The Effect of Bacterial, Viral and Fungal Infection on Mast Cell Reactivity in the Allergic Setting

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Abstract

Mast cells are well known for their role in allergic inflammation where, upon aggregation of the high-affinity immunoglobulin E receptor, they release mediators such as histamine that cause classical allergic symptoms. Mast cells are located in almost all tissues and are especially numerous in organs that interface with the environment. Given this strategic location and the more recent notion that they are endowed with receptors that recognize endogenous and exogenous danger signals such as pathogens, it is not surprising that they function as important cells in immune surveillance. When mast cells are activated by pathogens they modulate innate and adaptive immune responses. In allergy, infections might cause exacerbation of the allergic reaction by affecting the reactivity of mast cells. With new developments within the field of mast cell biology, we will better understand how mast cells execute their effector functions. This knowledge will also help to improve the management of allergic diseases.

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Introduction

Mast cells are one of our more evolutionary conserved inflammatory cell types, found even in simple organisms such as tunicates [1]. They act as surveillance cells, recognizing intruders or cell injury and rapidly respond by releasing mediators causing vascular effects, recruiting and activating other inflammatory cells, and by modulating an adaptive immune response. Depending on the nature of the receptor-ligand engagement, there will be a differentially regulated mast cell reaction to the insult, and thus, induction of diverse responses dictated by the specific trigger. In allergy, mast cells are infamous for their role as effector cells that cause a harmful reaction through the release of histamine, lipid mediators and cytokines. It is less familiar that mast cells appear to have an important role in host defence to pathogens [2]. Many types of parasites, bacteria, viruses and fungi are recognized by mast cells. This recognition takes place through 2 major ways: either via direct binding to the so-called pattern recognition receptors (PRRs) or via binding of antibody or complement coated bacteria by Fc receptors or complement receptors on the mast cell surface, respectively. The anti-microbial protective effects of mast cells are, at least

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in part, mediated by their fast release of proteases and cytokines that induce a rapid recruitment of neutrophils to the site of infection [2]. In addition, mast cells might also release antibacterial peptides, such as LL-37 [3], and the formation of extracellular traps [4] that can directly inhibit bacterial growth.

One of the best studied mast cell-mediated reactions is immunoglobulin E (IgE)-dependent mast cell activation where 2 high-affinity IgE receptors, FcεRI, are aggregated. How infection affects IgE-mediated mast cell activation is not clear, but it appears that there is a cross-talk between Toll-like receptors (TLRs) and FcεRI. There are *in vitro* studies suggesting that coactivation of TLRs and FcεRI augments the release of cytokines from mast cells without affecting the exocytosis of granule mediators [5]. Other studies have shown what appears to be opposite effects where costimulation of TLRs and FcεRI inhibits mast cell degranulation [6, 7]. *In vivo* studies in humans and mice suggest that infections promote the allergic response. Nigo et al. [8] described that lipopolysaccharide (LPS) treatment enhances allergic airway inflammation in a mast cell-dependent fashion. In a human study with rhinovirus, it was demonstrated that infection potentiates histamine release upon allergen provocation in allergic but not in non-allergic subjects [9]. Studies like these indicate that mast cell reactivity is altered by infection and suggest that this might play a role in exacerbations of allergic diseases. In view of the fact that both allergens and microbial antigens can trigger mast cell mediator release and that these triggering pathways are integrated, strategies to modulate these activities might be of therapeutic value. Herein, we will review mast cell biology, their function in host defence and how infection can affect IgE-mediated mast cell activation.

Mast Cell Distribution and Reactivity

In humans, mast cells are found in almost all tissues and are especially numerous in sites that are in contact with the environment, i.e. skin, respiratory tract, gastrointestinal tract and genitourinary tract. They are divided into 2 major phenotypes: the MC_T type expressing the protease tryptase and the MC_{TC} type containing both tryptase and chymase. The former corresponds to mucosal mast cells in rodents and the latter to the connective tissue mast cells. However, mast cells are a much more heterogeneous cell type than this simple categorization might indicate. Their reactivity to classical mast cell secretagogues, like substance P and compound 48/80, var-

ies as well as their sensitivity to mast cell inhibitors, such as cromolyn [10]. A recent study of human lung mast cells showed that within the MC_T and MC_{TC} populations, there is great diversity in the expression of different markers related to inflammation [11]. Within the lung, the number of mast cells varies in different compartments, with the small airways and alveolar parenchyma being the sites with the highest mast cell density. In these compartments, the majority of mast cells are of the MC_T type. In contrast, the mast cell population surrounding pulmonary vessels is composed of approximately 50% of each MC_T and MC_{TC}. Furthermore, the expression of proteins such as FcεRIα, leukotriene C₄ (LTC₄) synthase, renin and interleukin (IL)-9 varies greatly, not only between MC_T and MC_{TC}, but also within these 2 major mast cell phenotypes situated in the different lung compartments [11]. Whether this complex heterogeneity suggests the existence of more mast cell phenotypes than those two that we appreciate today, or it reflects an extended mast cell plasticity dictated by the surrounding cells is not fully known. Since not only the relation between MC_T and MC_{TC} changes with pathogenesis (e.g., in chronic obstructive pulmonary disease) but also the protein expression in the cells differs compared to a healthy lung, it may be that the microenvironment has a great impact on the phenotype of the cells [12].

So far, the expression of TLRs and other receptors of importance for immune surveillance have not been investigated in any detail on specific human mast cell phenotypes or on human mast cells in different tissue compartments. Such studies would be of great interest to better understand the function of mast cells in health and disease. However, the compiled knowledge on receptor expression on mast cells, both human and mouse, as such is rather massive. As discussed in this review, it appears that they have the capacity to express many, if not all, of the crucial receptors for recognizing pathogens.

Receptor-mediated activation of mast cells can have dramatic effects and can ultimately result in an anaphylactic reaction. The most studied and best described activation of mast cells is through the FcεRI [13]. Aggregation of FcεRI leads to immediate massive exocytosis and release of mediators stored in the granules, including histamine, proteases, heparin and certain cytokines. This is followed within minutes by secretion of leukotrienes and prostaglandins, and some hours thereafter, release of *de novo* synthesized cytokines, chemokines and growth factors. Of particular interest to understand mast cell biology and the function of this cell in host defence and pathogenesis in different diseases is the differential re-

Fig. 1. Activated mast cells release different profiles of mediators depending on the triggering factor. Mast cells can be activated by different means, including for instance FcεRI crosslinking (**a**), TLR activation (**b**) or CD30-CD30L interaction (**c**), leading to differential release of mediators. For example, antigens crosslinking FcεRI receptors on mast cells induce the activation of 3 separate activation routes, leading to degranulation, release of lipid mediators and de novo production and secretion of cytokines. In contrast, pathogen-derived compounds activating mast cells via

TLRs do not normally induce degranulation, but instead induce the secretion of lipid mediators and cytokines. Finally, a third response pattern can be observed in mast cells treated with the costimulatory molecule CD30, where mast cells respond by specific secretion of chemokines, but not by degranulation or release of lipid mediators. FcR = Fc receptor; PLCγ = phospholipase Cγ; NF-κB = nuclear factor-κB; MAPK = mitogen-activated protein kinase; PKC = protein kinase C; PLA₂ = phospholipase A₂; TRAF6 = tumour necrosis factor receptor-associated factor 6.

lease of mediators that takes place dependent on the trigger [14] (fig. 1). This means that a trigger acting through FcεRI gives rise to degranulation, lipid mediator secretion and release of de novo synthesized cytokines. In contrast, the costimulatory molecule CD30 induces a specific secretion of chemokines, without any preceding degranulation [15]. Most pathogens appear to not induce degranulation, but induce secretion of lipid mediators and de novo synthesis and release of cytokines [16]. Of importance is that different pathogens provoke a specific profile with diverse types of cytokines and/or chemokines released [16]. How the cell is triggered is of great significance for the outcome of the mast cell response.

With the many possibilities, a mast cell-mediated reaction can provoke, including vascular reactions, tissue remodelling, angiogenesis, immunomodulation, cell recruitment, activation of granulocytes and others, it is important to understand that a specific trigger generates a particular set of inflammatory mediators.

Mast Cells Express PRRs

During evolution, we have been equipped with a variety of defence mechanisms, warding us against the many different types of invading microorganisms. Lacking

such mechanisms, we would quickly succumb to an overwhelming pathogen burden. Aside from natural barriers like the skin, mucosal surfaces and low gastric pH, we rely on the innate immune system to rapidly detect and eliminate detrimental pathogens. Should a threat overwhelm the innate defence systems, an adaptive immune response can be called upon, thus helping to clear the infection.

The innate branch of the immune system heavily relies on PRRs to detect, identify and neutralize invading pathogens. PRRs are expressed on a variety of cell types, particularly on innate immune cells such as neutrophils, dendritic cells and macrophages, enabling these cell types to rapidly respond to invaders. To combat pathogens, the immune system has evolved several different types of receptors designed to identify microbial species. To date, there are 4 classes of PRRs described: TLRs, C-type lectin receptors (CLRs), Nod-like receptors (NLRs) and retinoic acid-inducible gene-I-like receptors (RLRs). The first TLRs were discovered in the early 1990s, paving the way for the understanding of pathogen recognition. So far, 13 TLRs are identified in humans and 12 in mice [17]. Ligands for the TLRs include bacterial, viral, fungal and also self-components. CLRs seem to, among other functions, have an important role in the defence against fungi, exemplified by the fact that dectin-1 and dectin-2 recognize β -glucan from fungi. NLRs and RLRs recognize bacterial and viral components, respectively, and are present intracellularly, scanning the cytoplasm for microbial components heralding infection.

Together, these 4 classes of receptors, along with their respective subclasses, recognize various and distinct molecular patterns present on microbes, including bacteria, viruses and fungi. Such microbial patterns are generally referred to as pathogen-associated molecular patterns (PAMPs). Although PAMPs come in many different shapes and variants, they are all composed of evolutionary conserved patterns common to certain classes of microbes. Importantly, many PAMPs are composed of microbe components crucial for their viability (e.g., bacterial flagellin and viral single-stranded RNA), thus rendering them more difficult to evolve in a direction that would facilitate immune system evasion. Once detected by PRRs, the presence of PAMPs triggers pathways leading to the induction of pro-inflammatory genes, often through nuclear factor- κ B activation, eventually resulting in the release of pro-inflammatory chemokines and cytokines. Mast cells are counted among the populations expressing different PRRs and are thus able to sense, recognize and respond to microbial intrusions (fig. 2).

Mast Cells in Bacterial Infections

The importance of mast cells in the defence against bacterial infections has been increasingly appreciated during the last decade. Upon bacterial recognition, mast cells can produce pro-inflammatory mediators, including chemokines, thus attracting neutrophils to the site of infection, which will ultimately eliminate the bacteria. In 1996, 2 ground-breaking studies were published providing direct evidence for the importance of mast cells in bacterial infections. In one of these, Echtenacher and colleagues [18] described a protective role for mast cells in acute bacterial peritonitis, assessed using a caecum ligation and puncture (CLP) model. In this model, mast cell-deficient WBB6F₁-Kit^{W/W^{-v}} mice displayed a significantly increased mortality rate after CLP, compared to their mast cell-sufficient congenital litter mates. Importantly, mast cell-deficient mice were protected if they were reconstituted intraperitoneally with mast cells prior to CLP.

Another intriguing study was published in the same issue of *Nature* by Malaviya and coworkers [19]. In their work, mast cell responses to intraperitoneal challenge with enterobacteria were investigated. Interestingly, mast cell-deficient mice were much less efficient in clearing the infection compared to mast cell-sufficient control mice or mast cell-reconstituted mice, again showing that mast cells are important for efficient bacterial defence mechanisms. In addition, mast cells have also been shown to be important for the defence against pathogens such as *Mycoplasma*, *Pseudomonas* and *Helicobacter* [20–22].

In the studies of Echtenacher et al. [18] and Malaviya et al. [19], mast cells seemed to mediate their protective role by driving tumour necrosis factor (TNF) production upon bacterial encounter, thus attracting neutrophils to the infected peritoneal cavity. It should also be noted that in a more recent study by Piliponsky et al. [23], the importance of mast cells in the survival of mice after CLP was confirmed in a different mast cell-deficient mouse strain, C57BL/6-Kit^{W-sh/W-sh}. However, TNF was not important for this mast cell-dependent survival, and in a more severe model of sepsis, the absence of mast cells and mast cell-derived TNF actually increased survival. Therefore, mast cell responses may result in divergent outcomes in various degrees of inflammation, and different models of mast cell-deficient mice may lead to contrasting conclusions in some systems.

A question arising from such studies is how mast cells actually recognize invading bacteria. One answer to this question is that mast cells express different PRRs, allowing them to detect various PAMPs present on bacteria.

Fig. 2. Mast cells are equipped with a variety of PRRs facilitating their role as sensors of extra- and intracellular pathogens. By expressing extra- and intracellular PRRs such as TLRs, NLRs and CLRs, mast cells can detect and respond to pathogens such as bacteria, viruses and fungi. RLRs may also be expressed by mast cells and enable viral recognition. When activated, mast cells induce differential responses that can include degranulation, lipid mediator release and/or de novo production and release of cytokines and chemokines, depending on the triggering factor. Dashed ar-

rows represent hypothetical pathways not yet fully investigated. FcR = Fc receptor; LRRs = lectin-like receptors; MAPK = mitogen-activated protein kinase; TRAF6 = tumour necrosis factor receptor-associated factor 6; NF- κ B = nuclear factor- κ B; TRAF = Toll/IL-1 receptor domain-containing adapter inducing interferon- β ; IRF = interferon regulatory factor; IFNs = interferons; RIG-I = retinoic acid-inducible gene; MDA5 = melanoma differentiation-associated gene 5.

For instance, it has been shown that TLR4 is important for the protective role played by mast cells in enterobacterial infections. In a study by Supajatura and colleagues [24], it was shown that TLR4 was required for mast cells to respond to LPS (a PAMP present on all Gram-negative bacteria) treatment by secreting cytokines. While mast cells from C3H/HeN mice which express functional TLR4 responded to LPS treatment with secretion of sev-

eral pro-inflammatory cytokines, mast cells from C3H/HeJ mice in which TLR4 contains a point mutation and is therefore defective lacked this response. Using a CLP model, the authors could also provide in vivo evidence for the importance of TLR4: mast cell-deficient mice reconstituted with TLR4-mutated mast cells displayed higher mortality compared to mice reconstituted with TLR4-intact mast cells.

In addition to TLR4, TLR2 has also been shown to be important for the mast cell ability to respond to bacteria. For instance, mast cells derived from TLR2^{+/+}, but not from TLR2^{-/-} mice degranulate in response to peptidoglycan (PGN; a PAMP constituting a part of the bacterial cell wall, especially thick in the cell wall of Gram-positive bacteria) [25]. When injected intradermally in wild-type mice, PGN caused skin reactions manifested due to increased vascular permeability. Interestingly, this response was neither observed in mast cell-deficient mice, nor in mast cell-deficient mice reconstituted with TLR2^{-/-} mast cells, suggesting that the vascular permeability was attributed to TLR2-expressing mast cells, which degranulate upon PGN recognition. Taken together, the above mentioned studies provide evidence for the fact that mast cells can detect and respond to bacterial infections, here exemplified by responses elicited towards LPS and PGN, detected by mast cells through TLR4 and TLR2, respectively.

Besides being able to detect bacterial PAMPs through TLRs, data from our group [26] suggest that human cord blood-derived mast cells can respond to the Nod1 agonist M-TriDAP, a PGN derivative, by secretion of pro-inflammatory cytokines and chemokines. Since Nod1 is an intracellular receptor, this suggests that mast cells are also able to detect and respond to intracellular bacteria. Interestingly, the pro-inflammatory response elicited upon M-TriDAP treatment could be augmented if M-TriDAP was combined with LPS, suggesting co-operation between intra- and extracellular PRRs on the mast cell.

Furthermore, mouse mast cells also recognize and phagocytose bacteria like *Escherichia coli* through binding of FimH, a mannose-binding lectin on the fimbriae of bacteria, to CD48 on the mast cell surface [19, 27]. Another Gram-positive bacteria, *Staphylococcus aureus*, is often associated with allergic diseases such as atopic dermatitis. The recognition of *S. aureus* by human mast cells appears to be mediated through binding to TLR2 and CD48, leading to the release of IL-8 and TNF [28]. How *S. aureus* infection and mast cell activation affects the course of the allergic disease remains to be elucidated.

Aside from detecting bacteria through PRRs, mast cells also have other means of fighting bacteria, including production and release of antimicrobial peptides. For instance, a study by Di Nardo and colleagues [3] revealed that cathelin-related antimicrobial peptide is important for mast cell antimicrobial activity. When cocultured with group A *Streptococcus* (GAS), wild-type mast cells were shown to be able to reduce bacterial growth. In contrast, mast cells generated from cathelin-related antimicrobial

peptide-deficient mice were approximately 50% less effective in reducing bacterial growth. Another more recent study investigated the susceptibility of mast cell-deficient C57BL/6-*Kit*^{Wsh/Wsh} mice to GAS infection in vivo [29]. After subcutaneous injections of GAS, the mice displayed larger lesions and larger lesion bacterial loads compared to wild-type mice. This effect could partly be attributed to a mast cell-derived cathelicidin peptide, providing further evidence for the role of mast cells as responders to bacterial intrusions. Also, the antimicrobial peptide LL-37 has been identified as a means through which mast cells combat infection. It was recently described that human lung mast cells could kill wild-type pneumococci after activation by pneumolysin, but had no effect on pneumolysin-deficient *Pneumococcus* [30]. This was caused by mast cell release of the cathelicidin LL-37, thus exhibiting a direct antimicrobial activity. Finally, von Kückritz-Blickwede et al. [4] have reported that human and mouse mast cells, like neutrophils, can release extracellular structures, which are composed of DNA, histones, tryptase and LL-37, which entrap and eventually kill bacteria. It has also been reported that mouse mast cells can act as phagocytes [31], thus killing bacteria within acidified vacuoles, much like professional phagocytes.

Conclusively, numerous previous and recent studies together demonstrate that mast cells constitute an important part of host defence, as they are capable of detecting and also eliminating bacterial threats.

Mast Cells in Viral Infection

Mast cells have only recently begun to be viewed as players in viral infection, and the beneficial role of mast cells in antiviral immune responses is quite a new area of research. Mast cells have been shown to express multiple PRRs that are activated by viral components. The 2 human cell lines LAD-2 and HMC-1 and primary peripheral blood-derived mast cells express TLR3, TLR7 and TLR9 [32]. These receptors enable mast cells to detect viral infection through the recognition of nucleic acids such as single- and double-stranded RNA and unmethylated CpG DNA sequences [17].

The pro-inflammatory function of virus-associated TLRs may explain why mast cells appear resistant to certain viral infections, as seen in the abortive infection of bone marrow-derived mast cells (BMMCs) with murine cytomegalovirus [33]. The synthetic double-stranded RNA agonist of TLR3, polyinosinic:polycytidylic acid [poly(I:C)], as well as vesicular stomatitis virus, stimu-

lates mast cell production of interferon- α , which is well known to induce an antiviral state in host cells [32, 34]. In mouse mast cells, poly(I:C) and Newcastle disease virus upregulate the costimulatory molecules CD28 and CD80, which may alter T-cell responses [35]. Newcastle disease virus infection also increases the expression of TLR3 and several key antiviral mediators including interferon- β , ISG15, CCL4, CCL5 and CXCL10. Furthermore, several studies have shown that mast cells are important for the recruitment of Langerhans cells, CD8⁺ T cells and natural killer (NK) cells, when stimulated with the TLR7 ligand imiquimod, the TLR3 ligand poly(I:C) and with double-stranded RNA reovirus, respectively [35–37]. Therefore, mast cells can produce antiviral cytokines and chemoattractants for key antiviral effector cells such as macrophages, NK cells and T cells in viral infection and may be important for directing their migration in the in vivo setting.

Mast cells have also been investigated in the context of dengue virus, which is a single-stranded RNA virus found in tropical areas of the world and is the etiological agent of dengue hemorrhagic fever. There is substantial work to support dengue virus infection via Fc γ receptors in human mast cell lines and primary cultures, resulting in higher numbers of infected cells and greater viral replication [38–40]. The fact that little to no dengue virus replication occurs in mast cells in the absence of anti-dengue virus antibodies, suggests that mast cells may be resistant during primary infections. Mast cells also produce several inflammatory mediators when infected with dengue virus, including the cytokines IL-1 β , IL-6 and granulocyte macrophage colony-stimulating factor [40]. These cytokines can effect endothelial activation, fever, plasma cell growth and macrophage differentiation, which are all important components of dengue disease. Additionally, dengue-infected human mast cells produce the chemokines CCL3, CCL4 and CCL5, which are known chemoattractants for monocytes/macrophages, dendritic cells, NK cells and T cells [39]. Clearly, mast cells are sites of productive virus infection and also sources of pro-inflammatory mediators that may contribute to dengue virus pathogenesis or viral clearance.

The initial investigation of mast cell responses to viruses initially began in the 1970s when their involvement in the pathogenesis of asthma was considered. Asthma is an inflammatory disease of the airways induced by hyperresponsiveness to environmental stimuli, resulting in bronchoconstriction, oedema and tissue remodeling. It is widely believed that mast cells play a role in the pathogenesis of asthma, due to the fact that many of their pre-

formed and de novo synthesized mediators such as histamine and leukotrienes can induce many asthmatic symptoms [for a review, see ref. 41]. Therefore, in this setting, mast cell responses may be detrimental to the host.

A clear link between viruses and asthma exists. In patients with existing asthma, symptoms are worsened during infection by pathogens such as rhinovirus and respiratory syncytial virus [for a review, see ref. 42]. Evidence of mast cell involvement in this mechanism began with the finding that mediators such as histamine are elevated in patients during virus-induced asthma exacerbations [43, 44]. Mast cell numbers can also be increased in the lung during viral infection of the airways [45]. Additionally, mast cells can undergo degranulation and histamine release when stimulated with numerous viruses of the airways, including influenza and respiratory syncytial virus [46]. All of these studies support a detrimental role for mast cells in virus-induced asthma exacerbations.

Overall, these studies clearly show that mast cells are armed with the tools necessary to detect and respond to viruses and that they can produce antiviral mediators and activate other arms of the immune system. However, they can also be targeted by multiple viruses, and their responses may in fact be harmful to the host in certain infections. More studies are necessary to truly understand the effect of mast cell responses on the overall clearance of virus and the resolution of infection.

Mast Cell Recognition of Fungi

Fungi belong to the group of pathogens capable of invading the body through mucosal surfaces and the skin. The fungal intrusion is likely detected through a combination of different receptors; however, today, most of the identified receptors involved in fungal recognition belong to the family of CLRs such as dectin-1, dectin-2, DC-SIGN (dendritic cell-specific ICAM-3-grabbing non-integrin), mannose receptor, Mincle receptor and TLR2, TLR4 and TLR9 [17]. Besides being important for the recognition of fungi, CLRs can also recognize other pathogens [47] as well as endogenous danger signals [48].

The function of mast cells in fungal defense is a rather new field of interest where more discoveries still lie ahead. However, recent studies identify mast cells as capable of recognizing fungal products such as zymosan (*Saccharomyces cerevisiae*) [49], mature fungal hyphae (*Aspergillus fumigatus*) [50] and also fungal cell extract (*Malassezia sympodialis*) [51, 52]. Earlier studies have used zymosan; however, not with the focus on the role of mast cells in

fungal infections, but rather as an inducer of peritonitis and other infectious conditions. Nonetheless, mast cells have been shown to play a role in zymosan-induced peritonitis in the mouse [53, 54].

The fungal intrusion typically activates the innate immune system to bind, phagocytose and kill the fungal cells, in combination with alerting the adaptive immune system to extinguish the fungal infection. The studies so far show that mast cells express receptors involved in fungal recognition such as dectin-1 [49, 51, 55], Mincle [51] and TLRs [52], and activation through these receptors results in production and release of cytokines and chemokines capable of promoting recruitment of inflammatory cells [49, 51, 52], production of leukotrienes [49, 52] and reactive oxygen species generation [55].

In the Northern hemisphere, skin commensal yeast *Malassezia* species have been isolated as contributing factors for the development of atopic eczema [56], and the involvement of mast cells in this disease raised the question whether they could be activated by the fungus [51, 52]. Both mouse and human mast cells do respond to extract from *M. sympodialis* [51, 52]. In mouse cells, the fungal extract directly induces release of cysteinyl leukotrienes, and additionally modulates simultaneous IgE receptor-dependent IL-6 production, as well as augments the degranulation of the cells through a TLR2/MyD88-dependent route [52]. Surprisingly, IgE sensitization of mouse mast cells leads to upregulated release of leukotrienes together with monocyte chemotactic protein 1 production and degranulation upon stimulation with the fungal extract. Human mast cells generated from atopic eczema patients, as well as from healthy controls, respond to the fungal extract alone, and in combination with activating IgE receptor crosslinking, by producing IL-6 together with IL-8 [51]. Mast cells (from atopic eczema patients and healthy controls) express dectin-1 and Mincle, as well as TLR2. Of particular interest, mast cells constitute the majority of the dectin-1-expressing cells in atopic eczema skin [51].

Fungi have for some time been considered to affect atopic diseases, either as a source of allergens or simply by augmenting immune reactions through increased production of proinflammatory cytokines and bronchoconstricting leukotrienes [57]. Furthermore, mast cells generated from patients with atopic eczema lack the ability to upregulate dectin-1 messenger RNA upon stimulation with *Malassezia* cell extract. This deficiency could reflect malfunctioning receptor(s) in atopic eczema patients causing hampered defense and difficulties to fight off commensal pathogens, eventually leading to perpet-

ual immune frustration [51]. Additionally, *A. fumigatus* induces IgE-independent degranulation of mouse mast cells, and may worsen chronic lung diseases [50].

The Effect of TLR Activation on Mast Cell Reactivity to Fc ϵ RI Crosslinking

The signalling pathways induced by ligation of TLRs and Fc ϵ RI are distinct, involving different adaptor and signalling molecules (fig. 2). Therefore, each activation event results in diverse mediator production. For example, human mast cell cytokine responses to poly(I:C) are distinct from Fc ϵ RI crosslinking in that TNF is not produced [32]. However, it is very likely that the receptors and mediators upregulated in response to ligands such as PAMPs may alter the result of IgE-mediated mast cell activation.

The majority of the research in this area has focused on bacterial products and cell wall constituents. LPS synergistically enhances BMMC production of the proinflammatory cytokines IL-6, IL-13 and TNF in response to IgE-mediated activation, with no alteration in degranulation or arachidonic acid metabolites [5]. The enhanced cytokine production was shown to be caused by a synergistic activation of mitogen-activated protein kinases, particularly JNK [5]. Furthermore, in vivo, cotreatment with LPS and antigen induces greater production of IL-5 and eotaxin compared to Fc ϵ RI-crosslinking alone [8]. These findings may have implications for antibody production and eosinophil recruitment, which could negatively impact the allergic condition. Surprisingly, IgE-sensitized mouse mast cells treated with LPS in the absence of antigen are also synergistically activated to produce IL-6 and TNF, suggesting that sensitized individuals may experience greater inflammation during bacterial infection than those who are not sensitized [58]. IgE and LPS costimulation can also rescue BMMCs from apoptosis due to IL-3 withdrawal, meaning that mast cells may survive longer in sensitized patients as well [59].

TLR2 recognizes a wide range of bacterial products and components and is expressed on human mast cells [60]. The TLR2 ligand PGN acts similarly to LPS by synergistically acting with Fc ϵ RI signalling to upregulate IL-6 and TNF production in BMMCs [5]. This synergism appears to be a consistent response to bacterial products, as the TLR2 agonist Pam₃CSK₄ lipopeptide also enhances IL-6 production during Fc ϵ RI crosslinking [6]. However, not all IgE-mediated mast cell responses are augmented by TLR2 activation. Mast cell degranulation is

inhibited by the TLR2 agonists PGN, lipoteichoic acid [7] and Pam₃CSK₄ [6]. This inhibitory effect has also been observed after stimulation of the rat cell line RBL-2H3 with the Gram-positive bacterium *Bifidobacterium* [61]. More importantly, Pam₃CSK₄ also inhibits the production of classical FcεRI-driven mediators such as IL-13 and LTC₄, suggesting that the recognition of bacterial infection may prevent IgE isotype switching, mucus and collagen production, as well as bronchoconstriction.

All of these studies combined provide strong evidence for an interaction between FcεRI and TLR signalling in mast cells. However, considering the contrasting effects of TLR2 and TLR4 stimulation, the overall outcome of mast cell responses to bacterial infection is likely to be highly dependent on the nature of the bacterial stimulus, and on the combination of signals delivered to the mast cell.

Very few studies in this area have focused on pathogens other than bacteria. Interestingly, low doses of extract from the skin commensal yeast *Malassezia* can augment IL-6 production by mouse mast cells, while high doses can inhibit this response, and both responses are dependent on TLR2 [52]. This perhaps indicates that mediator production by mast cells may be affected by the level of infection, and therefore, the potency of the stimulus. This yeast extract can also enhance degranulation driven by FcεRI crosslinking; however, these responses are TLR2 and MyD88 independent. In the class of parasites, filarial nematodes produce an anti-inflammatory glycoprotein called ES-62, which can bind to and activate TLR4 [62]. ES-62 inhibits the amount of degranulation and LTC₄ and prostaglandin D₂ production by FcεRI-crosslinked human mast cells. This may lead to an inhibition of allergic-type responses in parasite-infected patients and may also have detrimental effects on pathogen clearance, since Th2 responses are so important for immunity to parasites.

A study by Kulka et al. [32] demonstrated that the viral double-stranded RNA analogue poly(I:C) pretreatment inhibits human mast cell degranulation induced by FcεRI crosslinking in an in vitro adhesion model. This finding suggests that poly(I:C) recognition may reduce allergic responses and is in contradiction to previously mentioned studies showing that viral infection can induce mast cell degranulation [46, 63]. This may be explained by the simplicity of poly(I:C) as a model for viral infection. Alternatively, it may indicate that mast cell responses to viral infection are largely determined by localization of the pathogen, where infection of neighbouring cells, as detected by the uptake of double-stranded RNA into the

TLR3-containing endosomal compartment, induces an appropriate response compared to infection of the mast cell itself. Moreover, infections can directly induce IgE-mediated responses through the binding of superantigens to IgE on the human mast cell surface, thereby cross-linking the IgE receptors and release of mediators [64]. Examples of such superantigens are protein Fv, an endogenous protein synthesized in the human liver and increased during viral hepatitis, protein A of *S. aureus* and the envelope glycoprotein gp120 of HIV-1.

Conclusion

For many years, mast cells were more or less ignored, except by allergologists, since they were merely considered to be effector cells of allergic inflammation. During the last 2 decades, this view has dramatically changed. Today, we know that mast cells play an important role in both the initiation of an innate immune response and in the coordination of adaptive immune responses. This knowledge about mast cell biology has drawn attention to the role of mast cells in the pathogenesis of many diseases, beyond allergic inflammation, and also to the critical role of mast cells in host defence. The ability of mast cells to react to both harmless agents such as allergens and harmful pathogens might cause problems in allergic diseases, such as atopic eczema and asthma, where exacerbations are common in conjunction with infections. By furthering investigations into the role of mast cells in innate and adaptive immune responses, and the effect of infections on their reactivity, we will better understand the multifunctional role of mast cells in health and disease. Hopefully, this will also lead to new strategies to reduce allergic symptoms.

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