Mast cell-orchestrated immunity to pathogens

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Abstract | Although mast cells were discovered more than a century ago, their functions beyond their role in allergic responses remained elusive until recently. However, there is a growing appreciation that an important physiological function of these cells is the recognition of pathogens and modulation of appropriate immune responses. Because of their ability to instantly release several pro-inflammatory mediators from intracellular stores and their location at the host–environment interface, mast cells have been shown to be crucial for optimal immune responses during infection. Mast cells seem to exert these effects by altering the inflammatory environment after detection of a pathogen and by mobilizing various immune cells to the site of infection and to draining lymph nodes. Interestingly, the character and timing of these responses can vary depending on the type of pathogen stimulus, location of pathogen recognition and sensitization state of the responding mast cells. Recent studies using mast cell activators as effective vaccine adjuvants show the potential of harnessing these cells to confer protective immunity against microbial pathogens.

Mast cells are a key cell type of the haematopoietic lineage that has evolutionarily conserved functions in pathogen surveillance. They are dispersed throughout most tissues but are crucially located at the host's interfaces with the environment, such as the skin and mucosae, supporting a role in the recognition of pathogens or other signs of infection (FIG. 1). Mast cells and many of their products are best known for their association with pathological conditions such as asthma, allergy and anaphylaxis, in which aberrant, chronic or systemic activation of mast cells promotes harmful inflammatory sequellae and damage to host tissues. However, despite the potential detrimental effects that mast cells can have on immune homeostasis, these cells are indispensable to the host, as suggested by the observations that they are evolutionary preserved across many species and that humans that lack mast cells have never been described[1]. The first strong evidence that mast cells function in a protective capacity against infectious disease came from studies of host–parasite interactions[2,3], and an increasing amount of work supports their essential contribution to controlling a wide range of pathogenic infections, including those by parasites, bacteria and probably viruses. We now understand that mast cells function not only as sentinels but also as modulators of innate and adaptive immune responses, ultimately influencing disease outcomes.

In this Review, we discuss recent advances in our understanding of mast cell responses to pathogens. We first discuss the potential mechanisms by which mast cells can be activated by pathogens. We then describe the responses of mast cells, particularly with regard to the timing of the responses and the various roles they have in host defence, as sensors of pathogens, as effectors of adaptive immune responses and as modulators of local inflammation. Finally, we examine the evidence indicating that mast cells make meaningful contributions to controlling infectious challenges and discuss how mast cells might be harnessed during vaccination.

Cell biology of mast cells

Mast cells arise from bone marrow-derived precursors that circulate in the blood and become differentiated after entering tissues. They are long-lived cells, able to survive for months or years and, despite being terminally differentiated, they can proliferate in response to appropriate signals[4]. All mature mast cells reside in the body's tissues and have a common fundamental morphology with prominent electron dense granules in their cytoplasm. At the earliest stages of infection, mast cells are important for communicating the presence of a pathogen to many cell types located nearby in the site of infection and distally in draining lymph nodes (FIG. 2). To facilitate these interactions, mast cells

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are strategically located at the host–environment interface, proximal to both blood vessels (FIG. 1a,b) and lymphatic vessels (FIG. 1c), as well as to nerve fibres (FIG. 1d) and tissue-resident immune cells, including dendritic cells (DCs) (FIG. 1e).

Despite having a common lineage, granulated morphology and functions, mast cells are highly heterogeneous and phenotypically malleable cells[5,6], the intricacies of which have only begun to be defined, with little known about their distinct functionality. However, it is likely that this heterogeneity is shaped by the requirements of residing in a particular tissue or encountering unique pathogen challenges. On the basis of distinct staining properties, it was quickly recognized that rodent mast cells fall into two broad categories: mucosal and connective tissue mast cell types. These distinct mast cell types can now be further distinguished by several features, including granule composition, differing degranulation responses to pharmacological stimulation and the ability to proliferate in response to parasitic challenge[7]. This suggests that responses to other stimuli, including pathogens, might differ depending on the mast cell type.

The main protein components of mast cell granules are proteases. In mice, the granules of connective tissue mast cells contain two types of protease — tryptases and chymases — that are bound to heparin, whereas mucosal mast cells contain only chymases, which are bound to chondroitin sulphate[7]. Human mast cells also show heterogeneity with regard to these two main protease types, although with less stringent tissue-type specificity[8]. The storage of proteases varies not only between mast cell subtypes but also within an individual mast cell depending on the stimuli it receives. For example, in mouse mast cells, the expression of these proteases has been shown to be modulated at a transcriptional level by interleukin-10 (IL-10)[9], and treatment of human mast cells *in vitro* with IL-4 increased the relative amount of chymase incorporated into granules[10]. Two types of human mast cell, defined by relative tryptase and chymase content, also vary with respect to their expression of the receptor for complement component C5a (C5aR)[11]. Although other inflammatory mediators and surface receptors might also have tissue-type or activation-specific specificity, the varied composition of granules (particularly well characterized for proteases) shows the heterogeneity of mast cells.

Armed with granules containing preformed mediators, mast cells have the potential to be the first responders (within seconds to minutes) following recognition of an invading pathogen. The findings that mast cells can respond to their environment — not only by producing appropriate mediators for the pathogen they have encountered, such as selective cytokine production[12], but also by altering the transcription and storage of preformed mediators[9,10] — suggest that they can modulate their phenotype during the course of infection. They also have the ability to replenish their granules during an infection or after its resolution[7,13]. Altering the production of preformed mediators and, thereby, the composition of their granules, if shown to be beneficial

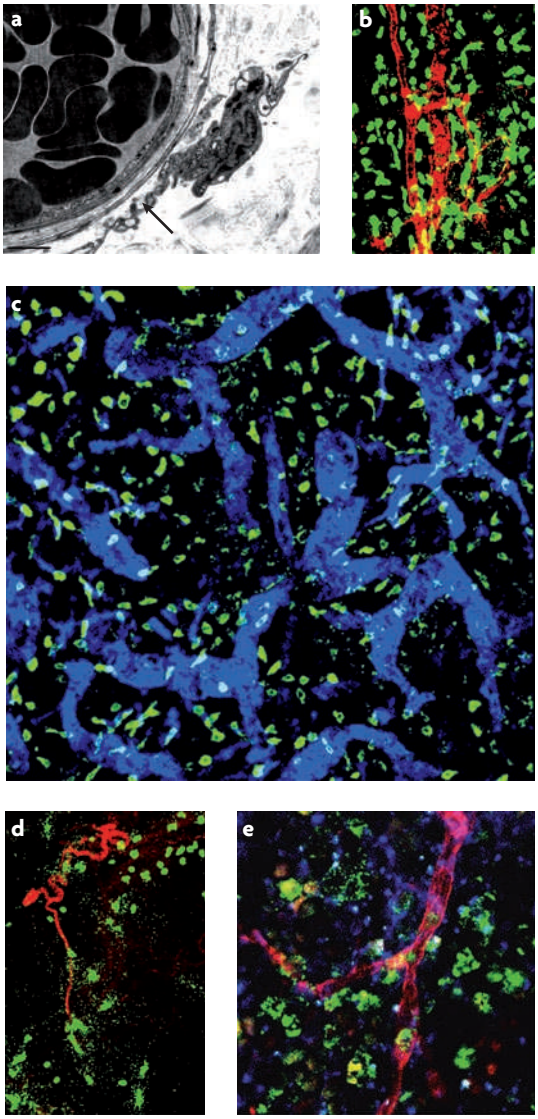

Figure 1 | **Mast cells are strategically located in host peripheral tissues. a** | A partially degranulated mast cell is visualized by electron microscopy, releasing granules near a blood vessel in mouse ear tissue activated by topical application of phorbol 12-myristate 13-acetate (PMA). The arrow indicates a granule that seems to be in the process of being released. **b** | Mouse ear tissue was stained in whole mount for CD31-expressing blood vessels (red) and mast cell granules, using a mast cell-specific fluorescent conjugated probe (green), after topical PMA treatment to activate mast cells. **c** | PMA-treated mouse ear tissue is stained to visualize LYVE1-expressing lymphatic vessels (blue) and mast cells (green). **d** | This image depicts tyrosine hydroxylase-expressing neurons (red) in close proximity to activated mast cells in compound 48/80-treated whole mounted bladder. **e** | Mouse ears were treated with PMA and prepared in whole mount for imaging by staining mast cells (green), CD31-expressing blood vessels (red) and CD11c-expressing dendritic cells (blue). For images in b–d, tissue was imaged at x10 magnification. Owing to whole mount preparation and large depth of field, overlap of stained elements can be seen.

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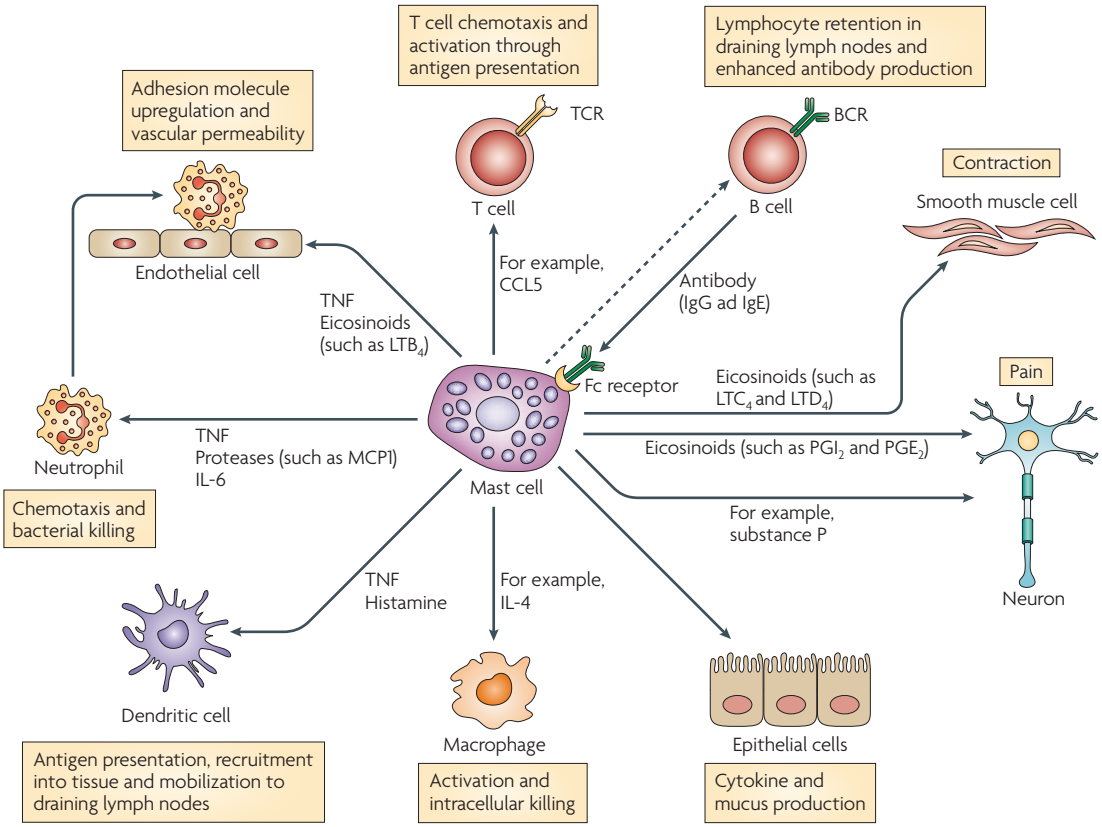

Figure 2 | **Cellular communication by mast cells promotes host defence.** Mast cells 'communicate' with various cell types, including immune cells (such as lymphocytes[50,67,71], macrophages[51], dendritic cells[41,46,49,61,68–70] and neutrophils[34,35,43,44,48,52]), epithelial cells[66], smooth muscle cells[45,63] and endothelial cells[49,57–60]. These interactions contribute to pathogen surveillance, antipathogen immunity and other mechanisms of eliminating microorganisms from the host. These cellular targets of mast cells are located both in the site of infection and in distant draining lymph nodes. Examples of functional consequences of mast cell communication are shown, as are examples of mast cell mediators that have been shown to contribute to the target cell response. BCR, B cell receptor; CCL5, CC-chemokine ligand 5; IL, interleukin; LT, leukotriene; MCP1, mast cell protease 1; PG, prostaglandin; TCR, T cell receptor; TNF, tumour necrosis factor.

in preventing or controlling reinfection, could be considered a form of immunological memory. This would allow the responses of pathogen-experienced mast cells to be refined by the infectious challenges they have previously encountered.

Mast cells as sentinels of infection

Direct recognition of microorganisms. At the initiation of infection, the first responsibility of mast cells is to recognize that pathogen invasion has occurred. Many cells, including DCs, epithelial and endothelial cells, can alert the host immune system to the presence of pathogens, whether in the skin, gut or other sites in the body that are exposed to the environment or susceptible to pathogen encounter. This can be achieved by directly recognizing pathogens through pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), which are activated in response to conserved pathogen-associated molecular patterns (PAMPs)[14]. Mast cells are well equipped for this task as, in addition to being able to recognize PAMPs, they can detect a range of products through the expression of other receptors that sense pathogens (for example, Fc receptors (FcRs), which bind pathogen-specific antibodies) and receptors for inflammatory factors produced at the site of infection. Direct pathogen recognition by mast cells occurs both in response to factors that are common to classes of pathogens (such as through TLRs) and those that are specific to only a certain infectious challenge (such as through binding of antibodies specific for pathogen-associated epitopes). Interestingly, mast cell responses to TLR triggering alone can vary depending on the PAMP stimulus. For example, lipopolysaccharide (LPS) stimulation of rodent mast cells through TLR4 promoted cytokine production in the absence of degranulation, whereas stimulation through TLR2 by peptidoglycan induced both degranulation and cytokine production[15]. In this study, responses to individual PAMP stimulation overlapped for some cytokines (stimulation of either TLR4 or TLR2 promoted the production of tumour necrosis factor (TNF), IL-6 and IL-13), but diverged for other cytokines (TLR4 stimulation resulted in IL-1β production but not IL-4 or IL-5 production, whereas TLR2 stimulation resulted in IL-4 and IL-5 production but not IL-1β production)[15]. Similarly, for mast cells derived from human cord blood, both peptidoglycan and LPS were shown to induce a T helper 2 (T_H2)-type

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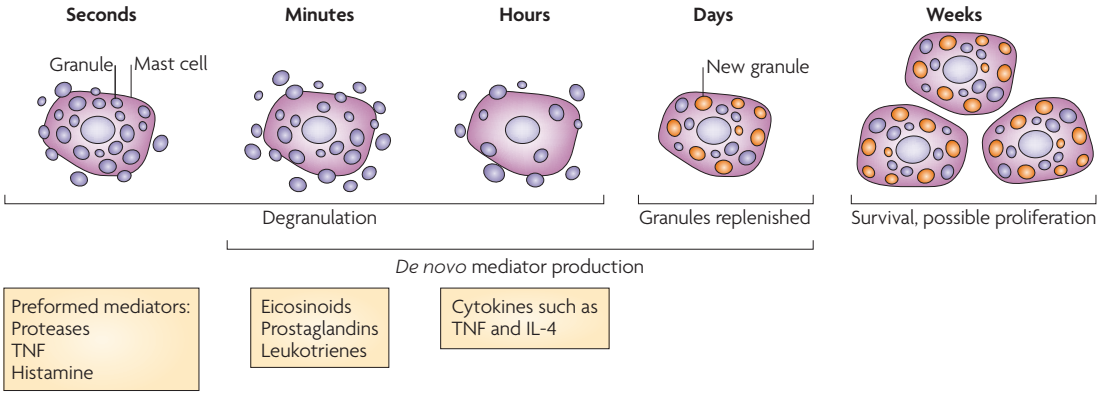

Figure 3 | **Timing of mast cell responses to pathogens.** Mast cells can respond quickly to pathogen challenges owing to the presence of preformed mediators in cytoplasmic granules that can be quickly released at a site of infection through the process of degranulation. Mast cells also begin to produce lipid-derived eicosinoid mediators in the initial minutes of activation, as transcription is not required for these mediators to be converted to an active form. In a second wave of the response, consistent with the processes that are initiated by other cells involved in pathogen surveillance, mast cells begin to release *de novo* synthesized mediators, including a large number of cytokines — such as tumour necrosis factor (TNF) and interleukin-4 (IL-4) — that are transcribed and translated in response to pathogens. They can also replenish their granules, possibly with altered contents, in response to inflammatory signals. Mast cells are unique in their ability to survive for prolonged periods after activation compared with other innate immune cell types that may begin to die during the contraction of the innate response. Finally, mast cells can survive in the tissues and might proliferate in response to appropriate stimuli.

cytokine response, however only peptidoglycan resulted in histamine release from intracellular stores[16]. In addition to conventional PRRs, other receptors on the surface of mast cells can be activated in response to pathogens; for example, CD48, which can detect the presence of fimbriated *Escherichia coli*, *Mycobacterium tuberculosis* and *Staphylococcus aureus*[17–19].

Activation by FcRs. Owing to the expression of multiple FcRs, including FcγRII receptors and the high-affinity receptor for IgE, FcεRI, mast cells can bind both IgG and IgE and become sensitized to antigens that have been previously encountered by the host. Subsequently, mast cells can become activated, resulting in degranulation following receptor cross-linking by polyvalent antigen[20,21]. Antibody-mediated mast cell recognition of specific antigens and the signalling events downstream of receptor cross-linking have been most thoroughly characterized in models of asthma and allergy, but are also likely to be relevant in the context of infection as suggested by one study examining parasite clearance[22]. Interestingly, it has been shown that FcεRI activation and TLR stimulation can have synergistic effects on cytokine production by mast cells, enhancing cytokine transcription through the cumulative increase in activity of mitogen-activated protein kinases (MAPKs)[23]. Mast cells can also be activated by FcR signalling triggered by bacterial superantigens, such as *S. aureus* protein A, which can to bind certain classes of antibodies, independent of antigen specificity[24].

Activation by pathogen-associated substances. Mast cells can also undergo degranulation in response to some exogenous stimuli that accompany pathogen injection into the skin or breaching of the skin barrier, such as components of wasp venom[25] or mosquito saliva[26]. Mastoparan, for example, is a 14-amino acid peptide found in wasp venom that efficiently induces mast cell degranulation[25]. Degranulation in response to mosquito saliva could have implications for immune defence against arboviruses or vector-transmitted parasitic diseases such as malaria, although this remains to be investigated.

Activation by endogenous inflammatory factors. Several host endogenous peptides, including neurotensin, substance P[27] and endothelin 1 (REF. 28), and by-products of inflammation, such as complement components, can also activate mast cells. Activation of mast cells through complement receptors, particularly C5aR, can result in degranulation[29]. This receptor was also identified as the main receptor responsible for mast cell detection of, and degranulation in response to, the yeast product zymosan, rather than TLR2 (which is generally ascribed the function of recognizing zymosan)[30]. The inflammation marker endothelin 1, which can have toxic side-effects during inflammation, is degraded by mast cell-derived proteases, showing an important feedback mechanism of mast cells in limiting potential detrimental effects of inflammatory processes through granule exocytosis[28].

Two waves of mediator release

As mentioned, activation of mast cells by pathogens can result in both degranulation and *de novo* cytokine synthesis. Degranulation involves the rapid (beginning within seconds to minutes following stimulation) release of pre-packaged, insoluble mediators into the surrounding tissue, a strategy that gives mast cell-derived products a temporal advantage over those produced by other immune surveillance cells (FIG. 3). Other sentinel cells, for example, Langerhans cells in

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the skin, tissue-resident DCs and various subtypes of epithelial cells show a comparatively delayed secretory response to pathogens owing to the requirement for *de novo* production of mediators. The two-phase process of mast cell secretion can affect the nature, duration and specificity of the host's responses to pathogen, for example through the contribution of mast cells to promoting the function of antigen-specific lymphocytes (discussed later).

Degranulation. As Paul Ehrlich, the first scientist to visualize and describe mast cells, noted, "the cell is chiefly of a chemical nature"[31], and so the earliest responses of mast cells depend on the structure and composition of their granules, which are formed and maintained as discrete insoluble structures in the cytoplasm by virtue of charge interactions. Following granule exocytosis, some granule-associated mediators, such as histamine, become soluble immediately, whereas most of the structure remains in an insoluble, particulate form. These exocytosed nanoparticles maintain their structure through tight interactions between negatively charged carbohydrate components, such as heparin, and positively charged proteins, most of which are proteases[7]. Granule proteins, such as TNF, can remain associated with the insoluble particles and be released slowly and for a prolonged time[32]. Moreover, these exocytosed particles can travel from the site of infection through lymphatic vessels to draining lymph nodes[32], suggesting that these structures have been physiologically tailored for long-distance delivery of inflammatory mediators. As the activity of a given amount of cytokine can be greatly enhanced when protected from degradation and dilution in the insoluble particulate structure, other cytokines may be identified that remain encapsulated within these particles and have functional effects beyond the site of inflammation, although limited quantities may make detection difficult.

Studies have begun to establish the importance of mast cell proteases to immune defence, including one study showing a role for mast cell protease 6 (MCP6; also known as tryptase-β2) in the host response to peritonitis[33]. Importantly, however, as proteases are structural components of granules, deficiencies in these proteins can result in altered granule contents and mediator storage. Despite this caveat to studying mast cell protease function, several lines of evidence implicate mast cell proteases in the recruitment of neutrophils to sites of infection[34,35]. Indeed, exogenous MCP6 can lead to the recruitment of neutrophils into the peritoneal cavity[32], and MCP6-deficient mice have impaired mast cell-mediated recruitment of eosinophils, resulting in defects in the control of a chronic parasitic infection[36]. Other mast cell-derived proteases, including human chymase, have also been shown to influence neutrophil recruitment[33]. Furthermore, mast cell proteases may have an important role in limiting the harmful effects of toxic host-derived by-products of inflammation, such as neurotensin (which causes hypotension during sepsis[37]) and endothelin 1 (REF. 28) (discussed earlier).

Many signalling mediators upstream of degranulation have been identified and these converge on a common requirement for generating a Ca^{2+} flux in the responding cell (reviewed in REF. 38). *In vivo*, integrated signalling pathways triggered by several stimuli contribute to degranulation. Indeed, exposure to a single stimulus, such as a PAMP, may not be sufficient to induce degranulation in experimental models, despite being able to contribute to degranulation *in vivo* and at the site of infection where many pathogen-derived products and pro-inflammatory endogenous products are present.

De novo *mediator production.* Similar to other cells, activation of mast cells by pathogens also induces *de novo* production of mediators such as cytokines and eicosinoids. Many studies have shown that *de novo* production of these mediators can vary greatly depending on the stimulus and the experimental conditions[39,40]. For example, IgE-mediated activation of human mast cells was shown to result in higher production of eicosinoids than the application of neurogenic peptides or pharmacological stimuli[40]. Leukotrienes, which are a type of eicosinoid, can be generated and quickly released by mast cells, owing to their lipid rather than protein structure and the requirement for only a catalytic conversion to the active form[41]. They function predominately at the local vascular endothelium, promoting the rolling and recruitment of neutrophils through the actions of P-selectin and neutrophil chemotaxis[42,43], and they contribute to defence against bacterial infection in mice[44]. Prostaglandins are another class of mast cell-produced, lipid-derived products that are produced quickly after mast cell stimulation. Few functions have been ascribed to them during infection, although in other models they, like leukotrienes, have been shown to contribute to vascular permeability, chemotaxis of various cells, mucus production and activation of nerve cells[41,45].

The list of cytokines and chemokines that mast cells can produce following stimulation is extensive and has been reviewed previously, with TNF, IL-4, IL-5, IL-6 and IL-3 being well characterized examples identified in several species[39]. Functions in host defence have not been ascribed to each mast cell-derived cytokine, so more work is needed to determine the individual contributions of these mast cell-derived factors to host defence as well as any synergistic effects they may have when produced in unique combinations. It is clear, however, that mast cell-produced cytokines and chemokines act both to activate local cells and to promote cell recruitment to sites of infection. For example, mast cell-derived TNF has been shown to promote DC activation and antigen presentation[46]. Mast cells are most often associated with T_H2-type inflammatory responses; however, more recently, mast cells were shown to be an important source of the T_H1-type cytokine IL-12 during peritonitis, which promoted neutrophil function and survival of infected mice[47]. Further actions of mast cell-derived cytokines can involve direct chemotactic responses, such as the TLR3-stimulated induction of cytotoxic T lymphocyte chemotaxis, or increased adhesion of leukocytes to the vascular endothelium,

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which occurs during *E. coli* infection through mast cell-derived TNF[48–50]. Recent studies have also shown that mast cell-derived cytokines can enhance bacterial killing: mast cell-derived IL-4 can promote macrophage killing of intracellular *Francisella tularensis*[51] and mast cell-derived IL-6 can enhance neutrophil killing of *Klebsiella pneumoniae*[52]. Furthermore, there is evidence that mast cell-derived cytokines can have an inhibitory function during infection; for example, intracellular IL-15 antagonizes the production of the chymase MCP2 (also known as MCPT2), which, in turn, adversely affected survival in a mouse model of sepsis[53]. The anti-inflammatory cytokine IL-10 is also produced by mast cells and can limit the extent of lymphocyte infiltration during contact hypersensitivity[54]; however, the potential contribution of mast cell production of IL-10 during infection has not been adequately investigated. As mast cells are increasingly being recognized for their negative regulatory or feedback responses[55], the contribution of this and other anti-inflammatory cytokines to counteracting either an already initiated immune response or other mast cell-associated processes, such as wound healing[4,56], should be a goal of future studies. Cumulatively, the range of possible mediators produced by mast cells allows them to follow a degranulation response with the production of factors that are suited to the activating stimulus, such as cytokines associated with the clearance of viral or bacterial infections. This flexibility allows mast cells to not only activate surrounding cells but also direct them towards appropriate containment and clearance of the particular pathogenic challenge.

Innate immune functions of mast cells

In models of bacterial pathogenesis, it has become clear that immune cell recruitment to sites of infection is facilitated by the location of mast cells in tissues. For the many mast cells located proximal to blood vessels, the release of factors, such as histamine, TNF, vascular endothelial growth factor (VEGF) and proteases, contributes to increased local vascular permeability and oedema at the site of infection[57–60]. Chemokine production by mast cells has been implicated in the recruitment of other participants in the inflammatory response, including eosinophils and natural killer (NK) cells, through CC-chemokine ligand 11 (CCL11; also known as eotaxin) and CXC-chemokine ligand 8 (CXCL8; also known as IL-8), respectively[12] (FIG. 4). Both mast cell-derived TNF and MCP6 have been reported to promote neutrophil recruitment in bacterial peritonitis models[34] and other inflamed tissues[52,61]. Recruitment of innate immune cells by mast cell products also probably occurs during viral infections, as it seems that the double-stranded RNA analogue polyinosinic–polycytidylic acid (polyI:C) promotes the production of a wide range of chemokines by human cord blood-derived mast cells, which in turn can induce NK cell chemotaxis[12] (FIG. 4).

Mast cells also produce products that have direct bactericidal activity. Both human and mouse mast cells can produce antimicrobial peptides known as cathelicidins. Mast cells from mice lacking the genes encoding these products were found to have defects in their ability to kill group A streptococci. Interestingly, these products seem to be produced constitutively by mast cells as well as induced in response to stimuli such as LPS[62]. In addition, mast cells produce compounds to aid bacterial killing after phagocytosis, including reactive oxygen species[4].

It is also important to consider the influence of mast cells on processes that impede pathogen colonization and promote their expulsion. For example, mast cells can influence the behaviour of nerve cells, the first cell type recognized as a target of mast cell signals, through products such as histamine (and serotonin in the case of mouse mast cells)[27]. Mast cells also communicate with smooth muscle cells[63]. Together, these interactions could potentially promote the elimination of pathogens from the body, such as that which occurs during mast cell-promoted expulsion of bacteria and fluids from the gut in response to cholera and clostridium toxins[64,65]. In addition, mast cells can promote mucus production by epithelial cells[66], a key process that immobilizes pathogens and aids in their clearance from surfaces such as the nasal mucosa, gut and bladder.

Promoters and effectors of adaptive immunity

After stimulation, mast cells shape the inflammatory milieu and control the activation state of many cells crucial for adaptive immunity[67]. In the site of infection, mast cell-derived TNF induces the upregulation of E-selectin expression by the local vascular endothelium, promoting the influx of monocyte-derived DCs, which are subsequently increased in draining lymph nodes[49]. The production of CCL20 by mast cells probably contributes to the recruitment of DC precursors from the blood and into the tissues[39]. Mast cells have also been shown to promote activation of Langerhans cells, a skin-resident DC subset, in response to the bacterial product peptidoglycan[68] or Gram-negative bacteria[49], which leads to increased numbers of Langerhans cells in the draining lymph nodes[49,68]. In addition, mast cell products can directly modulate DC activation and antigen presentation. For example, histamine has been suggested to promote antigen uptake and cross-presentation[69] and the upregulation of co-stimulatory molecules required for T cell activation[46]. Furthermore, mast cell products can promote DCs to acquire a T_H2 cell-inducing phenotype[70].

Mast cells might also promote the recruitment of effector T cells to sites of infection. In a viral peritonitis model, TLR3 activation on mast cells resulted in the upregulation of CXCL10 (also known as IP10) and CCL5 (also known as RANTES) and to the recruitment of $CD8^+$ T cells[50] (FIG. 4). This observation adds another facet to our understanding of how mast cells orchestrate the coordinated mobilization of immune cells during infection.

Moreover, mast cells themselves can present antigen to T cells. Early evidence suggesting that mast cells function as antigen-presenting cells (APCs) was provided by the findings that activated mast cells upregulate expression of MHC class II and co-stimulatory molecules and

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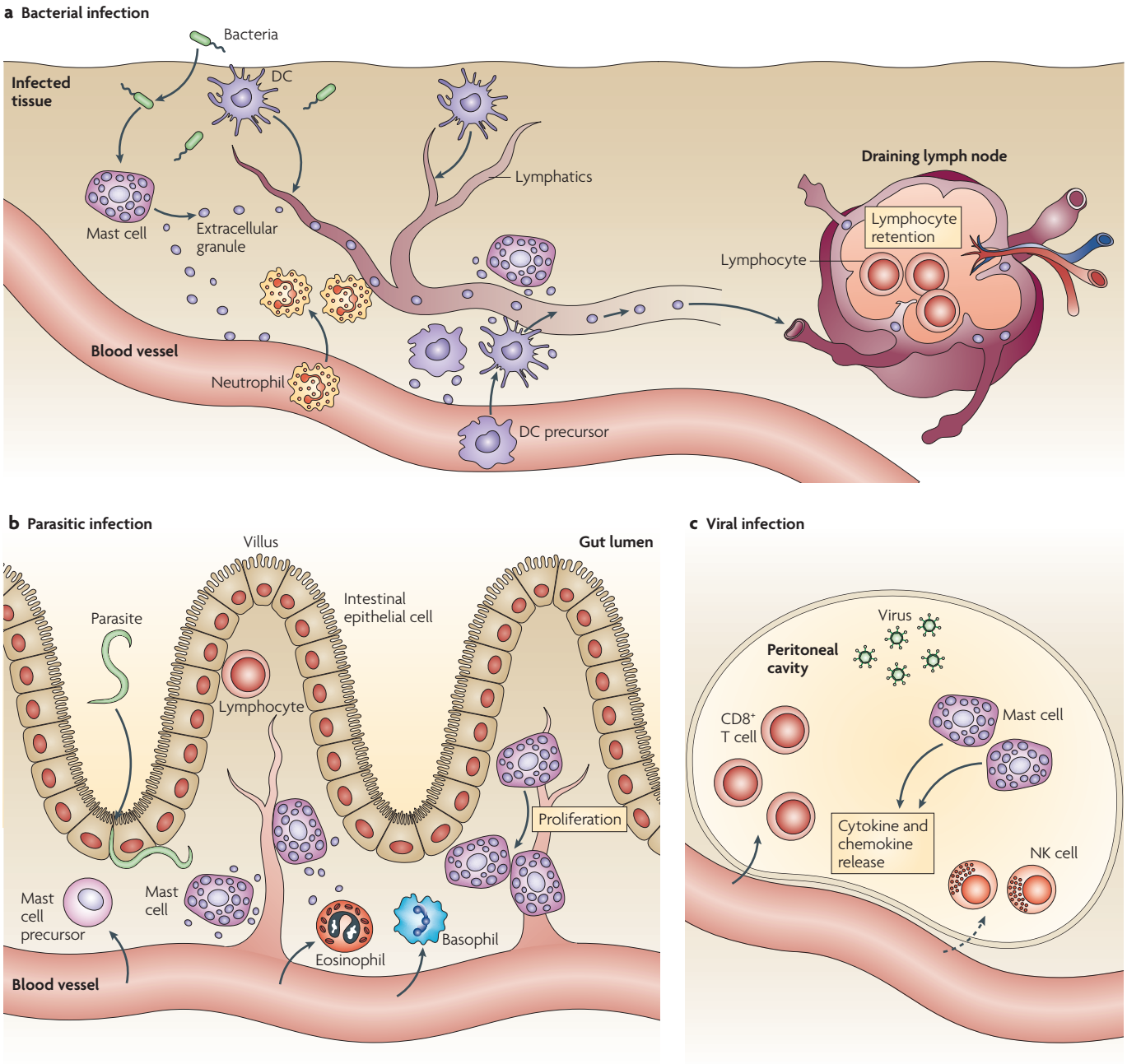

Figure 4 | **Cell trafficking responses induced or increased by mast cells.** Host control and clearance of invading pathogens requires the mobilization of many cell types, both into the site of infection for effective innate immune responses and into draining lymph nodes to initiate appropriate adaptive immune responses. The diverse and divergent cell types that are recruited into infected sites during various models of pathogenesis as a result of mast cell products collectively show the specificity of mast cell-promoted trafficking responses to individual pathogen challenges. **a** | After entry of a bacterial pathogen, mast cells can become activated and release products that promote many of the necessary cell trafficking events. In models of bacterial pathogenesis, neutrophils are recruited, which are largely responsible for pathogen clearance. Mast cells also enhance the trafficking of dendritic cells (DCs) through infected tissues by mobilizing the DC precursors from the blood and into infected tissues. The activation of several subclasses of DCs has been shown to occur as a result of mast cell activation during bacterial infections, resulting in enhanced trafficking of these cells from infected sites and into draining lymph nodes to initiate adaptive immune responses. Mast cell-derived particles from exocytosed granules can also flow into the lymphatics and travel to draining lymph nodes, where they promote the retention of lymphocytes during the process of lymph node hypertrophy. **b** | During infection with parasites, eosinophils, basophils and mast cell precursors have been reported to be recruited into sites of infection, such as in the gut. In addition, there is evidence that mast cells proliferate in parasite infection models. **c** | In viral infection, mast cell activation can promote the chemotaxis of $CD8^+$ T cells and natural killer (NK) cells to the peritoneal cavity or *in vitro*.

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that they have been visualized *in vivo* physically interacting with T cells[7]. However, the functional requirement of MHC class II-dependent antigen presentation by mast cells has yet to be fully evaluated *in vivo*. By contrast, there is now evidence that mast cells function efficiently as APCs for MHC class I-restricted $CD8^+$ T cells *in vivo*[71]. In this recent study, antigen-pulsed mast cells were shown to promote $CD8^+$ T cell activation, proliferation and production of T cell products such as IL-2 and granzyme B. This is the first report describing an important functional role for mast cells in antigen presentation[71] and, although shown in a model of autoimmunity, it raises the possibility that mast cells, acting as APCs, directly promote and modulate $CD8^+$ T cell function during infection.

Together with local responses to pathogens, mast cells have long-distance and long-term effects in the host by modulating draining lymph nodes and promoting the development of adaptive immunity to pathogens. As mentioned, mast cells can also influence cell trafficking to draining lymph nodes (FIG. 4). In an *E. coli* infection model, we showed that mast cell-derived TNF is required for normal lymph node hypertrophy, a crucial event in which draining lymph nodes double in size during the first 24 hours of infection owing to the retention of lymphocytes[49]. This process increases the probability that rare antigen-specific lymphocytes will be present in draining lymph nodes during the induction of adaptive immunity, probably improving the specificity of adaptive responses. Together, these two processes of DC trafficking from or through infected tissues to lymph nodes and lymphocyte sequestration in draining lymph nodes should increase the magnitude and specificity of the adaptive immune response by ensuring that appreciable peripheral antigen is shuttled to the draining lymph nodes and that, simultaneously, rare antigen-specific lymphocytes are retained there. We observed functional consequences of mast cell-promoted responses in enhanced humoral immunity to *E. coli* in wild-type mice compared with mast cell-deficient mice, including increased *E. coli*-specific antibody titres and protection after passive immunization[49], although it is currently unclear if mast cells have direct effects on B cells or antibody production.

As discussed previously, and best characterized in models of allergy, mast cells can function as effectors of adaptive immunity through their ability to become sensitized by binding antibodies through FcRs[7]. After a primary response, in which high-affinity pathogen-specific antibodies are present, antibody cross-linking of mast cell FcRs can result in faster responses of greater magnitude, allowing mast cells to participate in the enhanced immunity that results after immunological memory formation (FIG. 5). This may be particularly important during infection with some viral pathogens against which degranulation may not occur or may not be an immediate response. At least during chronic parasite infections, this sensitization can be important for ongoing control of latent infections[7,22,72], indicating that mast cells can translate a functional antibody response into protection against a pathogenic challenge.

Conversely, T cells can modulate mast cells, particularly through the production of chemokines such as CCL3 (also known as MIP1α) and CCL2, which probably contribute to mast cell degranulation, as well as through physical contact between mast cells and T cells[73]. *In vitro*, mast cell production of TNF and release of histamine can be enhanced by contact with T cells[73], suggesting that another feedback mechanism exists, by which the adaptive immune system might regulate mast cell function during an ongoing inflammatory process or infection.

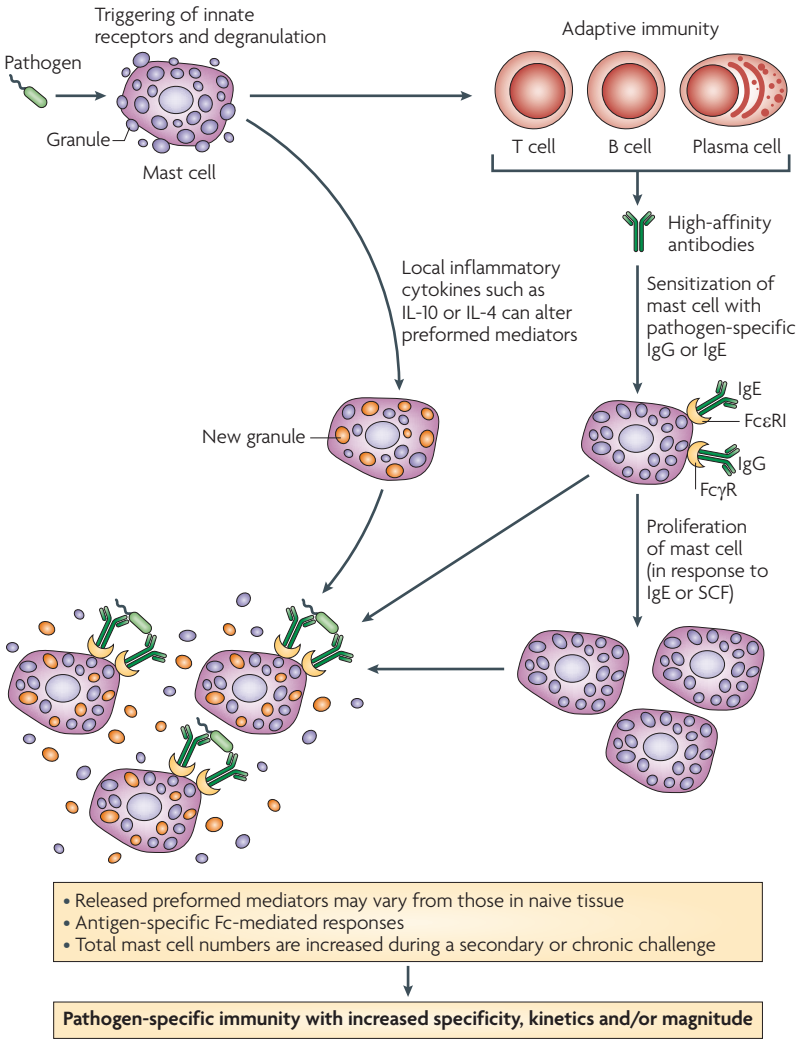

Figure 5 | **Pathogen-specific sensitization of mast cells enhances immune responses.** Tissue-resident mast cells are activated by many pathogens, and their products, through innate receptors and, as a result, promote adaptive immunity by modulating dendritic cell migration and cellular events occurring in distant lymph nodes. In addition, they can also be sensitized with various classes of immunoglobulins through Fc receptors (FcRs), enabling them to support more specific, amplified or quicker responses. This ability of mast cells to bind antigen-specific antibodies may be most crucial in secondary challenges, during chronic infection or in situations where innate recognition of a particular pathogen is not sufficient to initiate strong responses by mast cells. Furthermore, mast cells can replenish their granules after being activated and may alter the production of preformed mediators in response to the inflammatory milieu and the cytokines present there. This might result in a different degranulation response during the secondary challenge than during the initial challenge. IL, interleukin; SCF, stem cell factor.

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Pathogen-specific functional responses

The initial events following infection are key to containing rapidly replicating or host-adapted pathogens. Any delay in the initiation of antimicrobial efforts could potentially shift the advantage from the host to the pathogen, resulting in morbidity or mortality. Mast cells have been shown to initiate pathogen-specific programmes to functionally contribute to pathogen clearance and, in some experimental models, they have been shown to promote the survival of the host during an infectious challenge[48,74] (TABLE 1).

Parasites. The first observations that mast cells could have a role in the control of infection came from the use of models of helminth infection in the gut[2,3]. In these early studies, mast cells were observed to cluster around sites with parasites, although it was unclear at the time if these cells were proliferating or newly recruited, and they displayed an activated phenotype, with many cells undergoing degranulation[2]. It is now clear that the control or clearance of parasites by mast cells involves various mechanisms, including the recruitment of key immune cells, regulation of gut permeability and parasite expulsion, and containment of chronic infection[22,72,75–77]. Proliferation of mast cells during gut helminth infection was observed in one model to depend on the key mast cell growth factor stem cell factor (SCF; also known as KIT ligand)[78], and in another model to depend on IgE[79]. Studies of the requirement for mast cells during parasite infections have established that responses to pathogens vary greatly depending on the type of challenge. For example, a recent study examining hookworm infection indicated that expulsion of the parasite from the gut during a secondary challenge depended on basophils rather than mast cells, in contrast to expulsion during a primary challenge, which occurred much more quickly in mast cell-sufficient mice than their deficient counterparts[76]. In the case of infection with the parasite *Trichinella spiralis*, it was shown (using IgE-deficient mice) that IgE production by the host contributed to parasite expulsion from the gut[22]. In this study, the authors suggested that decreased levels of MCP1, previously shown to influence the speed of parasite expulsion from the gut, might explain these observations[22]. Mast cells also seem to be crucial for cutaneous immunity during the parasitic skin infection leishmaniasis, promoting protective immunity, including T cell function, and resulting in decreased skin lesion size[77]. The distinct functional roles of mast cells in various tissues are shown by these examples; however, the fundamental differences between responses require further characterization.

Bacteria. Mast cells are clearly essential for initiating both innate and adaptive immune responses to many bacterial pathogens and products, and for protecting the host from lethal infection[48,74]. Several of the earliest studies showed this by determining that mice deficient in mast cells show increased mortality after *E. coli* injection into the peritoneum than their wild-type counterparts[48,74]. In these peritonitis models, mast cells are functionally important for initiating innate immune responses to enterobacteria, particularly through their ability to recruit neutrophils to promote bacterial clearance[48]. These observations were reinforced by a recent study examining peritonitis caused by caecal ligation, however, during the most experimentally severe conditions, the mast cells in this study were no longer protective and the TNF they produced contributed to pathology[80]. Mast cells are crucial for containing bacterial infection and preventing dissemination in other tissues, and have been shown, for example, to promote the clearance of *E. coli* from the peritoneum and bladder[48,49] and *K. pneumoniae* from the lungs[48,52], as well as to limit skin lesions during *Pseudomonas aeruginosa* infection[81]. Based on the studies discussed above and others, the strongest functional evidence for the necessity of mast cell function to survival comes from studies involving bacterial challenges. Indirect activation of mast cells by host endogenous proteins can also highly influence the host response during bacterial infection, as shown by the contribution of mast cells to fluid production and neutrophil influx in the gut in response to neuron production of substance P, which occurs in the presence of toxin A from *Clostridium difficile*[82].

Viruses. The role of mast cells in viral infections is more enigmatic and has been less well studied. Many viral products can activate mast cells, in particular to promote cytokine production; however, the extent of mast cell degranulation in response to viral infection and any resulting functional implications are less clear. In support of direct recognition of viruses by mast cells, it has been shown that histamine release can occur in response to Sendai virus[83] and that the gp120 envelope protein of HIV can induce cytokine production by mast cells[84]. The ability of mast cells to promote the recruitment of $CD8^+$ T cells during viral challenge[50] (as previously discussed) or promote the production of type I interferons[85] suggests that mast cell recognition of virus may promote the types of cell-mediated responses that are associated with the clearance of intracellular viral infection. In both rodents and humans, mast cell numbers in the lungs are higher than uninfected controls following respiratory viral infections[86,87]. However, the interaction between viruses and mast cells does not always clearly favour the host. HIV, for example, is known to infect mast cells, and these cells may constitute a reservoir for latent virus within the host[88]. Other viruses that seem to infect mast cells include respiratory syncytial virus and dengue virus[89,90]; however, these studies were carried out in neoplastic cell lines and the consequences of these interactions remain to be explored *in vivo*.

Targeting mast cells in vaccines

Many parallels exist between the functions of mast cells *in vivo*, in particular their role in enhancing adaptive immune responses, and our requirements for efficient vaccine adjuvants. Thus, it was hypothesized that activation of mast cells during vaccination could potentially promote protective immunity. It has now been shown that addition of a mast cell activator, compound 48/80, to vaccine formulations can result in increased

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Table 1 | **Evidence for functional mast cell responses to pathogens**

Species	Tissue	Functional observation	Implicated factors	Refs
Parasites				
Trichinella spiralis (nematode)	Gut	Increased mast cell precursors in the gut	ND	93
		Parasite expulsion coincides with mucosal mast cell activation in rats	Parasite expulsion coincides with peak systemic MCP2 levels	2
		Reconstitution of mast cell-deficient mice with mast cells hastens parasite expulsion	Parasite expulsion delayed in mice lacking MCP1	72
		Mast cell defects result in increased numbers of larvae deposited in muscle cells	Eosinophil recruitment, but not parasite expulsion, delayed in the absence of MCP6	36
Nippostrongylus brasiliensis (hookworm)	Gut	Mast cell precursor mobilization from the blood and proliferation in the gut	IL-3 and IL-4	76,94,95
		Parasite expulsion coincides with mucosal mast cell activation in rats	Worm expulsion coincides with peak systemic MCP2 levels	2
		Accelerated expulsion of parasites during primary but not secondary infections in mast cell-sufficient compared with mast cell-deficient mice	ND	76
Strongyloides ratti (nematode)	Gut	Mast cell-deficient mice showed delayed parasite expulsion and higher peak larval numbers	ND	3
Strongyloides venezuelensis (nematode)	Gut	Mast cell-promoted parasite expulsion	IL-3 contributes to protection	96
Haemaphysalis longicornis (larval tick)	Skin	Decreased resistance to ticks in mast cell-deficient skin grafts compared with mast cell-sufficient skin grafts	ND	97
Leishmania major (protozoa)	Skin	Larger skin lesions with higher parasite burden and decreased cell recruitment in mast cell-deficient mice	Associated with reduced IL-12 in lesions	77
Plasmodium berghei (protozoa)	Parasitaemia	Mast cell-deficient mice have increased parasitaemia	Mast cell-derived TNF is protective	98
Bacteria				
Escherichia coli	Peritoneum	Mast cell-dependent recruitment of neutrophils and bacterial clearance	Dependent on mast cell-derived TNF and leukotrienes	44,48,74
	Skin	Mast cell-dependent lymph node hypertrophy, recruitment of DC precursors to site of infection and egress to draining lymph node	Mast cell-derived TNF increases E-selectin expression on vascular endothelium and lymph node hypertrophy	49,67
	Bladder	Enhanced bacterial clearance from bladder after passive immunization with sera from infected mast cell-sufficient mice compared with infected mast cell-deficient mice	Attributed to higher antigen-specific antibody titres in post-immune mast cell-sufficient animals	49,99
Citrobacter rodentium	Gut	Mast cell-deficient mice have decreased survival, increased histopathology and increased bacterial spread	ND	75
Mycoplasma pneumoniae	Airways	Mast cell-deficient mice have increased bacterial burden associated with increased lung pathology	ND	100
Francisella tularensis	Airways	Mast cell inhibition of bacterial replication in macrophages	Contact-dependent events and secreted IL-4	51
Klebsiella pneumoniae	Lung	Mast cell-dependent recruitment of neutrophils and bacterial clearance	Mast cell-derived TNF	48
	Peritoneum	Survival decreased in mice with mast cells lacking IL-6	Mast cell-derived TNF and IL-6 promotes neutrophil killing	52
Clostridium difficile	Gut	Toxin A from *C. difficile* induces mast cell-dependent neutrophil recruitment and intestinal fluid secretion based on studies with mast cell-deficient mice	Substance P-mediated activation of mast cells	82
Pseudomonas aeruginosa	Skin	Larger skin lesions in mast cell-deficient mice	ND	81
		Pronounced mast cell degranulation in wild-type mice and neutrophil accumulation	ND	

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Table 1 (cont.) | **Evidence for functional mast cell responses to pathogens**

Species	Tissue	Functional observation	Implicated factors	Refs
Bacteria (cont.)				
Caecal microflora	Peritoneum	Feedback inhibition of chymase transcription to constrict innate immunity	Intracellular IL-15	53
		Mast cell-dependent recruitment of neutrophils and bacterial clearance	Complement component C3 activation; mast cell-derived TNF is protective	101
		Decreased survival of mast cell-deficient mice	Reconstitution with wild-type but not IL-12-deficient mast cells corrects defect	47
Viruses				
Sendai virus	Lung	Mast cells increase after infection and airway hyperresponsiveness	ND	86,87
HIV	Systemic	gp120 protein acts as a superantigen and binds to IgE, activating FcεRI$^+$ cells	Release of IL-4 and IL-13 by FcεRI$^+$ cells	84,88
		Infected mast cells are an inducible virus reservoir		88
Newcastle virus	Peritoneum	Recruitment of CD8$^+$ T cells	Chemokine production including CCL5	50

CCL, CC-chemokine ligand; DC, dendritic cell; FcεRI, high affinity Fc receptor for IgE; IL, interleukin; MCP, mast cell protease; ND, not determined; TNF, tumour necrosis factor.

humoral immunity, which gives protection against a lethal viral challenge. When administered intranasally, these compounds can also promote antigen-specific IgA production, a key goal in the search for effective mucosal adjuvants[91,92]. It is likely that the mechanism of the enhanced responses is multifaceted and may involve many of the known interactions between mast cells and functional outcomes of adaptive responses including cellular mobilization, communication with draining lymph nodes and establishment of an environment at the site of vaccine administration that is similar to the environment in an infected tissue. These results indicate that mast cells can be intentionally activated to enhance protective host responses, including the production of high-affinity antibodies and immunological memory, and raise the possibility of incorporating mast cell activators in vaccine formulations to harness the inherent adjuvant activity of mast cell activation.

Concluding remarks

An increasing amount of experimental evidence supports the hypothesis that mast cells are essential for pathogen containment and/or clearance. They have the ability not only to affect immediate innate processes to clear pathogens but also to influence the long term host responses to pathogens. These mast cell contributions to adaptive immune processes occur directly (for example, through their antigen-presentation capabilities) or indirectly (such as through lymph node potentiation). The ability of mast cells to orchestrate complex cellular migration within tissues, from the blood and in distant lymph nodes (FIG. 3) is a key mechanism by which they bridge the processes of both innate and adaptive immunity and function to accelerate essential host programmes of defence. Further questions remain, however, particularly with regard to the functional contributions of mast cells acting as effectors of immunological memory during secondary challenges or during an ongoing process of chronic infection, topics that few studies have addressed. Future studies are also likely to provide more insight into the effects that the heterogeneity of mast cells within and between different tissues has on the functional outcomes of pathogen challenge, as current evidence discussed here suggests that mast cell responses may be highly specific, depending not only on the type of pathogen encountered but also on the tissue in which it is encountered. In addition, it will be important to assess the influence that a mast cell's experience of chronic or acute infection has on subsequent responses to pathogens, considering their long lifespan and phenotypic plasticity. The interaction between mast cell responses to pathogens and their involvement in other host inflammatory processes could be one area where these concepts are particularly applicable, as the complex interactions between chronic respiratory infections and airway hyperresponsiveness may show.

Mast cells have a kinetic advantage over other sentinel cells in initiating both innate and adaptive immune responses through their ability to store preformed mediators and release them nearly instantaneously into a site of infection. Furthermore, exocytosed granules seem to function as long distance delivery devices for their cargo, including inflammatory mediators. Long distance communication of cells is classically thought to be autocrine, in which large amounts of proteins, such as hormones, are released in a soluble form to achieve high enough serum concentrations to affect distant cell types. Yet, it now seems that mast cells have a newly discovered form of long distance communication that involves the targeted delivery of small quantities of mediators. Understanding this unique feature of mast cell biology raises the possibility of using this communication strategy for prophylactic or therapeutic potential. Thus, successful use of mast cell activators to enhance immune responses during vaccination confirms the potential contributions of mast cells to protection against subsequent challenges and opens the possibilities of targeting mast cells, or the processes they control, during rational vaccine design.

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Competing interests statement.
The authors declare no competing financial interests.

DATABASES
UniProtKB: http://www.uniprot.org
C5aR | CCL2 | CCL3 | CCL5 | CCL11 | CCL20 | CD48 | chymase | CXCL8 | CXCL10 | endothelin 1 | FcεRI | IL-1β | IL-3 | IL-4 | IL-5 | IL-6 | IL-10 | IL-13 | IL-15 | MCP1 | MCP2 | MCP6 | neurotensin | SCF | substance P | TLR2 | TLR3 | TLR4 | TNF

FURTHER INFORMATION
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