autoreactive T- and B-cell responses, whereas serum transfer models are used to study the effect of autoantibodies and chronic inflammation in arthritis. Next to variable models to study disease, there are now several models for mast cell deficiency, together resulting in seemingly contradictory results on the role pathogenic role of mast cells in autoimmune disease.

The role of mast cells in T cell-dependent autoimmunity has only been studied sparsely. A recent study on collagen induced arthritis showed that mast cell deficient mice display reduced arthritis severity and reduced numbers of collagen-specific Th1 and Th17 cells. Furthermore, mast cell numbers in draining lymph nodes were markedly increased upon immunization in wildtype mice, suggesting a direct role for mast cells in activation of autoreactive T cells (*104*). In contrast, mast cell deficiency in the diabetic NOD mouse, which depends on $CD4^+$ and $CD8^+$ T cells had no effect on disease pathogenesis (*105*). However, it is unclear to which extent the NOD mouse model reflects the autoreactive T cell response that occurs in type 1 diabetes patients (*106*).

In experimental autoimmune encephalitis, contrasting results have been obtained; whereas mast cells have been shown to contribute to autoreactive $CD8^+$ T cell responses, disease severity and recruitment of T cells to the CNS, another study reported no effect of mast cell deficiency on disease severity in the EAE model (*17, 107-109*). As described above, mast cells can contribute to T cell responses in various ways, including T cell activation and recruitment to lymph nodes, so it is conceivable that mast cells indeed play a role in these responses. More research into the function of human mast cells in the priming and activation of T cells is needed to get a better understanding of their capacity to contribute to T cell-dependent autoimmunity.

Autoantibodies

A major effector function thought to contribute to pathogenesis of autoimmune diseases is mediated by autoantibodies. An important group of autoantibodies in

rheumatoid arthritis targets citrullinated proteins (anti-citrullinated protein antibodies; ACPA). These antibodies recognize a variety of proteins or peptides in which the amino acid arginine is modified into citrulline through a posttranslational modification process mediated by Peptidyl Arginine Deiminase (PAD) enzymes. PAD enzymes are normally present inside cells and can be activated by high calcium levels when cells, such as neutrophils, undergo apoptosis, an event readily occurring during inflammation (*110*). PAD enzymes that are transported to the outside of cells can citrullinate extracellular proteins, although intracellular citrullinated proteins can also be released into extracellular space (*111*).

ACPA show a very high specificity for rheumatoid arthritis, and are present in the majority (~70%) of rheumatoid arthritis patients (*112, 113*). When ACPA antibodies are adoptively transferred into mice with a low-level synovial inflammation caused by anti-collagen antibodies, ACPA (reactive with citrullinated fibrinogen or collagen II) could enhance arthritis, implicating their direct involvement in the inflammatory process (*114, 115*).

There is not much evidence for a role of mast cells in the initiation of autoantibody production. In most immunization models, mast cell-deficient mice have similar levels of autoantibodies (*104*). However, mast cells express a variety of Fc receptors and complement receptors, making them an important effector cell type in the propagation of autoantibody-mediated inflammation. In mice, C5aR and Fcγ receptors on mast cells have been shown to play an important role in antibody-mediated arthritis and bullous pemphigoid (*116-119*). In these models, mast cells were shown to play an important role in recruitment of leukocytes, in particular of neutrophils. This is in line with their function observed in the Arthus reaction, where passive transfer of IgG antibodies and antigen lead to a mast cell-dependent neutrophil infiltration of the affected tissue (*120-122*). In contrast, some studies did not observe any effect of mast cell deficiency on autoantibody-mediated tissue inflammation (*17, 104, 123*).

Importantly, the expression of Fcγ receptors differs considerably between mouse and human mast cells (*124-127*), and, as a consequence, not much is known about the capacity of human mast cells to respond to immune complexes, especially in the context of autoimmune disease. Studies on the role of human mast cells in antibody-mediated inflammation may therefore provide more insight into the potential role of mast cells in autoimmune disease. As activation of immune cells through Fcγ receptors is an important effector mechanism of autoantibodies in autoimmune disease, it will be important to understand the capacity of mast cells to respond to Fcγ triggering. In the mouse, activation of mast cells through Fcγ receptors is known to lead to neutrophil

recruitment, however, not much is known about the role of Fcγ receptor activation of mast cells in autoimmune disease in humans.

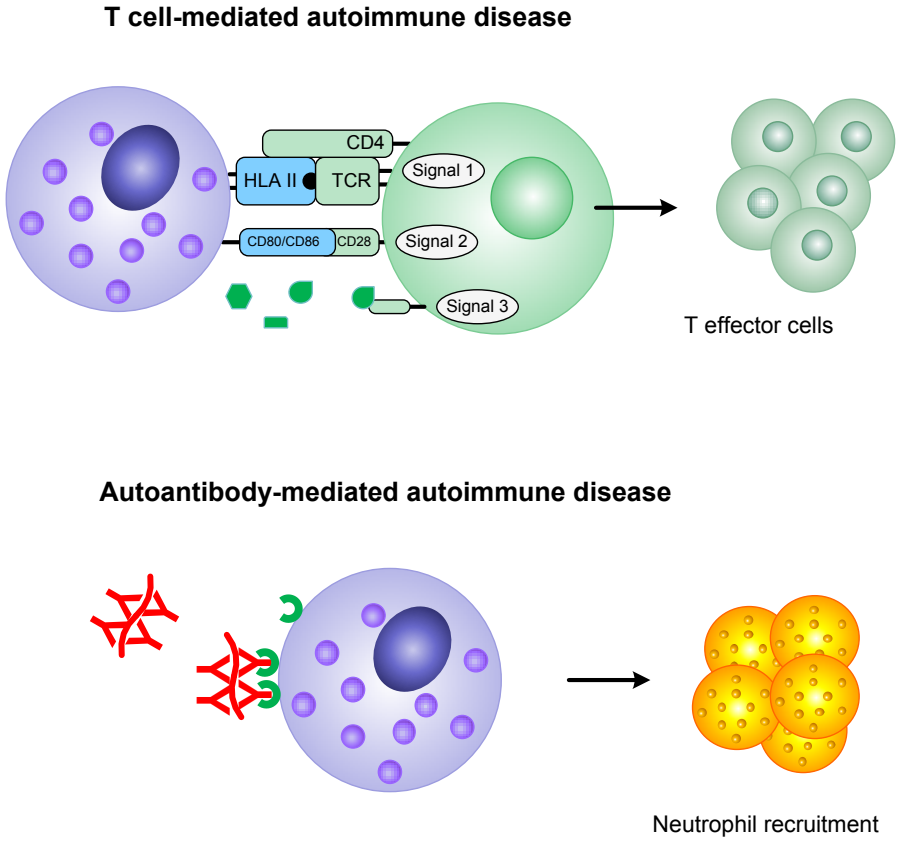

Figure 4: Potential role of mast cells in autoimmune disease. Mast cells have been implicated in T cell responses, therefore they could contribute to T cell-mediated autoimmune disease. Although not much is known about the interaction between mast cells and $CD4^+$ T cells in the human, there are suggestions that mast cells can function as antigen presenting cells, providing the 3 signals crucial for T cell activation: 1) Antigen presentation through HLA class II; 2) Co-stimulation, such as through CD28 or other molecules; 3) Skewing of T helper responses through production of cytokines.

CHRONIC INFLAMMATION

Repeated or continuous activation of the immune system can occur in a variety of conditions, including allergy and autoimmunity. In the case of allergy, after sensitization has occurred, repeated exposure to allergens, such as during yearly pollen season, can quickly induce reactivation of memory T cells and activation of innate immune cells through allergen-specific IgE. In the case of autoimmunity, release of self-antigens can lead to a

sustained activation of autoreactive T and B cells, as well as innate immune cells through antibody effector mechanisms. When the self-antigen or allergen cannot be eliminated, such responses can become chronic. Furthermore, the immune system contains amplification mechanisms to enhance inflammatory responses, and epitope spreading can contribute to further loss of tolerance in autoimmunity and allergy (*128-132*).

Not much is known about what exactly drives the incessant cycle of inflammation during these diseases, but it is likely that a proinflammatory cytokine environment, TLR ligands, antibodies and cellular effector mechanisms all cooperate to maintain chronicity. The danger model, as proposed by Matzinger, hypothesizes that self-antigen in itself is not enough to trigger autoimmunity, but that it needs to be accompanied by danger signals, such as occurring through tissue damage (*133*). In this context, it is interesting that most autoantibodies involved in chronic autoimmune disease have specificities for damage associated molecular patterns (DAMPs) (which I discuss in Chapter 12). This indicates that in autoimmune disease recognition of non-self through adaptive signals (e.g. antibodies) and innate danger signals often coincide, as they can be present even in the same molecule (*134*). In allergy, the danger signals can originate from infection (e.g. during asthma exacerbations) or tissue damage (during tissue remodeling in chronic allergy), although some allergens may also directly activate TLRs similar to self-antigens in autoimmune disease (*135*). Together, recognition of non-self in combination with danger signals can lead to a sustained response when tissue damage leads to additional release of DAMPs recognized by autoantibodies and TLRs (*136*).

The contribution of mast cells in the induction of tissue damage has not been widely studied. Although mast cells are not immediately viewed as a main cell type exhibiting cellular cytotoxicity, one study showed that mast cells can kill opsonized parasites, through release of tryptase (*137*). In addition, some studies have shown that mast cells exhibit cellular cytotoxicity, presumably through releasing their granule constituents such as TNF-α, granzyme B and possibly active caspase-3 (*138-142*). In addition, mast cell granule enzymes granzyme B, tryptase and chymase have been shown to degrade extracellular matrix proteins directly as well as indirectly through activating MMPs (*81, 143, 144*). Together, these processes may contribute to tissue and cellular damage, thereby inducing additional release of DAMPs and self-antigens which can be recognized by autoantibodies.

Furthermore, mast cells may directly release DAMPs upon cell death, for example through the formation of extracellular traps, or through necrosis (*145-148*). Besides direct effects of mast cells on tissue damage, their role in recruitment of neutrophils, activation of T cells and other immune cells can also contribute to inflammatory

responses, local cell death and release of DAMPs and self-antigens, thereby potentially contributing to the continuous amplification of local inflammatory responses.

However, not much is known about mast cell activation pathways in chronic inflammation, including their activation through TLRs, Fcγ receptors, as well as their intrinsic capacities to respond to these triggers in the context of continuous or repeated activation.

THIS THESIS

Although the studies mentioned above reveal a pivotal role for mast cells in a variety of immune responses, their role in autoimmunity has been studied sparsely. The objective of this PhD thesis is to understand mast cell (and basophil) functions and their role in autoimmune disease by focusing on three main aims:

1. To characterize the interaction between innate and Fc receptor triggers on mast cell and basophil function
2. To analyze the interaction between mast cells and $CD4^+$ T cells
3. To understand the function of mast cells in chronic inflammation

INNATE SIGNALS AND FC RECEPTOR TRIGGERING

First, it is important to characterize the specific pathways leading to mast cell activation, especially those pathways that operate in autoimmune diseases. The functional responses of human mast cells to triggering of Fcγ receptors, Toll-like receptors, or cytokines, have only sparsely been scrutinized. Furthermore, it is unknown how different activation pathways cooperate in the context of antibody-mediated responses. Such interactions are relevant for a variety of immune responses, including protective responses against pathogens, allergic reactions, as well as autoimmunity. Therefore, the first part of this thesis focuses on the interaction of these pathways. In Chapter 2 and 3, the activation of human basophils by TLRs and IL-33 was studied, in particular in combination with FcεRI-mediated activation. Furthermore, in these chapters, the effects of basophil activation on T helper cell skewing as well as monocyte activation are described. As basophils have recently emerged as important immunomodulatory cells, these studies will provide insight into their function, in particular in the context of IgE-mediated responses.

In Chapter 4-6, we describe the studies into the effect of the combined activation by innate signals (TLR, IL-33) and Fc receptor triggering in mast cells. In Chapter 4, the

cytokine profile of mast cells upon different TLR ligands in combination with FcεRI triggering is described, providing insight into the specific TLR ligands that could contribute to mast cell responses in allergy. In Chapter 5, I evaluated the activation of mast cells by anti-citrullinated protein antibodies, DAMPs and other TLR ligands. This study therefore aimed to gain understanding of activation pathways that contribute to synovial inflammatory responses in RA patients. In Chapter 6, we aimed to evaluate the role of IL-33 on mast cell activation, and the subsequent mast cell-monocyte interaction.

MAST CELL-T CELL INTERACTIONS

As mast cells are present at strategic locations of the environment/host interface where they can encounter pathogens, they have been implicated in the regulation of adaptive immune responses and the regulation of T cell immunity.

Besides their role in recruiting T cells to lymph nodes during bacterial infection, mast cells themselves were shown to be capable of migrating from the skin to the draining lymph nodes in murine models of contact hypersensitivity and UV radiation (*149, 150*), suggesting they may directly be involved in antigen presentation. Thus far, little information is available on the antigen-presenting capacity of human mast cells. Therefore, in Chapter 7 and 8 we studied whether mast cells possess the required molecular make-up, such as HLA-DR and costimulatory molecules, to activate T-cells through antigen presentation and co-stimulation.

As mast cells produce a variety of cytokines which can act on T cells, they have also been implicated in skewing of specific T cell responses. The effect of human mast cells on skewing of T helper cell responses and the effect of different modes of mast cell activation has not been elucidated. In Chapter 9 we therefore evaluated the effect of mast cells on Th cell responses.

CHRONIC INFLAMMATION

Finally, although mast cells are very potent cells in the context of acute inflammatory responses, only little is known about their function in the context of chronic inflammation. First of all, they are very long-lived (estimated >10 months in rats with a lifespan of 30-36 months) and therefore, can be influenced by inflammatory stimuli that are present during that time (*50, 151*). Further, as they enter a tissue as immature cell, their maturation depends on growth factors and cytokines in the tissue, allowing mast cell plasticity under the influence of local inflammatory conditions (*152, 153*).

However, as mast cells are usually considered in acute responses, not much is known about mast cell function under the influence of prolonged inflammatory stimulation or chronic infections. Therefore, the studies described in Chapter 10 were aimed at understanding mast cell function upon chronic Fc receptor triggering, providing detailed insight into changes in the mast cell transcriptome related to chronic allergy. Chapters 11 provides an overview of the role of mast cells in rheumatic disease, and Chapter 12 and 13 describe how autoantibodies are initiated and how chronic inflammation is propagated in autoimmune disease, also providing a perspective on novel therapeutic targets for the treatment of these diseases.

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