

CHAPTER 11

MAST CELLS AND IMMUNOREGULATION/ IMMUNOMODULATION

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Abstract: Mast cells often represent one of the first cells of the immune system to interact with environmental antigens, invading pathogens or environmentally-derived toxins. Mast cells also can undergo alterations in phenotype, anatomic distribution and numbers during innate or adaptive immune responses. In addition to their well-known roles as effector cells during IgE- and antigen-induced allergic reactions, mast cells can be activated by many other signals, including some that are derived directly from pathogens or which are generated during innate or adaptive immune responses. Mast cells also express many costimulatory molecules with immunoregulatory activities and can secrete many products that can positively or negatively regulate immune responses. In this chapter, we describe mouse models used for analyzing mast-cell function in vivo and illustrate how such models have been used to identify positive or negative immunomodulatory roles for mast cells during specific innate or adaptive immune responses. We also briefly describe some of the mast-cell functions, products and surface receptors that have the potential to permit mast cells to promote or suppress immune responses that can either enhance host defense or contribute to disease.

INTRODUCTION

Mature mast cells are long-lived tissue resident cells distributed widely throughout vascularized tissues. Large numbers of mast cells can be found near body surfaces in the skin, airways and gastrointestinal tract.¹⁻³ Mast cells, with dendritic cells (DCs) and monocytes, thus potentially represent one of the first cells of the immune system to interact

with environmental antigens and allergens, invading pathogens or environmentally-derived toxins. Mast cells can re-enter the cell cycle and proliferate following appropriate stimulation and increased recruitment and/or retention and local maturation of mast-cell progenitors can also contribute to the expansion of mast-cell populations in tissues.¹⁻⁴ Expansion of mast cell numbers, local changes in their tissue distribution and alteration in their phenotypic characteristics can occur as a result of persistent inflammation and tissue remodeling.¹⁻⁴

In addition to their well-known function in IgE-dependent responses, mast cells can respond to a variety of innate signals derived from pathogens, venomous animals, or the activation of complement, or from other host cell types, such as neurons.⁵⁻⁹ Moreover, mast cells are equipped with a wide spectrum of costimulatory molecules that have immunoregulatory functions and also represent a potential source of many potent chemical mediators, growth factors, chemokines and cytokines, some of which can be rapidly released upon mast cell activation. With properties such as these, mast cells have the potential to regulate the transition from innate to acquired immune responses through effects that can either enhance or suppress the development, survival, proliferation, migration, maturation, or function of other immune cells.^{2,3,6,7,10-13}

During individual biological responses, mast cells can function as effector cells, immunoregulatory cells, or both (Table 1). Mast cells can function as effector cells during innate^{6,8,11,14,15} or acquired^{6,7,10,12,13,16} immune responses. “Effector functions” of mast cells include killing pathogens,^{11,14,15} degrading potentially toxic endogenous peptides¹⁷⁻¹⁹ or components of venoms^{18,20} and regulating the numbers, viability, distribution, phenotype or “non-immune” functions of structural cells, such as fibroblasts and vascular endothelial cells. Mast cells can exert effector functions through the direct or indirect actions of a wide spectrum of mast-cell-derived products and such effects can be observed in both innate^{6,11,14} and acquired^{6-8,10,12,13} immune responses.

Mast cells also can influence many aspects of the biology of immune cells, defined herein as cells of hematopoietic origin that participate in innate or acquired immune responses, including granulocytes, monocytes/macrophages, DCs, T cells, B cells, NK and NKT cells, etc. Effects of mast cells on the recruitment, survival, development, phenotype or function of immune cells are herein defined as “immunomodulatory functions”.

Through effector and/or immunomodulatory functions, mast cells can promote the initiation and increase the magnitude of, the inflammation, tissue remodeling and, in some cases, tissue injury associated with immune responses, including innate or adaptive immune responses to pathogens as well as allergic or autoimmune disorders.^{6-8,10-16,21} In vitro studies suggest that mast cells also can promote the development and extent of acquired immune responses through functions such as antigen presentation and many different effects on the biology of DCs, T cells and B cells.^{12,15,22} However, few of these potential functions have been confirmed in vivo.

Given the many mechanisms by which mast cells can enhance the initiation or magnitude of immune responses, mast cells are often thought of as cells whose primary role is to “turn immune responses on”. However, several lines of recent evidence indicate mast cells can also reduce the inflammation, tissue remodeling and tissue injury associated with immune responses.^{12,13,23,24} Accordingly, a new picture of the mast cell is emerging: these cells have the potential to help turn immune responses off, as well as to turn them on. T cells specialized to down regulate immune responses are referred to as T regulatory (T_{Reg}) cells.²⁵ However, there so far is no evidence for the existence of a specific developmentally and phenotypically distinct “subset” of “immunoregulatory mast cells” specialized to down- regulate or suppress immune responses. Accordingly, we herein will refer to anti-inflammatory or

Table 1. Effector and immunomodulatory functions of mast cells^a***Effector functions^b***

Promote clearance of pathogens by phagocytosis and/or secretion of antimicrobial peptides
 Degrade potentially toxic endogenous peptides and components of venoms
 Increase vascular permeability (e.g., by histamine)
 Stimulate bronchial smooth muscle–cell contraction (e.g., by leukotriene C₄)
 Promote fibroblast collagen synthesis (e.g., by tryptase)

Immunomodulatory functions^c*Examples of positive immunomodulatory functions*

Promote the migration, maturation, differentiation and function of immune cells via secretion of factors such as TNF, chemokines, histamine, LTB₄ and proteases
 Present antigen to T cells (via MHC class I or II molecules) or enhance Antigen presentation by capturing IgE-bound-antigen via FcεRI and then undergoing apoptosis
 Promote B-cell IgE production (through IL-4, IL-13 and CD40L)
 Promote B-cell IgA production (through IL-6 and mast cell-B cell proximity)
 Promote expression of TSLP on epithelial cells (for example, by TNF, IL-4 and IL-13)
 Promote recruitment of immune cells by production of TNF and other mediators that upregulate adhesion molecule expression on vascular endothelial cells
 Promote T_H2 responses via effects of prostaglandin D₂ on DC maturation
 Promote airway smooth muscle production of chemokines and cytokines (via TNF, IL-4 and IL-13)
 In the presence of physiological levels of IgE, promote sensitization in certain models of CHS (via increased migration of skin DCs and perhaps other functions)
 In response to certain activators of mast cells used in conjunction with vaccines, enhance development of protective adaptive immune responses to pathogens

Examples of negative immunomodulatory functions

Suppress sensitization for CHS (via UVB-induced production of histamine)
 Promote peripheral tolerance to skin allografts (via mechanisms that remain to be defined)
 Mediate Anopheles mosquito-bite-induced suppression of development of certain T-cell-dependent responses (via effects of mast cells that remain to be defined)
 Suppress many features of certain models of severe CHS, in part via effects of IgG1/mast cell-FcγR-dependent production of IL-10
 Mediate jet fuel-induced suppression of CHS (via PGE₂ and perhaps IL-10)
 Suppress many features of the pathology of chronic low-dose UVB irradiation of the skin, in part via effects of 1α,25-dihydroxyvitamin D₃-mast cell-VDR-dependent production of IL-10

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Table 1. Continued

Suppress cytokine production by T cells and monocytes (via IL-10)
Suppress production of pro-inflammatory cytokines and chemokines by keratinocytes (via IL-10)
Enhance ability of DCs to reduce T-cell proliferation and cytokine production (via IL-10)

a) This table is modified from Galli SJ et al. Nat Rev Immunol 2008; 8:478-86 (ref. 13) with permission from Macmillan Publishers Ltd. Please see the original source for relevant references.

b) Effector functions include the non-immunomodulatory physiological or pathological functions of mast cells or the direct regulation of “nonimmune” cells, such as vascular endothelial cells, epithelial cells, fibroblasts, nerve cells and muscle cells.

c) These are effects on other immune cells (such as DCs, T cells, B cells, monocytes/macrophages and granulocytes) and effects on structural cells (such as vascular endothelial cells, epithelial cells and smooth muscle cells) that alter their ability to influence immune cells.

immunosuppressive functions of mast cells as “negative immunomodulatory” functions and to those functions that enhance the initiation, magnitude or duration of immune responses as “positive immunomodulatory” functions” (Table 1).

In this chapter, we highlight mouse models used for analyzing mast-cell function in vivo and illustrate how such models have been utilized to identify immunomodulatory roles for mast cells during specific immune responses. We also briefly describe some of the mast-cell functions, products and surface receptors that have the potential to contribute to the mast cell’s ability to promote or suppress immune responses (reviewed in refs. 6,7,9-11,13,16).

MOUSE MODELS OF MAST-CELL FUNCTION

Mast-Cell Knock-In Mice

Although mice that specifically lack only mast cells have not been reported, *c-kit* mutant mice, which are deficient in mast cells but have several other phenotypic abnormalities, are available for analyzing the in vivo functions of mast cells.^{1,6,26} The most commonly used animals for such studies are the WBB6F₁-*Kit*^{W/W-v} mice and C57BL/6-*Kit*^{W-sh/W-sh} mice.^{6,7,26-28} *Kit*^W is a point mutation that produces a truncated Kit, lacking the transmembrane domain, that is not expressed on the cell surface; *Kit*^{W-v} is a (Thr⁶⁶⁰→Met) mutation at the *c-kit* tyrosine kinase domain that substantially reduces the kinase activity of the receptor; and *Kit*^{W-sh} is an inversion mutation that affects the transcriptional regulatory elements upstream of the *c-kit* transcription start site on mouse chromosome 5 (reviewed in refs. 6,7,28).

Adult WBB6F₁-*Kit*^{W/W-v} mice and C57BL/6-*Kit*^{W-sh/W-sh} mice are profoundly deficient in mast cells and melanocytes.^{1,6,26,27} WBB6F₁-*Kit*^{W/W-v} mice exhibit several other phenotypic abnormalities, such as macrocytic anaemia, reductions in numbers of bone-marrow and blood neutrophils, sterility and an almost complete loss of interstitial cells of Cajal.^{1,6,26} By contrast, C57BL/6-*Kit*^{W-sh/W-sh} mice are neither anaemic nor sterile, but have increased numbers of bone-marrow and blood neutrophils, enlarged spleens and mild cardiomegaly.^{26,28-30}

Because the *c-kit*-related phenotypic abnormalities that affect lineages other than mast cells are generally milder in C57BL/6-*Kit*^{W-sh/W-sh} mice than in WBB6F₁-*Kit*^{W/W-v} mice and because C57BL/6-*Kit*^{W-sh/W-sh} mice are fertile and, like many other mutant mice to which they might be bred, are fully on the C57BL/6 background, they are becoming increasingly popular for studies to elucidate the roles of mast cells in vivo. Herein, we will refer to both WBB6F₁-*Kit*^{W/W-v} and C57BL/6-*Kit*^{W-sh/W-sh} mice as “*c-kit* mutant mice”.

Differences in the biological responses in *c-kit* mutant mice compared with wild-type mice may be due to any one of the genetic abnormalities in these animals and may not be due to the loss of mast cells. However, the lack of mast cells in *c-kit* mutant mice can be selectively repaired by the adoptive transfer of genetically-compatible, in-vitro-derived wild-type or mutant mast cells.^{1,6,26,27} Such in vitro-derived mast cells, for example bone-marrow-derived cultured mast cells (BMCs), can be administered to *c-kit* mutant mice intravenously, intraperitoneally or intradermally, or directly injected into the anterior wall of the stomach, to create so-called “mast-cell knock-in mice”. These mast-cell knock-in mice can then be used to assess the extent to which differences from wild-type mice in the expression of biological responses observed in *c-kit* mutant mast-cell-deficient mice reflect their lack of mast cells.

Transgenic Mice with Deletion/Mutation of Mast Cell-Specific Products

The role of specific mast-cell-associated mediators can be investigated in vivo by testing animals in which that mediator has been knocked out. To the extent that the mediator is selectively expressed by mast cells and if its deletion does not significantly influence the expression of other mast cell products, then one can draw conclusions about the role of that mast cell mediator in vivo. For example, in mice that lack mouse mast-cell carboxypeptidase A (mMC-CPA, also known as mast cell-CPA and CPA3; a highly conserved secretory granule protease), the expression of mouse mast-cell protease 5 (mMCP-5) is also reduced because it requires mMC-CPA for proper packaging in the cytoplasmic granules.³¹ This problem can be circumvented by using mice in which mMC-CPA has been mutated specifically to eliminate its catalytic activity, a change that preserves mMC-CPA's ability to ensure proper packaging of mMCP-5 in the mast cell granule.²⁰ Mice that lack mast cell protease-1 (MCPT-1),³² MCPT-4,³³ tryptase beta 2 (TPSB2; also known as MCPT-6),³⁴ or mast cell-CPA/CPA3,³¹ or that have a mutated form of mast cell-CPA/CPA3 that essentially lacks enzymatic activity,²⁰ have been used to analyze whether the absence of these proteases (or their enzymatic activity) influences other aspects of mast-cell phenotype, such as content of other stored mediators, and to define the functions of such proteases in vivo.

Transgenic mice expressing Cre-recombinase under the control of “mast cell specific” promoters recently have been generated.³⁵⁻³⁷ Such “mast cell cre mice” are being crossed with other transgenic mice in which the genes of interest are “floxed” in attempts to reduce the expression of specific gene products only (or, at least, predominantly) in the mast cell lineage. Such approaches may prove to be useful in attempts to analyze to what extent mast cells represent important sources of products (including those with potential effector and/or immunomodulatory functions) that can also be derived from other cell types. “Mast cell cre” mice could also be mated to other transgenic mice in which important mast cell survival factors are floxed in order to ablate mast cells selectively. This approach may permit the generation of “improved” mast cell-deficient mouse models that are independent of *c-kit* mutations. However, time will tell whether various “mast cell cre” mice achieve

truly mast-cell-specific expression of Cre recombinase activity, or can be used to ablate all mast cell populations without affecting other cell lineages.

Other Approaches

Pharmacological approaches or those based on the use of antibodies to deplete mast cells or to neutralize their products may also provide useful information, but are limited by the specificity of the drug or antibodies chosen. Some agents, such as antihistamines, block the effects of that mediator whether it is secreted from mast cells or other cells. Antibodies that neutralize SCF³⁸ or block Kit³⁹⁻⁴¹ can result in the depletion of mast cells *in vivo*, but may also influence other cell types that express Kit.

Drugs (or antibodies) that only interfere with mast-cell activation would be highly desirable for experimental studies and, possibly, for evaluation as therapeutic agents. One drug, disodium cromoglycate, is widely characterized as a “mast-cell stabilizer” (i.e., an agent that blocks the release of mast cell mediators that occurs upon appropriate activation of the cell) and sometimes is used to suppress mouse mast-cell function *in vivo*,^{42,43} but its molecular targets are not fully defined. However, these targets are not restricted to mast cells⁴⁴ and the drug also influences granulocyte and B-cell function.⁴⁵ Given the current limitations of using pharmacological or antibody-based approaches to eliminate mast cells or specifically to block their functional activation, we think that genetic approaches, including those employing mast cell knock-in mice, mice deficient in specific mast-cell-associated mediators and, when they have been fully validated, approaches that genetically delete specific mediators selectively in mast cells, are the most definitive way to identify and characterize mast cell functions *in vivo*.

MAST CELL ACTIVATION

Mast cells can be activated by a wide range of stimuli, including those that activate immunoglobulin Fc receptors (FcεRI, FcγR), complement receptors (C3aR, C5aR) and microbial pattern-recognition receptors (PRRs, such as TLRs). Mast cells also respond to many other signals, including neuropeptides, cytokines, growth factors, toxins, venoms or venom components and physical stimuli. These stimuli trigger mast cells to release a diverse array of biologically active products, many of which can potentially mediate pro-inflammatory, anti-inflammatory and/or immunosuppressive functions.¹³ The strength and nature of the responsiveness of mast cells to various activating stimuli may be influenced by intrinsic or microenvironmental factors that affect the expression pattern or functional properties of the surface receptors or signaling molecules that contribute to such responses.^{6,46-48} Furthermore, mast cells can participate in multiple cycles of activation for mediator release and can be differentially activated to release distinct patterns of mediators or cytokines, depending on the type and strength of the activating stimuli.^{6,49}

Activation via FcεRI and Other Fc Receptors

Mast cells and basophils are the two major cell populations in the mouse that constitutively express on their surface large numbers of the high affinity receptor for IgE, FcεRI and the number of surface FcεRI is up-regulated by increased concentrations of IgE.³ Aggregation of FcεRI by binding of bi- or multi-valent antigen to surface- FcεRI

-bound IgE on mast cells initiates complex intracellular biochemical events that lead to degranulation and secretion of mast-cell-derived products and mediators. This FcεRI-dependent mast cell activation response results in rapid release (in minutes) of preformed cytoplasmic granule-associated mediators (such as histamine, heparin and other proteoglycans, proteases) and certain cytokines (TNF, VEGF), the secretion of de novo-synthesized lipid mediators (including cysteinyl leukotrienes [LTs] and prostaglandins [PGs]) and the production, with a prolonged kinetics, of many cytokines, chemokines and growth factors.^{3,6,50}

Aggregation of only a small fraction of the mast cell's FcεRI is sufficient to trigger mast cell activation and mediator secretion; as a result, individual mast cells can be simultaneously sensitized to respond to many different specific Antigens.³ As discussed above, the extent to which mast cells secrete various types of mediators can vary according to the strength of the activation signal, with release of some cytokines occurring at lower antigen concentrations than the concentration required to induce substantial degranulation and release of stored mediators.⁴⁹ For example, low occupancy or weak stimulation of FcεRI can induce mast cells to produce "pro-allergic" chemokines, while mast cell IL-10 production requires strong and prolonged stimulation of FcεRI.⁵¹ The extent of mast cell activation in response to FcεRI stimulation can also be positively or negatively regulated by the cells' exposure to ligands for many other receptors.^{6,49,50}

Antigen- and IgE-dependent mast cell activation is widely regarded to be a, if not *the*, major initiator of the clinical signs and symptoms of an allergic reaction and can also contribute to later consequences of allergen exposure, by promoting local inflammation and by directly or indirectly enhancing certain aspects of tissue remodeling. In addition to FcεRI, mast cell activation can also be triggered by Fcγ receptors. IL-4 primed-mouse mast cells can produce TNF, IL-10 and VEGF upon IgG1/FcγRIII dependent activation^{24,52,53} and aggregation of FcγRI on IFNγ-treated human mast cells can induce histamine release.⁵⁴

TLRs and Other Innate Receptor-Mediated Activation

Mast cells activated by innate signals also can influence the development of acquired immune responses.^{7,13,55-62} Mast cells express many Pattern Recognition Receptors (PRRs).⁶³⁻⁶⁵ Protein or mRNA of 10 TLRs (TLRs 1–10) have been detected in human or mouse mast cells.⁶⁵ Mast cell responses to TLR agonists can vary among different populations of mast cells. For example, the stimulation of TLR4 on mouse mast cells by LPS resulted in cytokine production without degranulation, while TLR2 activation led to both cytokine release and degranulation.⁶⁶ Mast cells activated by TLR3 stimulation (e.g., with Poly(I:C) or Newcastle disease virus) produced antiviral cytokines (IFN-β, ISG15) and chemokines (IP10, RANTES) without undergoing degranulation.⁶⁷ Human and mouse Mast cells express NLRs (the second major class of cytoplasmic innate immune sensors), such as NOD receptors and NLRP proteins,⁶⁸⁻⁷¹ which can be activated by intracellular pathogens and "danger signals".⁷²⁻⁷⁴ Expression of various PRRs thus permits mast cells to respond to both Pathogen-Associated Molecular Patterns (PAMPs) and "danger signals" resulting from cell stress or injury. Moreover, studies in mice indicate that activation of mast cells via the NLRP3 inflammasome can contribute to IL-1β overproduction and chronic urticarial rash in subjects with cryopyrin-associated periodic syndrome, a disorder associated with NLRP3 mutations.⁷¹

In conclusion, mast cell secretion of cytokines and other mediators in response to nonFcR-dependent innate stimuli, such as to TLR agonists, complement products and certain inflammasome activators, has the potential to influence the phenotype, maturation and function of Antigen Presenting Cells (APCs) and other immune cells and thus to modulate the subsequent T-cell or B-cell responses.^{6-8,12}

IMMUNOMODULATORY EFFECTS ON DENDRITIC CELLS

Both in vitro and in vivo evidence indicate that mast cells have the potential to influence DC migration, maturation and function.^{6,7,13} Histamine, abundantly stored in mast cell granules and rapidly released by the process of mast cell degranulation, can induce chemotaxis,⁷⁵ alter the pattern of secreted cytokines⁷⁶⁻⁷⁸ and enhance expression of costimulatory and MHC class II molecules in DCs;⁷⁷ and histamine-conditioned DCs have been shown to preferentially induce T_H2 polarization.^{76,77} In vitro coculture of IgE+antigen-activated umbilical cord blood-derived human mast cells with human monocyte-derived DCs induced maturation of DCs, but potently suppressed IL-12p70 production by DCs and thus promoted their T_H2 polarization.⁷⁹ Other mast cell products, such as PGD₂,⁸⁰ or PGE₂,⁸¹ have also been shown to inhibit IL-12 production by DCs and to induce maturation of DCs toward an effector DC2 phenotype, again leading to the polarization of naive T cells to T_H2 cells. These in vitro observations are in accord with in vivo findings showing that antigen immunization in conjunction with the administration of agents that induce mast cell degranulation suppresses antigen specific T_H1 responses while enhancing T_H2 responses.⁸² Many other mast cell products, such as PGD₂,^{80,83,84} LTB₄,⁸⁵ and PGE₂, TNF, IL-1, IL-16, IL-18 and CCL5 (reviewed in ref. 7), have also been shown to modulate DC migration, differentiation, maturation and function.

Another mechanism by which mast cells can regulate DC function is via exosomes, small vesicles implicated in the transfer of materials among cells. Mecheri and his colleagues have shown that endocytosed antigens are accumulated in association with heat shock protein hsp60 and hsp70 in mast cell exosomes. Such mast cell-derived exosomes are highly potent in inducing maturation (CD40 and CD80 expression) and functional activation (secretion of IL-12p70) of DCs and can potentiate the efficiency of antigen presentation by DCs.⁸⁶

Relatively few studies have investigated the ability of mast cells to influence DC biology in vivo. In a model of contact hypersensitivity (CHS) to oxazolone, Bryce et al reported evidence that the emigration of skin Langerhans Cells (LCs) from the epidermis in response to epicutaneous application of oxazolone was impaired in mast-cell-deficient WBB6F₁-*Kit*^{W/W-v} mice and was enhanced by antigen-independent effects of IgE.⁸⁷ Moreover, this group showed that mice lacking IgE exhibited impaired elevation of mRNA for the mast-cell-associated product, MMCP-6 (also known as MCPT-6 or TPSB2), as well as for several products (that can be produced by mast cells and other cell types) known to influence DC biology (such as IL-1, IL-6, CCL2 and TNF), one hour after the epicutaneous application of the hapten oxazolone.⁸⁷ These results suggest that the binding of IgE to FcεRI on dermal mast cells, even in the absence of antigen known to be recognized by that IgE, in some way can “prime” such mast cells to participate more effectively in the induction of DC migration and perhaps other functions that promote sensitization to the hapten.⁸⁷ Histamine derived from IgE- and antigen-stimulated mast cells in mouse skin can promote the H2 receptor-dependent migration of LCs to draining lymph nodes (LNs)⁸⁸

and TNF derived from mouse mast-cells can contribute significantly to the initial stages of FITC-induced migration of cutaneous and airway DCs.⁸⁹ Administration of peptidoglycan can result in mouse mast cell-dependent LN hypertrophy, LC mobilization⁹⁰ and recruitment of plasmacytoid and CD8⁺ DCs to draining LNs.⁹¹ While peptidoglycan-induced LN hypertrophy was TNF-independent in this model, optimal LC migration required TNF.⁹⁰ Activation of dermal mast cells by a TLR7 agonist also has been shown to promote the emigration of LCs, via a process partially dependent on mast-cell-derived IL-1.⁵⁶

IMMUNOMODULATORY EFFECTS ON LYMPHOCYTES

Evidence for the migration of mast cells to draining LNs during immune responses and the close proximity of mast cells and lymphocytes at sites of tissue inflammation^{6,12,92,93} suggest that mast cells and lymphocytes may influence each other's functions by bidirectional cell-cell interactions.

Regulation of Mast Cell Functions by T Cells

Upregulation of IL-8 mRNA transcription has been demonstrated in mast cells that were in contact with the membrane of activated T cells.⁹⁴ Incubation of mast cells with activated lymphocytes induced mast cell degranulation and release of granule-associated mediators/proteases (histamine, β -hexosaminidase, metalloproteinase-9 and tissue inhibitor of metalloproteinase [TIMP]1), TNF and other cytokines.⁹⁵⁻⁹⁸ ICAM-1/LFA-1 dependent cell-cell contact is necessary for anti-CD3-activated T cells to augment Fc ϵ RI-dependent mast cell (BMCMC) degranulation and histamine release⁹⁶ and the interactions between LT α 1 β 2 and/or LIGHT on activated T cells and LT β receptors expressed on mast cells are important for mast cell cytokine release induced on contact with activated T cells.⁹⁸

Mast Cell Effects on T-Cell Functions

Mast cells can also modulate T-cell functions. Antigen processing and presentation has been proposed as one mechanism by which mast cells might regulate T-cell responses.^{7,92,99} Mecheri and his colleagues demonstrated upregulation of MHC class II in BMCMCs upon stimulation with LPS and reported that BMCMCs can take up, process and present antigen peptide to antigen specific T hybridoma cells.²² Rat peritoneal mast cells (PMCs)¹⁰⁰ and human mast cells¹⁰¹ were also later reported to be capable of antigen presentation. MHC class I molecules are expressed on mouse BMCMCs and on human mast cells isolated from lung, liver, uterus and skin⁹⁹ and, in vitro, mouse BMCMCs can stimulate bacterial antigen-specific CD8 T-cell activation by presenting bacterial antigens through MHC class I.¹⁰² Recently, Stelekati et al showed that BMCMCs can present antigen to CD8 T cells in a MHC I-restricted manner, resulting in IL-2, IFN γ and MIP-1 α production and promoting CD8 T-cell degranulation and cytotoxicity.¹⁰³ Although less efficiently than professional APCs, MHC II-expressing mast cells can upregulate CD69 expression, proliferation and cytokine production in effector T cells.^{104,105}

The expression of MHC molecules and antigen presenting functions in mast cells appear to be under complex control: MHC II expression is down-regulated by IL-3, but upregulated by IL-4 and IFN- γ .^{101,104,106} GM-CSF, while not influencing expression of MHC II, can increase CD80 and CD86¹⁰⁷ expression and substantially enhance the

antigen-presenting capacity of IL-4-treated mast cells. By contrast, IFN γ almost completely abolished mast cell antigen-presenting function.¹⁰⁶ In another study, Delta-like 1 (DII1)/Notch signaling was shown to induce the expression of MHC-II and upregulate the expression of OX40L on mast cells, thus promoting the ability of MHCII+OX40L^{high} BMCMCs to enhance naïve T-cell proliferation and their differentiation into IL-4, IL-5, IL-10 and IL-13 secreting T_H2 cells.¹⁰⁸ Treatment of cultured mast cells (BMCMCs and spleen-derived mast cells) with LPS/IFN γ also enhanced mast cell expression of MHC-II and the inhibitory costimulatory molecule PD-L1, but not positive costimulatory B7 family members CD80 and CD86 and promoted the mast cells' ability to process and present antigen directly to previously activated effector CD4 T cells and, to a lesser degree, to naïve T cells. Furthermore, LPS/IFN γ -primed mast cells stimulated expansion of antigen-specific Foxp3⁺ T_{Reg} cells preferentially over naïve T cells.¹⁰⁵ This finding may have implications for understanding the mechanisms by which mast cells can exert anti-inflammatory functions in certain T-cell mediated acquired responses.^{24,109}

Although MHC class II molecules are not expressed on most "resting" mouse or human mast cells, expression is upregulated in mast cells that have been isolated from pathogen-infected tissues and/or stimulated by tumor necrosis factor (TNF), IFN γ or bacterial lipopolysaccharide (LPS).^{99,105} Kambayashi et al showed that s.c. injections of LPS increase mast cell numbers in the draining LN and that these mast cells expressed MHC II, PD-L1, CD80 and CD86.¹⁰⁵ Freshly isolated peritoneal mast cells and IFN γ /IL-4-primed mast cells, derived from peritoneal cell culture, have been shown to process protein antigen and to form functional immunological synapse with and induce activation of, effector CD4 helper T cells, but not naïve T cells.¹⁰⁴ The cognate interactions at the immunological synapse between T helper cells and antigen-presenting mast cells and the formation of T-cell polarization at the contact of T cells and mast cells has been visualized by confocal laser scanning microscopy.¹⁰⁴ Such direct contacts of mast cells and T helper cells also render mast cells more susceptible to Fc ϵ RI-dependent degranulation.¹⁰⁴

Mast cells can also influence T-cell activation by the release of exosomes. Exosomes derived from IL-4-treated mouse BMCMCs induce lymphocyte proliferation and IL-2 and IFN γ production in vitro. Injection of such mast-cell-derived exosomes, which contain costimulatory molecules (such as MHC II, CD86, CD40, CD40L, LFA-1 and intercellular adhesion molecule 1), can induce lymphocyte proliferation and cytokine production in vivo.¹¹⁰

Mast cells can enhance antigen presentation indirectly in vitro by internalizing antigen bound to Fc ϵ RI-associated IgE; this mechanism is independent of mast-cell MHC class II expression, but requires that such mast cells undergo apoptosis and then are phagocytised by other antigen-presenting cells.¹¹¹ Targeting antigens to IgE or IgG bound to mast cells can enhance the efficiency of antigen presentation,^{112,113} which might be mediated by the mechanism described by Kambayashi et al,¹¹¹ or by the transfer of antigen to other APCs via mast-cell-associated exosomes.

As noted above, mouse and/or human mast cells can express many costimulatory molecules including members of the B7 family—inducible T-cell costimulator ligand (ICOSL), PD-L1, PD-L2, CD80 (also known as B7.1) and CD86 (also known as B7.2)—members of TNF–TNF receptor families—OX40, CD153, CD95, 4-1BB and glucocorticoid-induced TNF-receptor-related protein (GITR)—and CD28 and CD40 ligand (CD40L).^{12,114} Moreover, mast cells can exhibit costimulatory function in vitro. For example, engagement of the costimulatory ligand OX40L expressed by human¹¹⁴ or mouse¹¹⁵

mast cells and OX40 expressed by T cells is required for optimal mast-cell-dependent enhancement of T-cell proliferation¹¹⁴ or cytokine production.¹¹⁵

Many secreted products of mast cells can influence T-cell activation. For example, histamine, LTB₄, PGD₂ and TNF can promote the migration, recruitment, maturation and activation of lymphocytes; and PGD₂ can enhance cytokine production by T_H2 cells.¹¹⁶ Histamine promotes T_H1-cell activation through H1 receptors but conversely can suppress both T_H1 and T_H2 cell activation through H2 receptors.¹¹⁷ Mast cells are also sources of many cytokines, such as IL-2, IL-4, IL-6, IL-10, IL-12, IL-13 and transforming growth factor- β (TGF- β), that can influence the polarization of naïve T cells toward T_H1, T_H2, T_H17 and T_{Reg} cells¹² and can modulate the function of distinct T-cell subsets.

Taken together, mast cells can potentially modulate T-cell-dependent acquired immunity by antigen presentation to T cells or potentiate the efficiency of antigen presentation by other APCs. Mast cells can enhance T-cell function by direct cell-cell contact, by exosome-mediated effects and/or by their secreted products. Although there is not yet clear evidence that native populations of mast cells can perform these functions in vivo, migration of mast cells to draining LN has been shown in many innate and acquired immune responses, including CHS,¹¹⁸ UVB irradiation,¹¹⁹ EAE,¹²⁰ cutaneous infection with *Leishmania major*,¹⁰⁵ injection of LPS¹⁰⁵ and in a model of anti-GBM (glomerular basement membrane) induced glomerulonephritis.¹²¹ These observations suggest that mast cells derived from sites of antigen challenge have the potential to influence T-cell function within LNs, as well as at the sites in which such mast cells ordinarily reside.

Effects on CD8 T Cells

Several recent studies have specifically examined the regulation of CD8 T-cell function by mast cells. Mouse BMCMCs can stimulate bacterial antigen-specific CD8 T-cell activation by presenting bacterial antigens through MHC I.¹⁰² A recent study by Stelekati et al confirmed that BMCMCs can present antigen to CD8 T cells in an MHC I-restricted manner, resulting in IL-2, IFN γ and MIP-1 α production and can promote CD8 T-cell degranulation and cytotoxicity.¹⁰³ Intraperitoneal injection of Poly(I:C), a TLR3 ligand, induces CD8 T-cell recruitment and promotes the expression of MHC II, CD80, CD28 and complement receptors by PMC.⁶⁷ Heib et al found that mast-cell-deficient *Kit^{W-sh/W-sh}* mice exhibited an impaired peptide-specific cytotoxic T-lymphocyte response after transcutaneous peptide immunization together with TLR7 ligands (used as adjuvants) and that, in mice containing mast cells, treatment with ligands for TLR7 induced mast cell IL-1-dependent LC emigration and mast cell TNF-dependent LN hypertrophy.⁵⁶

Effects on B-Cells and Immunoglobulin (Ig) Production

Several mast cell products, including IL-4, IL-5, IL-6, IL-13, CD40L and rat mast-cell protease I can influence B-cell development and function, including IgE production.^{122,123} Using a coculture system, Merluzzi and colleagues recently showed that BMCMCs can promote the survival and activation of naïve B cells and promote the proliferation and differentiation of activated B cells toward IgA secreting plasma cells.¹²⁴ These effects of mast cells require both cell-cell contact and mast-cell-derived IL-6.¹²⁴ Mast-cell-derived exosomes, by effects on DC maturation and APC efficiency, can elicit specific antibody production in naïve mice in the absence of conventional adjuvants.⁸⁶ In a mouse model of urinary tract infection with uropathogenic *E. coli* in WT mice, *Kit^{W-sh/W-sh}* mice and

Kit^{W-sh/W-sh} mice engrafted with WT or TNF^{-/-} mast cells, mast cells and mast cell TNF were found to promote a protective humoral response against *E.coli* infection.⁶² Furthermore, McLachlan et al showed that antigen vaccination administered with mast-cell-activating compounds greatly enhanced the production of antigen specific IgG (with s.c. injection) and IgA (with intranasal exposure) and the enhanced production of Ig was correlated with DC and lymphocyte recruitment to the LN and with enhanced resistance to vaccinia virus infection.⁵⁸ However, mast cells appear to make little or no contribution to antibody production in other experimental settings, in which both naïve and antigen-challenged mast-cell-deficient mice exhibit normal levels of antibodies.⁷

IMMUNOMODULATORY FUNCTIONS IN VIVO

Some of the immunomodulatory functions of mast cells that have been proposed based on in vitro studies have been confirmed in vivo using mast-cell knock-in mice or mice lacking specific mast-cell-associated proteases or lacking specific protease enzymatic activity. In many of these studies, the end points assessed included the recruitment of particular immune cells, such as granulocytes, DCs or various subpopulations of lymphocytes. Many of these studies also showed that the lack of mast cells, or a specific mast-cell product, decreased the pathology associated with the immune response or impaired its effectiveness in promoting host resistance to infection (reviewed in ref.13). While most of these studies focused on the pro-inflammatory functions of mast cells, several recent studies provided compelling evidence that mast cells can also down-regulate inflammatory responses and promote tissue homeostasis in certain experimental settings.

Host Defense against Infections

Work by many groups, using both mast cell knock-in and mast-cell-associated protease-deficient mice, has shown that mast cells can modulate host resistance and survival during several examples of bacterial infections.^{8,11,19,30,34,59,125-128} The beneficial role of mast cells in host defense against acute bacterial infection was first demonstrated by two seminal studies in 1996.^{125,126} In the cecal ligation and puncture (CLP) model, which is considered by some investigators to be the “gold standard” mouse model of sepsis (reviewed in refs. 17,19,30,125), host resistance and survival can be enhanced by mast cells and TNF. Treatment with SCF, that increases mast cell numbers, can provide further protection in the CLP model.¹²⁹ Activation of mast cells mediated by TLR4,⁶⁶ complement receptors,^{130,131} or endothelin-1 (see ref. 17) has been shown to contribute to the ability of mast cells to enhance host resistance in CLP models. On the other hand, Smad3 and IL-15 expression in mast cells function to inhibit mast cell-mediated protection against bacterial infection.^{132,133} There is evidence that MCPT-2 can contribute to neutrophil recruitment and host survival during CLP, but that, in wild-type mice, mast-cell production of intracellular IL-15 limits the mast cell’s ability to produce this protease in that setting.¹³³ Mast-cell-deficient *Kit^{W/W^v}* mice engrafted with Smad3^{-/-} mast cells had significantly improved survival after CLP compared to *Kit^{W/W^v}* mice engrafted with wild-type mast cells, which exhibited higher production of pro-inflammatory cytokines in the peritoneal cavity.¹³²

Several mast cell functions have been implicated in host defense. These include enhancement of the recruitment or function of granulocytes,^{11,125-127,134}

phagocytosis-dependent bactericidal activities (reviewed in refs. 8,59,135,136) and proteolytic degradation of endogenous mediators which would otherwise be elevated to toxic levels, such as endothelin-1 (see ref. 17) and neurotensin.¹⁹ Secretion of proteases/enzymes and an antimicrobial peptide¹³⁷ and formation of extracellular traps that contain antimicrobial peptides, histone, DNA and tryptase,¹³⁸ have also been proposed as potential mechanisms by which mast cells exert protective functions. For example, mast-cell-derived proteases can promote host defense following intraperitoneal injection of the bacteria *Klebsiella pneumoniae* and TPSB2-deficient mice have both decreased neutrophil recruitment into the peritoneal cavity and significantly increased mortality.³⁴ Mast cells can also contribute to host defense by modulating acquired immunity to pathogens. They can process bacterial antigen and present it to T cells,¹⁰² recruit lymphocytes and induce LN hyperplasia by TNF production.¹³⁹ Mast cells and mast cell-TNF have been shown to recruit T cells, DCs and other inflammatory cells into inflamed tissues and the draining LN after *E. coli* infection⁶² and mosquito bites.¹⁴⁰ Kunder et al have reported that one mechanism by which mast cells regulate lymphocyte recruitment is by the delivery of an inflammatory signal, consisting of exocytosed heparin-containing cytoplasmic granules that also contain other mast cell mediators, particularly TNF, to draining LNs via lymphatic vessels.⁶⁰

Such observations support the conclusion that mast cells can have important sentinel and effector roles during bacterial infection, which help to promote clearance of the bacteria, protect the host from pathology and enhance survival. However, there is evidence from work in mast-cell-engrafted C57BL/6-*Kit*^{W-sh/W-sh} mice that mast-cell-dipeptidyl peptidase-I can have effects in a severe model of CLP that decrease levels of IL-6 and reduce survival.¹⁴¹ Moreover, another study in mast-cell-engrafted C57BL/6-*Kit*^{W-sh/W-sh} mice indicates that in certain severe bacterial infections, including a model of severe CLP, mast cell production of TNF (and perhaps other mast cell functions) can exacerbate inflammation and mortality.³⁰ Another setting in which mast cell responses to bacteria may contribute to pathology is in atopic dermatitis, an allergic disorder in which the majority of patients have colonization of the skin with *Staphylococcus aureus*, a source of peptidoglycan which could mediate TLR2-dependent activation of mast cell cytokine production.^{59,142} Considerations such as these suggest that, depending on the setting, including the severity and/or type of infection or the presence of another disorder, mast cells can either promote health or increase pathology during host responses to bacteria. Intrinsic properties of the mast cell, such as genetic predispositions to produce larger or smaller amounts of TNF and other cytokines, or the presence of other abnormalities in the host (e.g., C57BL/6-*Kit*^{W-sh/W-sh} mice have increased numbers of neutrophils),²⁸⁻³⁰ also may influence whether the role of mast cells in particular bacterial infections are beneficial or harmful.

In addition to their role in bacterial infections, mast cells can promote host resistance to certain parasite infections. However, the mechanisms involved have not been fully defined and may be complex, involving both local and systemic mast-cell-dependent effects (reviewed in refs. 6,8,143,144). For example, a deficiency in TPSB2 (or in IgE) was associated with markedly reduced recruitment of eosinophils to the sites of larvae deposition in skeletal muscle during the chronic phase of *Trichinella spiralis* infection, but was not associated with a detectable abnormality in the intestinal expulsion of the parasite.¹⁴⁴ In a model of cutaneous parasite infection by *Leishmania major*, mast cells contributed to the control of skin lesions by priming antigen-specific T cells and enhancing the recruitment of pro-inflammatory neutrophils, macrophages and DCs. Interestingly,

local mast cell activation at the infected sites is sufficient for the induction of systemic protection in this model.¹⁴³

There is evidence that mast cells participate in host responses to certain viruses, but their precise roles in such settings are not yet clear.^{8,145} Rodent^{11,59,67,134} and human^{11,63,146} mast cells express TLRs (e.g., TLR3) which can be activated by viral double-stranded RNA to release various chemokines and cytokines, including interferon- α and $-\beta$.⁶³ Some of these mast-cell-derived products may contribute to host defense against viruses.^{8,11,63,67} Co-stimulation of rodent and human mast cell populations in vitro via the Fc ϵ RI and certain TLRs can enhance the cells' secretion of various pro-inflammatory mediators, suggesting one mechanism whereby bacterial or viral infections might exacerbate allergic diseases and other IgE- and mast-cell-associated disorders in vivo.^{59,147,148}

Innate Responses to UVB Irradiation

Mast cells have been implicated in ultraviolet (UV)-B-induced immunosuppression and many mast-cell-associated mediators (histamine, PGE₂, serotonin, PAF, TNF, IL-4, IL-10) are produced in response to UVB irradiation (wavelengths: 280-320 nm) of the skin.^{119,149} Mast cells seem to play two roles during UVB-induced inflammation. ET-1, a mediator produced upon UVB irradiation, activates mast cells to potentiate skin inflammation following a single exposure to UVB.¹⁵⁰ By contrast, mast cells are anti-inflammatory in a model of chronic low-dose UVB-irradiation,^{24,151} at least in part by their ability to produce IL-10 and perhaps other anti-inflammatory cytokines. After a series of exposures to UVB, mast cells limited multiple aspects of the inflammatory responses and tissue pathology, including numbers of granulocytes, macrophages and T cells at the reaction sites, as well as the local tissue swelling, epidermal hyperplasia and epidermal necrosis.^{24,151}

Hart et al²³ showed that the ability of a single high dose of UVB irradiation of the skin to induce systemic immunosuppression of contact hypersensitivity (CHS) responses to the hapten 2,4,6-trinitrochlorobenzene was markedly reduced in (C57BL/6 x DBA/2) F₁-*Kit*^{W^{-f}/W^{-f} mice but was restored following mast-cell engraftment. Several lines of evidence suggested that histamine was a major mediator of this UVB-induced, mast-cell-dependent effect.¹⁴⁹ Mast cells were probably also responsible for the finding that UVB irradiation suppressed delayed-type hypersensitivity (DTH) responses to allogeneic spleen cells in *Kit*^{W^{-f}/+} mice (which contain dermal mast cells) but not in *Kit*^{W^{-f}/W^{-f} mice (which do not contain dermal mast cells). Alard et al showed that mast cells, probably via the secretion of TNF, are required for local UVB-induced immune suppression.¹⁵² CXCR4 dependent migration of mast cells to the LN upon UVB treatment is critical for the UVB-induced immune suppression.¹¹⁹ More recently, Biggs et al provided evidence that activation of mast cells through their vitamin D receptors (VDRs) by physiologically active vitamin D₃ (i.e., 1 α ,25-dihydroxyvitamin D₃) is required for optimal release of mast-cell-derived IL-10, which in turn contributes to the mast cell's ability to suppress inflammation and skin pathology at sites of chronic low-dose UVB irradiation (Fig. 1).¹⁵¹}}

T-Cell-Dependent Responses

EAE

In the mouse model of experimental autoimmune encephalomyelitis (EAE) induced by myelin oligodendrocyte glycoprotein (MOG), mast cells can increase the incidence

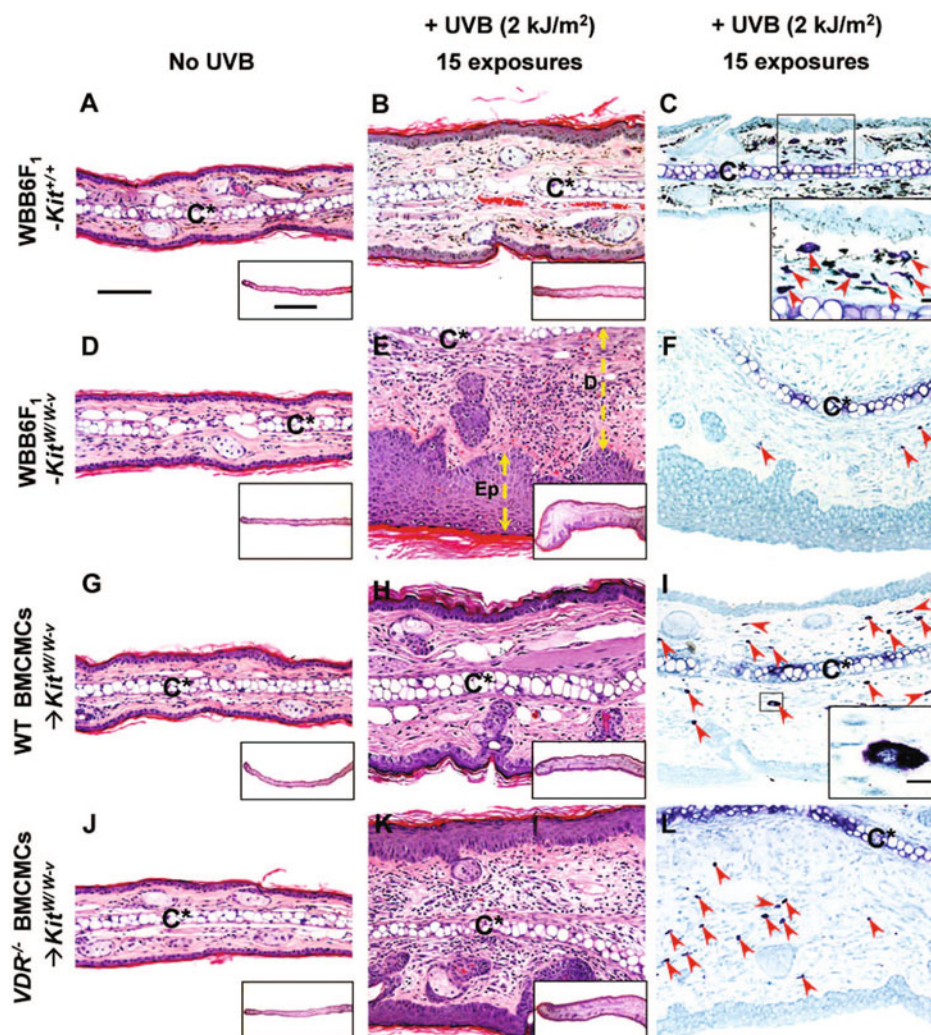


Figure 1. Mast cells and mast cell VDR expression are required to limit pathology induced by chronic low-dose UVB irradiation of ear skin. Cross-sections of ears obtained from WBB6F₁-*Kit*^{+/+} (wild-type) mice (A-C), WBB6F₁-*Kit*^{W/W-v} (*Kit*^{W/W-v}) mice (D-F), WT BMCMCs → *Kit*^{W/W-v} mice (G-I), or *VDR*^{-/-} BMCMCs → *Kit*^{W/W-v} mice (J-L) at 24 h after the final exposure to 2 kJ/m² UVB (15 exposures, 2 d apart; B,C,E,F,H,I,K,L); sections stained with haematoxylin and eosin (A,B,D,E,G,H,J,K), or toluidine blue (C,F,I,L). Mice that did not receive UVB irradiation were also killed for analysis of skin histology at the end of the experiment (A,D,G,J). C*, cartilage. Double-headed arrows indicate thickness of dermis (D) or epidermis (Ep). Red arrowheads indicate mast cells (C,F,I,L). Scale bars in A = 100 μ m (or 1000 μ m in inset) (for A,B,D,E,G,H,J,L inset), or 10 μ m (in C,I insets). Results are representative of those obtained in the 3 different experiments performed, each with 3-4 mice analyzed per group. Reproduced with permission from Biggs L et al. J Exp Med 2010; doi:10.1084/jem.20091725.

and severity of the disorder.^{103,153,154} Remarkably, it appears that mast cells do not have to be within the CNS to exert at least some of their important effects in MOG-induced EAE. Therefore, although systemic engraftment of mast-cell-deficient *Kit*^{W/W-v} mice with

in vitro-derived BMCMCs does not result in the appearance of mast cells in the CNS of these mice, they exhibit a CNS disease severity that is similar to that in wild-type mice. Two mechanisms have been proposed to explain how extra-CNS mast cells can influence autoimmune responses to MOG. One mechanism is through the production of IL-4 in the LNs which enhances the development of encephalogenic T_H1 cells.¹⁵⁵ Mast cell antigen presentation that enhances the priming of MOG specific CD8 T cells has recently been shown to also contribute importantly to EAE pathogenesis.¹⁰³ More recently, Brown's group reported that, in this model of EAE, mast cells in the meninges of the brain can enhance blood-brain-barrier permeability and local inflammatory cell infiltration to CNS, including the recruitment of neutrophils by mast cell-derived TNF.¹⁵⁶

Mouse Models of Asthma

Various mouse models have been used to elucidate the roles of mast cells in allergic airway inflammation. Depending on the mode of allergen sensitization, the contribution of mast cells is most obvious when the mice are sensitized and/or challenged with low doses of antigens, with adjuvant-free antigen, or sensitized with antigen by inhalation route (reviewed in refs. 157,158). In mouse models of asthma in which mast cells enhance the response, mast cells¹⁵⁸⁻¹⁶³ and mast-cell-derived TNF significantly contribute to both airway hyperreactivity and airway inflammation.^{159,158,160-163} Some of the effects of TNF probably reflect its ability to promote T-cell recruitment and T_H2-type cytokine production.¹⁶¹ Another mechanism which may contribute to "mast-cell-dependent" effects on T cells during models of allergic inflammation is FcεRI-dependent mast cell secretion of LTB₄, with subsequent recruitment of effector CD8 T and CD4 T cells to sites of airway inflammation.¹⁶⁴⁻¹⁶⁶ Mast cells also contribute to a model of T_H17-cell-dependent, neutrophil-associated lung inflammation in ovalbumin (OVA)-challenged, OVA-specific T-cell receptor transgenic mice.¹⁶⁷

Moreover, in a mouse model of chronic allergic inflammation of the airways, mast cells are required for the full development of several features of tissue remodeling, including increased numbers of mucus-producing goblet cells in the airway epithelium and increased lung collagen deposition, changes accompanied by a mast-cell-dependent exacerbation of airway hyperreactivity to methacholine.¹⁵⁸ In this mouse model of chronic asthma, mast cell activation mediated by antibody dependent- and independent-mechanisms contributes to the full manifestation of the airway disease.¹⁵⁸ Finally, in one model of allergic inflammation of the airways, mice lacking the mast cell chymase, MCPT-4, compared to the corresponding wild-type mice, exhibited more substantial increases in airway hyperreactivity to methacholine, increased airway inflammation and thickening of bronchial smooth muscle.¹⁶⁸ These findings indicate that at least one mast cell product may help to limit the pathology associated with allergic inflammation.

The Two Faces of Mast Cells in CHS

Under some experimental conditions, mast cells are required for the optimal elicitation of the inflammation associated with mouse models of hapten-induced CHS (reviewed in ref. 55). Mast cells and IgE can contribute to LC emigration and promote effective sensitization to the chemical hapten oxazolone,⁸⁷ as well as contribute to the effector phase of the responses, including the elongation of cutaneous nerve fibers.¹⁶⁹ However, mast cells can also have negative immunomodulatory functions in some models of CHS. Mast cells markedly limited the magnitude and duration as well as promoted the resolution, of

CHS responses induced in mice by the hapten 2,4-dinitro-1-fluorobenzene) (DNFB) or by urushiol, which is the hapten-containing sap of poison ivy or poison oak.²⁴ In these responses, mast cells substantially inhibited the development of pathology, including the leukocyte infiltration, epidermal hyperplasia and epidermal necrosis associated with these responses. Several lines of evidence indicated that mast cell production of IL-10, mediated at least in part by antigen-specific IgG1 binding to Fc γ RIII on mast cells, is essential for mast cell anti-inflammatory function in these responses.²⁴ Although the pathways that link mast-cell-derived IL-10 (or other mast cell mediators that are relevant in this setting) to the observed tissue changes remain to be defined, mast cells and mast-cell-derived IL-10 may influence these responses through a complex combination of direct and indirect effector and immunoregulatory functions.

In another study, the nature of the mast cells contribution to Ox-induced CHS was found to depend on the dose of hapten used to immunize the mice. Norman et al showed that mast-cell-deficient *Kit^{W/W-v}* mice, when compared to wild-type mice, exhibited reduced CHS responses (associated with little or no local production of IL-10) when immunized with low doses of Ox but more severe reactions (associated with local production of IL-10) when immunized with high doses of Ox. This study suggests that, depending on the strength of immunization, mast cells can regulate the magnitude of the T-cell-associated CHS responses by altering the cytokine microenvironment.¹⁷⁰

Antibody-Dependent Responses

Mast cells can contribute substantially to the disease pathology induced by auto-antibodies. In a mouse model of anti-glucose-6-phosphate isomerase (GPI)-induced destructive arthritis, work in *Kit^{W/W-v}* mice indicated that mast cells can contribute to the initiation of joint inflammation by Fc γ R-dependent release of IL-1.^{171,172} *Kit^{W/W-v}* mice also did not develop wild-type levels of joint swelling in a model of arthritis elicited by injection of antitype II collagen mAbs followed by LPS. By contrast, *Kit^{W-sh/W-sh}* mice developed features of arthritis in this model that were similar to those of the corresponding wild-type mice.²⁹ The differences in the susceptibility of these two strains of mast-cell-deficient mice to the autoantibody-induced joint disease may be due to the numbers of neutrophils in these animals. *Kit^{W/W-v}* mice have reduced numbers of bone-marrow and blood neutrophils, whereas *Kit^{W-sh/W-sh}* mice have increased numbers of bone-marrow and blood neutrophils (which may diminish the importance of any contribution of mast cells in this strain). Mast cells have been shown to recruit neutrophils in a mouse model of bullous pemphigoid (an autoimmune disease of the skin) elicited by injection of anti-hemidesmosomal IgG.¹⁷³

On the other hand, mast cells can exert anti-inflammatory and protective functions in some models of antibody-mediated diseases. In a model of experimental anti-GBN glomerulonephritis, *Kit^{W/W-v}* mice developed more severe glomerular damage, with more intense T-cell and macrophage infiltration, as well as more severe proteinuria and higher mortality, than wild-type mice or mast cell-engrafted *Kit^{W/W-v}* mice.¹²¹ Although mast cells are not detectable in the kidneys after induction of this model of glomerulonephritis, mast cell tryptase (mouse transmembrane tryptase [mTMT]) was detected in the draining LNs. These results suggest that whatever anti-inflammatory functions mast cells exert in this model reflect the actions of mast cells in draining LNs and/or other sites distant from the organ-related pathology, not mast cells at the site of inflammation.¹²¹ Moreover, there is evidence that at least one mast-cell-derived product, MCPT-4, can promote pathology in this model,¹⁷⁴ suggesting that mast cells can have complex roles in this and perhaps

other immune responses, with some of their mediators having pro-inflammatory effects and others having anti-inflammatory functions.

OTHER NEGATIVE IMMUNOMODULATORY FUNCTIONS OF MAST CELLS

Mast cells also contribute to other models of immunosuppression. Depinay et al¹⁷⁵ reported that the bites of *Anopheles* mosquitoes can impair the development of antigen-specific T-cell responses in a model of DTH to ovalbumin in mice, but only if mast cells are present in the bitten skin. How mast cells mediate immunosuppressive function in this model remains to be elucidated. Limon-Flores et al¹⁷⁶ used *Kit^{W-sh/W-sh}* mice to show that PGE₂ production by mast cells and mast cell migration to draining LNs, were critical for observing the mast-cell-mediated inhibition of CHS responses elicited by epicutaneous application of jet fuel to mouse skin.¹⁷⁶ IL-10 also has been implicated as contributing to immunosuppression in this model.¹⁷⁶ However, while the jet fuel-induced immunosuppression of CHS in *Kit^{W-sh/W-sh}* mice containing IL-10-deficient skin mast cells was greater than that seen in mast-cell-deficient *Kit^{W-sh/W-sh}* mice, it was not as substantial as that in wild-type mice or in *Kit^{W-sh/W-sh}* mice engrafted with wild-type mast cells.

In addition to determining whether mast cells are important sources of IL-10 or PGE₂ (or other factors that can mediate immunosuppressive effects) in other examples of immunosuppression, it will be of interest to assess whether mast cells and mast-cell-derived products have important effects on T_{Reg} cell numbers, phenotype and/or function in models of CHS or chronic UVB irradiation, or in any of the many other settings in which T_{Reg} cells are thought to have an important role.^{177,178}

Studies that investigate the potential interactions between mast cells and T_{Reg} are of great interest in helping to unravel the mechanisms of mast-cell-dependent immunosuppression.^{179,180} The first in vivo study of this type was that of Lu et al,¹⁰⁹ who showed that mast cells were essential for the optimal induction of peripheral tolerance to skin allografts, which requires the participation of CD4⁺CD25⁺FoxP3⁺ T_{Reg} cells. T_{Reg} cells are a source of IL-9 and IL-9 can mediate the suppression of alloreactive CD8 T cells and act as a mast-cell survival and/or growth factor that can enhance mast-cell function (reviewed in ref. 109). Local production of IL-9 (by T_{Reg} cells and/or other sources) may have contributed to the development and perhaps influenced the function, of mast cell populations within the tolerant allografts.

Consistent with the observation that mast cells can promote peripheral tolerance to skin allografts in mice, evidence derived from studies of *c-kit* mutant *Kit^{W^s/W^s}* versus wild-type rats (in which grafts from the non-inbred *Kit^{W^s/W^s}* and wild-type rats were transplanted into *Kit^{W^s/W^s}* and wild-type rats, respectively) suggests that mast cells may favor the survival of heterotopic cardiac grafts in rats.¹⁸¹ However, work in C57BL/6-*Kit^{W-sh/W-sh}* versus wild-type mice indicates that the presence or absence of mast cells in the recipient mice (which received cardiac allografts derived from other, mast-cell-containing, mouse strains) makes little or no difference in the features of either an acute or chronic model of cardiac allograft rejection.¹⁸² Moreover, local or systemic mast cell degranulation can impair T_{Reg} function and lead to the T-cell-dependent acute rejection of established tolerant skin allografts.¹⁸³ The latter finding is in accord with the results of an in vitro coculture study showing that mast cells can inhibit T_{Reg} suppression, but promote T effector cell expansion and T_H17 cell differentiation via an IL-6 and cell proximity dependent

(OX40/X40L) manner.¹⁸⁴ Taken together, the results of both in vivo and in vitro data suggest that multiple factors may be able to influence whether mast cells have positive, negative, or neutral effects on allograft survival.

In mice, mast cell IgE-dependent effector function can be modulated by T_{Reg} . In a mouse model of IgE-mediated passive systemic anaphylaxis, assessment of histamine levels in the serum showed that mast cell activation in response to challenge with IgE and specific antigen was significantly increased, relative to values in wild-type mice, either in wild-type mice that had been depleted of T_{Reg} in vivo or in OX40-deficient mice.¹⁸⁵ In vitro studies showed that T_{Reg} can directly inhibit FcεRI-dependent mast cell degranulation (but not mast cell production of IL-6 or TNF) through cell–cell contact involving interactions between OX40 expressed on T_{Reg} and OX40 ligand expressed by mast cells.¹⁸⁵ This study defined a novel, T_{Reg} -dependent mechanism, which can suppress mast cell degranulation and which could serve to limit anaphylaxis and perhaps other IgE-dependent responses.

CONCLUSION

In summary, mast cells can exert positive immunoregulatory functions in vivo that can either enhance host defense or promote disease, that reflect actions of the mast cell's stored mediators and/or cytokines and that can be mediated by functions of mast cells that reside either at the site of the immune response or at peripheral sites, such as within LNs. As outlined above, mast cells can also have negative immunomodulatory functions, in addition to their well-established roles as effector cells. Understanding in detail how individual positive or negative immunomodulatory functions can be induced or suppressed in various mast cell populations will continue to be of considerable interest.

However, understanding the specific roles of mast cells in immunomodulation during particular immune responses in vivo may be quite challenging. For example, it already is clear that mast cells can have either positive or negative immunomodulatory functions in what would appear to be very similar settings, such as in different mouse models of CHS.^{7,10,87,186,187} One may even speculate that immune responses will be found in which mast cells first promote the sensitization phase of the response, then help to initiate the local inflammation that occurs when the host subsequently is exposed to specific antigen and finally help to limit the extent of and/or resolve, the ensuing inflammation and associated tissue pathology.

In support of this hypothesis, both in vitro and in vivo data strongly suggest that one of the mechanisms that promote the mast-cell-dependent IL-10 production that in turn limits certain CHS responses is the activation of mast cells by immune complexes of specific antigen and IgG1.²⁴ These antigen-specific IgG1 antibodies develop, probably by mast cell-independent mechanisms, in response to the initial exposure to hapten during the sensitization phase of CHS.²⁴ Thus, in this model, the development of an aspect of the humoral component of the immune response to hapten challenge (i.e., antigen-specific IgG1) results in the generation of a signal (i.e., antigen-IgG1 immune complexes) that promotes an anti-inflammatory phenotype in the mast cells resident at the site of the local reaction.

The notion that mast cells might first promote the sensitization and/or elicitation phases of an immune response and then help to limit or resolve the local tissue alterations induced by antigen challenge, is consistent with the hypothesis that one key function of

this cell type is to promote homeostasis—even in those instances when the mast cells also have a major role in perturbing homeostasis in order to promote host defense.¹⁷⁻²⁰

It will be of great interest to define in detail how and under which circumstances, the positive and negative immunomodulatory functions of mast cells can significantly influence the development, magnitude or kinetics of innate or acquired immune responses. It will also be of interest to assess whether such mast-cell functions can be manipulated to achieve therapeutic ends, such as the enhancement of immune responses that promote health or the suppression of those that result in disease.

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