

Authors' addresses

Yoseph A. Mekori^{1,2}, Dean D. Metcalfe³,

¹Department of Medicine, Meir General Hospital, Kfar-Saba, Israel.

²The Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel.

³Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA.

Correspondence to:

Yoseph A. Mekori

Department of Medicine B

Meir General Hospital

44281 Kfar-Saba

Israel

Fax: 972 9 7423218

e-mail: ymekori@netvision.net.il

Summary: Mast cells are known to be the main effector cells in the elicitation of the IgE-mediated allergic response. The specific location of mast cells within tissues that interface the external environment, and the extent of their functional capacity, including the ability to phagocytose and to produce and secrete a wide spectrum of mediators, have led investigators to propose a potential role for mast cells in innate immune responses. Certain microorganisms have been found to interact either directly or indirectly with mast cells. This interaction results in mast cell activation and mediator release which elicit an inflammatory response or direct killing leading to bacterial clearance. The *in vivo* relevance of these *in vitro* observations has been demonstrated by the use of complement-deficient and/or mast cell-deficient and mast cell-reconstituted mice. It thus has been shown that both C3 and mast cell- and tumor necrosis factor- α -dependent recruitment of circulating leukocytes with bactericidal properties are crucial to a full response in certain models of acute infection. Modulation of mast cell numbers *in vivo* was also found to affect the host response against bacterial infection. Thus, mast cells do have a role in innate immunity in defined animal models of bacterial infection. Whether mast cells participate in innate immune responses in the protection of the human host against bacteria remains to be determined.

Introduction

Mast cells are known to be essential resident effector cells in the elicitation of the allergic response. IgE-sensitized mast cells, upon encounter with specific antigen that is recognized by surface receptor-bound IgE, secrete and generate bioactive mediators. These mast cell-derived mediators then generate the early initiation phase (vascular reaction and exudation) and contribute to the late phase (leukocyte accumulation and wound healing) of allergic inflammation (1–3).

Mast cell precursors, originating from pluripotential CD34⁺ c-kit⁺ CD13⁺ cells in humans or from Thy-1^{lo} c-kit^{hi} cells in the mouse, circulate in the blood and lymphatics and migrate into tissues, where they gain their mature morphologic and functional characteristics under the influence of local microenvironmental factors (1–7). Specific cytokines have been shown to differ in their effects on human and rodent mast cells. Interleukin (IL)-3 and the c-kit ligand, also known as

stem cell factor (SCF), are the principal cytokines that promote rodent mast cell proliferation and maturation. IL-3 has a minimal direct effect on human mast cell proliferation as opposed to SCF which, upon interacting with its tyrosine kinase c-kit receptor, induces human (bone marrow-, cord blood-, or peripheral blood-derived) mast cell growth and differentiation (8, 9). Several other cytokines have been found to promote or augment murine mast cell proliferation or differentiation (or both), including IL-4, IL-9 and nerve growth factor. In humans, however, IL-4 as well as IL-13 have been reported to inhibit, and in some instances to enhance, SCF-dependent mast cell differentiation. Granulocyte-macrophage colony-stimulating factor (GM-CSF) inhibits the development of mast cells from both mouse and human marrow (1, 3, 10).

Although mast cells in different tissues share many characteristics, they are not a homogenous population. They exhibit histochemical heterogeneity based on the cytoplasmic granule protein content. This was first observed in murine mast cells and later on in human mast cells and was found to reflect not only variation in staining properties of granules in mast cell populations at different anatomic sites, but also variations in cell size, cytoplasmic granule ultrastructure, mediator content, sensitivity to stimulation by secretagogues or growth factors, and susceptibility to various pharmacologic agents (1, 3, 11).

Mast cells reside at specific locations within the microenvironment, frequently poised strategically in tissues that interface with the external environment, and often in proximity to blood vessels and nerves. These cells are capable of reacting, both within minutes and over hours, to a variety of physical, biological and chemical stimuli with either local or systemic effects. Mast cells also react in a co-ordinated and graded manner which appears to ensure an appropriate response. There is also a more sinister side to these well engineered residents of the microenvironment, in that well meaning effector mast cell functions may result in annoying to catastrophic allergic reactions and perhaps contribute to the pathology of autoimmune diseases (12, 13). The locations of mast cells and the unique extent of their functional diversity no doubt account for the tendency of investigators to implicate mast cells in various biological responses other than IgE-dependent allergic responses.

It has been argued that mast cells represent a host defense system that evolved solely as part of protective mechanisms directed against parasitic infections (1, 14). This hypothesis as the only justification for this effector system appears no longer tenable. The body of information on the origin, differentiation and biologic potential of mast cells now suggests that these cells are more central to innate and adaptive immune responses than previously believed. Today, mast cells are considered to be

involved in multiple biologic processes. They are known to phagocytose particles, process antigens, produce cytokines and release vasoactive substances. Mast cells exhibit an array of adhesion and immune-response receptors that empower them with an advanced capability to react to multiple non-specific and specific stimuli. It thus seems clear that mast cells are incorporated into inflammatory processes dependent upon antigen-specific immunoglobulin-dependent responses in acquired immunity. But equally interesting is the potential role of mast cells in innate immunity, a possibility enhanced by the prominent location of mast cells at and within body surfaces that interface with the external environment.

Mast cell activation and mediator release

Mast cell activation may be initiated upon interaction of a multivalent antigen (allergen) with specific IgE antibody attached to the cell membrane via the high affinity receptor, FcεRI. Cross-linkage of IgE by the interaction of allergen with specific determinants on the Fab portion of the molecule brings the receptors into juxtaposition and initiates mast cell activation and mediator generation and release. Experimentally, IgE cross-linkage may be induced artificially by the use of anti IgE antibodies and FcεRI aggregation may be directly achieved using antibodies against the IgE receptor itself (1–3, 15, 16). Activation of mast cells then results in three types of biologic effects. 1) Mast cells undergo regulated secretion in which preformed contents stored in their granules are rapidly released by exocytosis. Mediators released include histamine, proteoglycans, proteases and cytokines such as tumor necrosis factor (TNF)-α and IL-16. 2) Mast cells enzymatically synthesize lipid mediators derived from precursors stored in cell membranes and in lipid bodies. These variably include, depending on the circumstances, the arachidonic acid metabolites prostaglandin (PG) D₂, leukotriene (LT) C₄ and platelet-activating factor (PAF). 3) Mast cells initiate transcription, translation and secretion of a diverse array of cytokines, including TNF-α, GM-CSF, SCF, IL-3, IL-4, IL-5, IL-6, IL-10, IL-13, IL-14 and IL-16, and expression of certain chemokines, such as macrophage inflammatory protein (MIP)-1α, MIP-1β, T-cell activation gene 3, lymphotactin and JE/monocyte chemotactic protein-1, again depending upon the mast cell type and stimulus (1–3, 16, 17).

Apart from activation via FcεRI, mast cells are also activated to variably release some of the same mediators following aggregation of surface FcγRIII molecules (13, 15) and following exposure to a range of small peptides, including the neuropeptides substance P and calcitonin gene related peptide (18, 19), endothelin (20), and β-chemokines such as MIP-1α and mono-

cyte chemoattractant peptide (MCP)-1 (21, 22). Other plasma factors generated during the inflammatory response such as C3a and C5a (termed anaphylatoxins) (23) and subfragments of fibrinogen and fibronectin (24) also recruit and activate mast cells. Even several prototypic allergens, such as phospholipase A₂ in bee venom or proteases associated with dust mite emanations, have been found to induce mast cell degranulation and IL-4 synthesis and release in the absence of antigen-specific IgE (25). Recently, heterotypic aggregation with activated T cells has been found to induce mast cell activation and mediator release (26, 27). This would thus allow mast cells to contribute to a lymphocyte-dependent targeted response.

Morphologically, degranulation produced through immunologic and non-immunologic pathways appears similar. However, biochemical processes that lead to mediator release as well as the nature of mediators released may differ. Also, the kinetics of mediator release as well as the types and amounts of the products released vary depending on the inciting stimulation (26). Thus, it has been suggested that some of the mast cell's adaptive functions may reflect the cell's ability to release, in localized microenvironments or in submaximal amounts, many of the same mediators and cytokines that are produced widely, rapidly and in large quantities during systemic allergic reactions (28).

Some of the stimuli that lead to mast cell activation and mediator release can be generated in the context of innate immune responses (reviewed in (28)). Notably these agents include specific microorganisms and their products, as well as C3a and C5a generated by non-specific activation of the complement system. Mast cells thus exhibit two basic mechanisms of microbial recognition (Fig. 1) (reviewed in (29)). The first is opsonin-dependent recognition. Here, microbes coated with complement component such as the iC3b are recognized by the C3 receptor expressed on the mast cell membrane. Mast cell binding to *Salmonella typhimurium* and to the helminth *Schistosoma mansoni* has been reported to involve this mechanism (30, 31). The second pathway involves opsonin-independent recognition in which specific receptors on mast cells and complementary ligands on the microorganism surface are thought to be involved. Examples of this mechanism of recognition of microbes include the binding of *Escherichia coli*, *Klebsiella pneumoniae* and *Leishmania* parasites to mast cells (32–34). It has been reported that mast cells specifically bind FimH, a 29-kDa mannose-binding lectin presented on type 1 fimbriae expressed by *E. coli* and other enterobacteria (32, 35). The specific receptor on the mast cell surface that binds FimH has recently been identified as the mannose-containing glycosylphosphatidylinositol-anchored receptor molecule CD48 (36). Thus, antibodies to

CD48 specifically blocked binding of FimH-expressing bacteria to the surface of mast cells, and FimH-expressing, but not FimH-deficient, bacteria mediated mannose-sensitive binding to recombinant CD48 (36). In the case of *Leishmania major* and *infantum*, rapid binding of live, but not attenuated, stationary metacyclic promastigote forms to mast cells have been noticed at 1 h of co-culture (34). This interaction is believed to be opsonin independent, as the level of parasite binding remained the same in serum-free conditions.

Significant mediator and cytokine release has been associated with microbe–mast cell interactions (Fig. 1). Thus, the mast cell–*Leishmania* aggregation was accompanied within minutes by a significant release of preformed mediators such as the granule-associated β -hexosaminidase and TNF- α , followed by the induction of TNF- α synthesis within hours (34). Fimbriated *E. coli* and several additional gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus faecium* were also found to induce histamine release as well as TNF- α production upon binding to murine peritoneal- or human cord blood-derived mast cells (33, 35). In certain cases, the magnitude of mast cell degranulation was directly proportional to the number of adherent bacteria on the cell surface in the initial period of the interaction (35). These effects were mediated, in the case of *E. coli*, by interaction with surface CD48, since antibodies directed against CD48 inhibited both the adherence of FimH-expressing *E. coli* to mast cells and the TNF- α response to the bacteria (36). As was shown for the *Leishmania* interaction with murine bone marrow-derived mast cells (BMMC), the interaction of certain bacteria with human cord blood-derived mast cells also resulted in both immediate (within 30 min) and late (after 3–6 h of co-culture) TNF- α release, reflecting substantial *de novo* synthesis and release of this cytokine (33).

Soluble mediators derived from infectious agents, such as toxins and cell wall constituents (i.e. lipopolysaccharide (LPS)), also activate mast cells. The effects of these microbial products are significant but varied. For example, staphylococcal enterotoxin B induces serotonin release from both murine and rat mast cells upon binding to a specific site on these cells (37). In contrast, both cholera toxin and the toxin A from *Clostridium difficile* failed to induce histamine release from rat peritoneal mast cells while variably influencing the release of certain cytokines. Thus, 10 ng/ml of *C. difficile* toxin caused a significant release of TNF- α at 4 h of incubation, while the cholera toxin significantly enhanced IL-6 production by mast cells but inhibited TNF- α production (38, 39). A differential effect on degranulation and cytokine release was also noticed when the effect of enterobacterial LPS on rat peritoneal mast cells was investigated. A substantial expression of IL-6 was seen which

was however not preceded by degranulation and histamine release (40). This bacterial constituent probably interacts with mast cells through pattern recognition receptor(s) (41).

Biological functions of mast cells

Mast cells are regarded as critical effector cells in the inflammatory reaction underlying the Th₂- and IgE-mediated immediate hypersensitivity responses. The biological manifestations of these responses represent the net effect of the various mediators produced and secreted by mast cells upon activation by the aggregation of FcεRI. These outcomes include bronchoconstriction and increased gastrointestinal motility due to smooth muscle contraction, changes in blood vessel tone, increased vascular permeability and mucus production by goblet cells. However, numerous observations support the possibility that mast cells also actively participate and contribute to the development and modulation of other inflammatory and physiological processes. The remarkable variety of mediators derived from mast cells and the recognition that mast cells release these mediators in response to an array of biologically distinct mechanisms in addition to FcεRI aggregation support this argument. Furthermore, changes in mast cell numbers in various anatomic sites and/or evidence of degranulation have been observed in a wide spectrum of innate, adaptive and pathological immune responses and in a large number of disease-related processes. These include delayed hypersensitivity reactions, parasitic or bacterial infections, fibrosis, autoimmune pathology, neoplasia, inflammation of the rheumatoid synovium and inflammatory bowel diseases (reviewed in (1, 42)).

An additional reason for attributing an important role for mast cells in various immune responses is that mast cell mediators have immunoregulatory or immunomodulatory effects. Histamine has been reported to have effects *in vitro* that are considered immunosuppressive, several of which are thought to be mediated by H₂ receptor-bearing suppressor T cells (43). Serotonin inhibits lectin-stimulated lymphocyte proliferation, possibly by downregulating the expression of IL-2 receptors on the surface of responder cells (44). Products of arachidonic acid oxidation dampen immune responses. As one example, PGE₂, produced upon activation of rat mast cells, has been reported to diminish IL-2 production *in vitro* (45). Heparin has been implicated in the suppression of immune responses by interfering with mitogen- or antigen-induced blastogenesis and by inhibiting migration of effector T cells to sites of antigen challenge, possibly due to its inhibitory effect on TNF-α production (46, 47). In contrast, LTB₄, PAF and several cytokines and chemokines produced by mast cells augment immune res-

ponses due to their potent chemotactic effects (1, 3, 16). Finally, the wide spectrum of cytokines and chemokines produced and secreted by mast cells contribute numerous possibilities for exerting important proinflammatory or other immunoregulatory roles.

In addition to classical IgE-mediated allergic responses, mast cells have been found to have a role in a range of other inflammatory reactions. For example, mast cells or their products have been shown to exert immunomodulatory roles by interacting directly with cells of the adaptive immune system (26, 27, 48) and presenting exogenous antigens to T lymphocytes *in vitro* (49). These effects result in T-cell proliferation and cytokine release, polarization of T cells towards the Th2 phenotype and induction of IgE production by B cells (reviewed in (27)). Relevant to innate immunity, mast cells have been shown to be pivotal in mediating leukocyte recruitment *in vivo* (50–52), to participate in acute inflammation (53, 54) and to play a role in defense against bacterial infections (55, 56). These effects may be attributed to the wide range of soluble mediators released from mast cells, as well as direct contact between mast cells and other immune cells.

Mast cells and leukocyte recruitment

Mast cells are strategically poised at perivascular sites to regulate inflammatory responses, and their effect on cell migration has been extensively investigated. The contribution of mast cells to neutrophil and mononuclear cell recruitment in IgE-induced late allergic responses has been verified by the use of mast cell-deficient mice (52, 57). Unlike normal mice, IgE-mediated stimulation in mast cell-deficient mice is not associated with a late significant cellular infiltration. This data is consistent with evidence that mast cell products released and generated during degranulation, including TNF-α, IL-1, IL-4, IL-6, IL-8, IL-13, PAF and LTC₄, are variously capable of affecting endothelial cells. Indeed, the antigen-dependent, IgE-mediated mast cell-induced late phase cutaneous response, which includes a profound leukocyte influx into mouse skin 6–12 h post-degranulation, is significantly reduced by anti-TNF-α antibody, suggesting that TNF-α contributes significantly to the delayed cellular infiltration in this response (57).

The capacity of mast cells to modulate endothelial cell function *in vivo* has also been supported by reports of increased E-selectin expression following dermal mast cell stimulation, mediated largely by TNF-α (58). Marked expression of both intercellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM) is similarly observed 6 h post-intradermal allergen challenge in atopic individuals (59). Increased bronchial ICAM-1 expression following allergen inhalation in

a sensitized animal relates to cellular infiltration and bronchial hyper-reactivity (60).

Activated mast cells, or their conditioned medium, induce T-cell adhesion to human endothelial cells or fibroblasts, a critical step in the promotion of local inflammation (59). This effect is associated with a significant increase in VCAM-1 and ICAM-1 expression as early as 4 h and which peaks 16 h post-exposure to mast cell degranulation products. The role of VCAM-1 and ICAM-1 in T-cell binding to endothelial cells was confirmed by the use of blocking antibodies. Thus, antibodies to ICAM-1 or VCAM-1 significantly reduced adherence, suggesting that lymphocytes required expression of both receptors for maximal adherence. The ICAM-1 upregulation by mast cell-derived mediators was almost completely neutralized by antibody to TNF- α while the expression of VCAM-1 was partially neutralized. These data implicate mast cell-derived TNF- α as having an important role in mediating ICAM-1-associated inflammation. Other mast cell-derived mediators such as IL-4 and IL-13 may further contribute in a synergistic manner with TNF- α to stimulate VCAM-1 expression (61).

Mast cell-derived cytokine-induced expression of adhesion molecules should be a relatively slow process, requiring protein synthesis for detectable expression. However, mast cell-dependent rolling and adhesion have been observed as early as 10 min after reperfusion of post-ischemic intestine, invoking a role for early mast cell-associated leukocyte recruitment in this inflammatory process (50). Indeed, it has been shown that mast cell degranulation induced leukocyte rolling on, and adhesion to, mesenteric venule endothelium within 30 min. The increase in leukocyte flux was blocked with the H1 blocker diphenhydramine and an anti-P selectin antibody. A PAF antagonist and an anti-CD18 (a ligand of ICAM-1) antibody decreased adhesion, but not leukocyte flux. These observations again support the conclusion that mast cells at perivascular sites have the capacity to initiate an early multistep process in leukocyte recruitment. This includes histamine-induced, P selectin-dependent leukocyte rolling and PAF-associated, CD18-dependent leukocyte adhesion. Thus, mast cell activation by a variety of secretagogues stimulates the leukocyte-endothelial cell interaction by the production and release of proinflammatory mediators. This in turn upregulates the expression of adhesion molecules necessary for cell migration from the intravascular compartment into sites of antigen challenge (50, 51).

Leukocyte recruitment to sites of inflammation, after encountering activated endothelial cells, is further influenced by specific chemoattractants released by neighboring cells. While mast cells clearly participate in this leukocyte recruiting process, and supernatants of activated mast cells are known to

have lymphocytic chemotactic activity, little has been known, until recently, of the specific molecules released by mast cells that are chemotactic for leukocytes. Such studies have now demonstrated that activated human leukemic mast cells release IL-8 (62). Similarly, mast cell-derived tryptase is known to induce both mRNA expression and secretion of IL-8 from human umbilical vein endothelial cells (63). Activated murine mast cells also release lymphotactin, a lymphocytic chemokine with preferential activity for CD8⁺ T cells. More recently, it has been shown that human BMMC and human lung mast cells are a source of IL-16. Upon treatment with phorbol myristate acetate (PMA) or the anaphylatoxin C5a, IL-16 mRNA levels increase. This is followed by the release of IL-16 protein. The secreted IL-16 is biologically active and is a chemoattractant for CD4⁺ T cells (64).

Phagocytosis and antigen presentation by mast cells

The ability to phagocytose and thereby to contribute to host defense was first attributed to mast cells a short time after their identification at the end of the 19th century. This phagocytic function of mast cells has now been analysed in some detail, at least *in vitro* (30, 32, 33, 65). FimH⁺ adherent bacteria were found to trigger mast cell phagocytosis, and subsequent killing of the bacteria was observed within acidified vacuoles and through the release of superoxide anions (32). Cord blood-derived cultured human mast cells phagocytose various gram-negative and gram-positive bacteria, a process that is associated with a release of TNF- α and subsequent killing of the phagocytosed bacteria. After 1 h of incubation, the viability of adherent *S. aureus* and *E. coli* was found to be 25% and 45% respectively (33). There are several additional mechanisms by which mast cells may kill microorganisms. These include both oxidative and non-oxidative systems, as well as secretion of granule-associated proteolytic enzymes such as chymase and protease II (29, 65).

Mast cells are able to present antigens to T cells in either an MHC class I or class II-restricted and co-stimulatory molecule-dependent fashion (49, 66). Both rodent and human mast cells have been reported to express MHC class II molecules, and to process and present immunogenic peptides to CD4⁺ cells, inducing lymphocyte proliferation and cytokine production. Mast cells are also capable of processing antigens following phagocytosis of a number of pathogenic gram-negative enterobacteria for class I MHC presentation to CD8⁺ T cells (66). This finding, and the ability of mast cells to produce IL-4, is of particular interest in the context of class I-restricted presentation to cytotoxic T cells (CTL). IL-4 has been shown to stimulate both proliferation and differentiation of CTL precursors and to

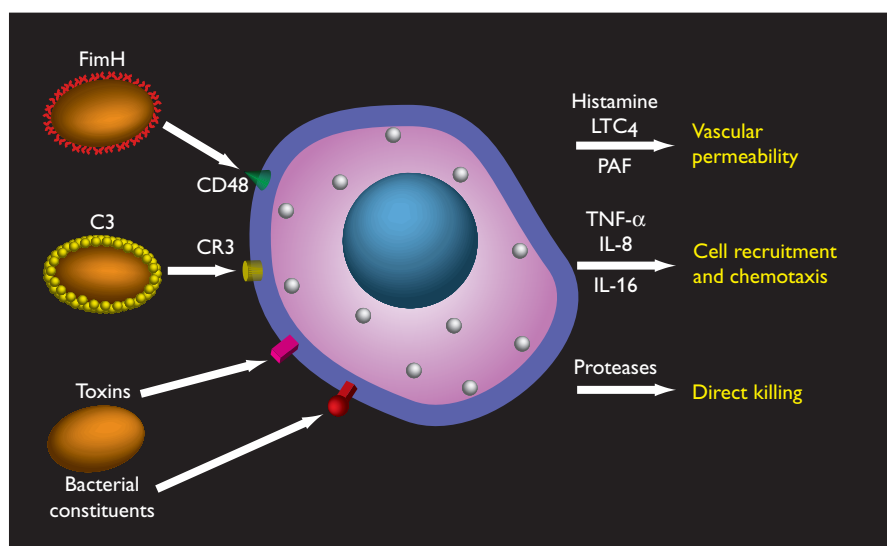


Fig 1. Mast cell–microorganism interactions. Microorganisms such as bacteria or parasites or their products interact with mast cells through specific receptors. This interaction results in mast cell activation and mediator release which elicit an inflammatory response or direct killing, leading to bacterial clearance.

enhance the CTL response to alloantigens in a mixed lymphocyte reaction. Thus, mast cells may be involved in the generation of CTL responses to bacterial antigens *in vivo* (66).

The contribution of these findings to innate or acquired immune responses has to be cautiously evaluated. This is because there has been little analysis of the phagocytic effect and antigen processing by mast cells *in vivo*, especially in comparison to those other “professional” phagocytes or antigen-presenting cells (28). Moreover, the capacity of mast cells to phagocytose and kill bacteria may be subverted by certain bacteria and parasites to their advantage (29), rendering these cells as facilitators rather than inhibitors of acute infections. Nonetheless, the ability of mast cells to immediately respond to specific microorganisms and antigens points to a linkage between the initial activation of cells of the innate immune system and subsequent adaptive immune responses (67, 68).

Mast cell participation in innate immune responses *in vivo*

Convincing proof for the importance of mast cells in specific immunologic or pathological responses *in vivo* has been difficult to obtain (28). Many mast cell-associated mediators are produced by other cell types. As a result, evidence that one of these mediators has a critical role in a particular biological response is not by itself sufficient proof that mast cells are important in that response. A similar argument may be made about pharmacological experiments employing antagonists of specific mast cell-associated mediators or inhibitors of mast cell activation. As valuable as this approach is for evaluating the function of each individual mediator, resulting data may provide incom-

plete or, in some cases, misleading information as to the role of the intact mast cell, since other target cells may also be implicated (69). Mast cell activation also results in the elaboration of several mediators, which may have diverse and sometimes opposing biological effects, further complicating the analysis of mast cell functions *in vivo*. It is therefore difficult to predict the net effect of mast cell degranulation. Finally, mast cell heterogeneity, which includes variations not only in morphology but also in mediator content and response to secretagogues and drugs, makes it difficult to offer succinct generalizations about mast cell function in biological responses.

The availability of genetically mast cell-deficient WBB6F₁-Kit^W/Kit^{Wv} (W/W^v) mice bearing mutations in c-kit has enabled investigators to attempt to evaluate the contribution of mast cells to various biological systems (28, 70). These mice are also anemic and lack germ cells and melanocytes. These defects reflect consequences of the mutations affecting c-kit, which result in a marked reduction in the signaling that depends on kit receptor tyrosine kinase in kit-bearing cells (9). Nonetheless, the multiple consequences of mutations in c-kit must be taken into account when interpreting data obtained in this model.

The experimental approach using mast cell-deficient mice is to compare the expression of a biological response in these mice to their normal littermate +/+ controls. If a significant difference between these two groups is demonstrated, the next step is to determine whether it is specifically related to mast cells. This question can be addressed by testing whether the difference in results obtained with the W/W^v and +/+ mice is abolished after the mast cell compartment in W/W^v mice is

restored by local reconstitution of the target organ with cultured mast cells (70). One may also substitute cultured mast cells with products known to affect the cells' development, survival and/or function (71).

In recent years, there have been several studies using mast cell-deficient mice to investigate the potential role of mast cells in innate immune responses. In these studies, mast cells have been found to amplify many features of immunologically non-specific acute inflammatory responses, and to contribute to innate host defense mechanisms against bacterial infection by affecting the recruitment of circulating leukocytes with bactericidal properties to the site of infection.

Mast cells in acute inflammation

Studies in mast cell-deficient and reconstituted mice have demonstrated that mast cells in specific, albeit limited, models of inflammation amplify many features of immunologically non-specific acute inflammatory responses. These reactions are characterized by an influx of leukocytes (predominantly neutrophils), edema, and changes in the caliber and permeability of the microvasculature. Using the experimental approach described above, mast cells have been shown to be required for the full expression of the tissue swelling and leukocyte infiltration associated with the response to the epicutaneous application of PMA or to the intraperitoneal injection of thioglycollate (53, 54). Mast cells have also been found to be important for the induction of a neutrophil influx in immune complex-mediated responses. This effect has been attributed to the ability of mast cells to produce and secrete TNF- α , as TNF- α levels were significantly reduced in the mast cell-deficient W/W^v mice in association with the decrease in local neutrophil accumulation. Mast cell reconstitution normalized TNF- α levels and neutrophil mobilization (72). This induction of immune complex-mediated mast cell-dependent inflammation was shown to involve IgG Fc receptors. This suggests that mast cells may not only contribute to but also initiate these responses, as mouse mast cells express Fc γ RII and Fc γ RIII (13).

Mast cells and their mediators also play a central role in the elicitation of a polymorphonuclear (PMN) leukocyte influx in zymosan-induced acute peritonitis in mice. This effect has been attributed to the local production of the CXC murine chemokine KC and the CC chemokine MCP-1. Although mast cells accounted for a large part of the release of these two chemokines, since their removal inhibited both PMN accumulation and chemokine production, the possibility could not be excluded that other mediators secreted by mast cells may have caused KC and MCP-1 release from another resident cell types (73).

Mast cells in acute infection

By using mast cell-deficient and reconstituted mice, several groups have recently shown that mast cells represent a central component of host defense against bacterial infection. It has been shown that mast cell- and TNF- α -dependent recruitment of circulating leukocytes with bactericidal properties is crucial to a full response. The TNF- α released by mast cells is apparently in response to contact with the bacterial component FimH, serving an important role in the generation of the associated influx of neutrophils to the site of infection which results in bacterial clearance (55, 56). Thus, as shown in two different models of acute bacterial infection, W/W^v mast cell-deficient mice were less efficient in clearing the pathogenic bacteria from the peritoneum in cecal ligation and puncture (CLP)-induced peritonitis and from the lungs in mice intranasally challenged with *K. pneumoniae*. Infected W/W^v mice showed a significant increase in mortality compared to their normal littermate +/+ controls or to mast cell reconstituted mice (55, 56). The limited bacterial clearance from the infected lungs in W/W^v mice directly correlated with impaired neutrophil influx. Moreover, local concentrations of TNF- α were found to be higher in +/+ and in mast cell-reconstituted W/W^v mice, and administration of antibodies against TNF- α was associated with decreased neutrophil flux (into the peritoneum) and increased mortality in +/+ and mast cell reconstituted mice which were intraperitoneally challenged with type 1 fimbriated enterobacteria (55). Evidence was provided that the TNF- α was secreted from mast cells upon contact with the pathogenic bacteria via FimH and, indeed, FimH-deficient mutant bacteria evoked a limited neutrophil response in +/+ mice compared to the wild-type bacteria (55). A more recent study has demonstrated that mast cell degranulation and bacterial clearance in acute peritonitis induced by CLP is complement dependent. C3-deficient mice exhibited reductions in peritoneal mast cell degranulation, production of TNF- α , neutrophil infiltration into the peritoneum and bacterial clearance (74). These complement-deficient mice exhibited a significantly higher rate of mortality compared to wild-type controls. Thus, certain types of bacterial infections may induce local complement-dependent mast cell degranulation which leads to mast cell mediator release and a TNF- α -mediated influx of neutrophils with bactericidal activity. Enhancement of neutrophil infiltration in these settings may reflect a number of different actions of TNF- α , such as upregulation of E selectin on venular endothelial cells, induction of neutrophil chemoattractants such as IL-8 and direct upregulation and activation of integrins on leukocytes. The precise mechanism of complement activation in this model system of acute peritonitis as well as the

mechanisms of complement-dependent mast cell activation remain to be determined.

In support of the above observations, there have been two recent reports demonstrating that manipulation of local mast cell numbers and/or function improves the animal's ability to express innate immunity. Thus, $\beta 2$ integrin Mac-1 (CD11b/CD18, CR3)-deficient mice have a significant reduction in peritoneal mast cell numbers and exhibit increased mortality from acute bacterial peritonitis which is associated with an increased bacterial burden. The neutrophil accumulation in the peritoneum is also diminished in the Mac-1 mutants as compared to the wild-type mice (75). By using the same experimental model of CLP-induced acute bacterial peritonitis, it has been demonstrated that repetitive treatment of normal or W/W^v-mast cell reconstituted mice with SCF results in increased peritoneal mast cell number and improved survival after acute peritonitis. There was no significant change in either mortality rate or neutrophil influx in mast cell-deficient W/W^v mice treated with SCF, indicating that the effect of SCF treatment reflects, at least in part, the action of SCF on mast cells (71).

The case of mast cell involvement in the host defense against parasitic infections is diverse (reviewed in (1, 14)). Mast cell hyperplasia and activation are observed in intestinal helminthic infections. Moreover, there is evidence of local mast cell activation at sites of infection, and the elevation of systemic mast cell products has been associated with the expulsion of certain parasites. Mast cell precursors have been shown to migrate from the peripheral blood and bone marrow to the intestine during parasitic infections (76). The proliferation of mast cells in the intestine appears to be T-cell dependent and involves the cytokines IL-3, IL-4, IL-5 and IL-9 (77). During primary infection, mast cells may be activated by parasite components, and later in the course of infection, by parasite-specific IgE (1, 25). Mast cell products may either cause direct damage to the parasite (65, 78) or lead to expulsion by promoting mucus hypersecretion and increased intestinal motility (14, 79), as well as by affecting ion transport and fluid influx (80). However, there does not seem to be a strict requirement for mast cells *in vivo* in the successful resolution of these infections. Depending on the system employed, mast cells thus enhance parasite expulsion to varying degrees (1).

Conclusions

It is intriguing to speculate that at least one reason that mast cells appear concentrated on surfaces that interface the external environment is that they play a prominent role in innate host

defense mechanisms against infectious organisms. This hypothesis is further strengthened by the demonstration that mast cells *in vitro* may be activated by direct interaction with bacteria and in some cases through specific receptors. Further, this interplay results in the release of mediators, including TNF- α , that are suspected of having a major role *in vivo* in host defense (Fig. 1). When this hypothesis is taken into animal studies in defined models of infection, there is supporting evidence that mast cells do indeed play a role in innate immunity in, for example, the protection of mice against peritonitis in a model that involves bowel puncture. What is needed now is to show that mast cells do indeed participate in an innate immunity in the protection of the human host against bacteria.

Unfortunately, there is no published information to date that clearly implicates mast cells in protection of the human organism against bacteria. Such evidence will be difficult to obtain. Patients experiencing profound neutropenia, and patients with defects of neutrophil function, have led to clear and convincing evidence that neutrophils are an essential element in host defense against bacterial diseases. There are no known equivalent model systems that implicate mast cells. There is no human disease known to be associated with a defect in mast cell function or that results in mastocytopenia; increased susceptibility to infections results from mastocytopenia and would thus support the role of mast cells in innate defense mechanisms against bacteria. In fact, one could argue the opposite. Mast cells remain in normal numbers in situations where bacterial infections are common, particularly in individuals undergoing chemotherapy and where a falling neutrophil count is often implicated. Mast cells have not been reported to increase, nor to be shown to be activated in human peritonitis; overwhelming sepsis is not associated with evidence of mast cell hyperplasia or systemic release of mediators. There simply are no adequate studies of mast cell activation in tissues where pyogenic inflammation is present. Thus, the possibility does remain that mast cells may participate in innate host defense mechanisms against bacteria but are not essential for this protection, nor sufficient in themselves. It is clear that innovative studies in human diseases need to be performed to further explore the hypothesis that mast cells are involved in innate immunity and confirm the elegant *in vitro* studies and animal models of infection that have suggested this as a real possibility.

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