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Mast cells in the promotion and limitation of chronic inflammation

Summary: Observations of increased numbers of mast cells at sites of chronic inflammation have been reported for over a hundred years. Light and electron microscopic evidence of mast cell activation at such sites, taken together with the known functions of the diverse mediators, cytokines, and growth factors that can be secreted by appropriately activated mast cells, have suggested a wide range of possible functions for mast cells in promoting (or suppressing) many features of chronic inflammation. Similarly, these and other lines of evidence have implicated mast cells in a variety of adaptive or pathological responses that are associated with persistent inflammation at the affected sites. Definitively characterizing the importance of mast cells in chronic inflammation in humans is difficult. However, mice that genetically lack mast cells, especially those which can undergo engraftment with wildtype or genetically altered mast cells, provide a means to investigate the importance of mast cells and specific mast cell functions or products in diverse models of chronic inflammation. Such work has confirmed that mast cells can significantly influence multiple features of chronic inflammatory responses, through diverse effects that can either promote or, perhaps more surprisingly, suppress aspects of these responses.

Keywords: acquired immunity, IgE, inflammation, innate immunity, T cells, cytokines

Introduction

During his light microscopic examinations of the tails of living tadpoles, the mesentery of a rabbit with peritonitis, and other normal or inflamed tissues from different animals and humans (1), von Recklinghausen probably was one of the first to observe what later came to be called mast cells. However, in these studies, the cells that in retrospect probably were mast cells were not described in a way that would permit their clear distinction from other cells that exhibited overlapping light microscopic features. By contrast, Paul Ehrlich (2) provided the first description of some of the distinctive histochemical features of the cells that he was shortly thereafter to name *Mastzellen* (or well fed cells) (3, 4). These histochemical and morphological features permitted Ehrlich to distinguish mast cells from the 'plasma cells' that had been described by Waldeyer (5). Ehrlich also noted that human tissues affected by

chronic inflammatory disorders (he referred simply to 'chronic inflammation' and also mentioned 'brown pulmonary induration' and 'carcinomas') exhibited striking increases in mast cell numbers (4).

The application of these initial and later more sophisticated morphological approaches, including transmission electron microscopy, to the analysis of human and experimental animal tissues has resulted in a long list of conditions in which mast cell numbers are reported to be increased, mast cell anatomical distribution or phenotype is altered, and/or mast cell activation is evident (6–15) (Fig. 1). Many of these conditions also are associated with evidence of chronic inflammation,

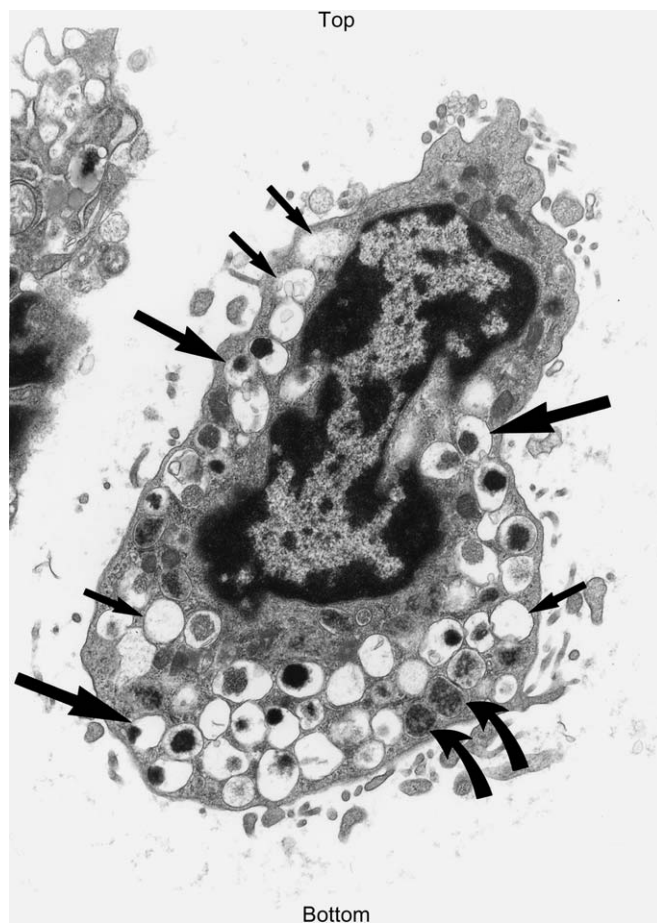


Fig. 1. Transmission electron micrograph of a mast cell exhibiting piecemeal degranulation, identified in an ileal biopsy in a patient with Crohn's disease. The mast cell exhibits only a few cytoplasmic granules (curved arrows) that appear to be filled with heterogeneous, electron-dense material and thus resemble the cytoplasmic granules observed in the so-called 'resting' (non-activated) mast cells. The great majority of the cytoplasmic granules appear empty (small arrows) or contain small amounts of electron-dense material (large arrows), findings characteristic of mast cells that exhibit evidence of piecemeal degranulation. Original magnification is $\times 11\,400$. The electron micrograph, kindly provided by A. M. Dvorak (11), is reproduced with the permission of the publisher.

which can be defined as inflammation that is persistent (usually for days, weeks, or even longer), that often includes prominent infiltrates of T lymphocytes and monocytes/macrophages (but can also include many eosinophils, neutrophils, basophils, and B cells, including plasma cells), and that frequently is associated with changes in the structure and function of the affected sites (16–18). Some chronic inflammatory processes are the consequence of or are influenced by acquired immune responses, whereas others are not (16, 17, 19, 20).

The list of agents that can stimulate immunological or non-immunological activation of mast cells continues to grow (7, 8, 13, 14, 21–23) (Table 1). In parallel, our understanding of both the diversity of functions that can be mediated by the secreted products of mast cells and of mast cell functions that can be expressed independently of the secretion of stored or newly synthesized products continues to increase (7, 12, 14, 22, 24–26). As a result, it has become possible to think of an extremely broad spectrum of mechanisms by which mast cells might influence the development, extent, or duration of chronic inflammatory responses (7, 13, 14, 21–23, 26). However, it can be quite challenging to prove that mast cells can perform such proposed functions *in vivo* and even more difficult to assess the biological importance of such mast cell-dependent contributions.

In this article, we review some basic principles of mast cell biology and then describe one approach for investigating mast cell function *in vivo*: analyzing models of adaptive or pathological responses in *c-kit* mutant mast cell-deficient mice, the corresponding wildtype mice, and *c-kit* mutant mice that have been engrafted with wildtype or genetically altered mast cells. We then describe how this approach has been used to study the roles and importance of mast cells in a variety of biological responses that are associated with chronic inflammation. Finally, we provide our perspective on the important issue of whether one can use findings regarding mast cell function derived from studies in mice to draw inferences about mast cell function in humans.

Basic biology of mast cells

Mast cell development, anatomical distribution, and phenotype

Mast cells are widely distributed throughout virtually all vascularized tissues, where they normally reside in close proximity to blood vessels, nerves, smooth muscle cells, mucus-producing glands, and hair follicles and where they can be especially numerous in anatomical sites that are directly

Table 1. Mechanisms by which mast cells can be activated* (12, 22, 40, 247)

By immune receptors	
IgE	By FcεRI; IgE can also bind to IgG receptors (FcγRII and FcγRIII) and to galectin-3, which are expressed on some mast cell populations
IgG ₁	Mouse: FcγRIII; humans: FcγRI after treatment with IFN-γ
Ig-binding superantigens	Endogenous (e.g. protein Fv in Hepatitis B virus and Hepatitis C virus infections), bacterial (e.g. <i>Staphylococcus aureus</i> Protein A and <i>Peptostreptococcus magnus</i> protein L), or viral (e.g. HIV gp120)
Products of complement activation	
C3a, C5a, C3b, and C4b	By their respective receptors: C3aR, C5aR, CR3, and CR4
Ligands of Toll-like receptors	
Peptidoglycan	TLR2
Double-stranded viral RNA, polyI:C	TLR3
LPS (lipopolysaccharide)	TLR4/CD14
Flagellin	TLR5 (human mast cells)
Single-stranded viral RNA	TLR7
CpG-DNA (cytosine and guanine, separated by a phosphate)	TLR9
Bacteria and their products	
<i>E. coli</i> FimH	CD48
<i>Pseudomonas aeruginosa</i>	Human mast cells
Virulence factors/toxins	e.g. <i>Clostridium difficile</i> toxin A, cholera toxin, VacA (cytotoxin of <i>Helicobacter pylori</i>), streptolysin O
Viruses	
Respiratory syncytial virus, influenza virus, Dengue virus, Sendai virus	By TLR and possibly by other mechanisms
Parasites	
<i>Schistosoma mansoni</i>	Activation by cercariae
<i>Leishmania major</i>	Activation by living promastigotes
Cytokines and inflammatory mediators	
Tryptase	Functional PAR2 has been shown in human skin mast cells and HMC-1 (248)
Stem cell factor	By c-Kit
IL-1, IL-12	Secretion of selective mediators (in human mast cells)
TNF	In human mast cells
Prostaglandin E ₂	Secretion of selective mediators (in human mast cells, by EP ₂) or potentiation of IgE/Ag-induced mediator release (in mouse, by EP ₁ /EP ₃)
LIGHT	Activation by LTβR in the presence of ionomycin or activated T cells (174)
CD30	Reverse signaling by CD30L induces selective secretion of chemokines (249)
Adenosine	Increase of IgE/antigen-induced degranulation by A ₃ R
Endogenous peptides	
Nerve growth factor, substance P, CGRP, and other neuropeptides	By respective G protein-coupled receptors on mast cells
ET-1	By ET _A > ET _B
Neurotensin	e.g. β-defensin 2 and LL-37
Anti-microbial peptides	
Venoms or venom components	
Sarafotoxin 6b	Component of <i>A. engaddensis</i> venom, by ET _A (43)
Phospholipase A ₂	Component of many different animal venoms (250)
Mast cell degranulating peptide	And other components of honeybee venom (251)
Numerous venoms or venom components	From scorpions, the platypus (123), frogs (252), ants (253), sea urchins (254), Portuguese man-of-war (255), etc.
Physical stimuli	
UV light, cold, heat, pressure, vibration	Possibly through direct and indirect effects on mast cells (256, 257)

*This is not a complete list. Receptors other than the ones mentioned also may be involved in the activation of certain mast cells by some of the listed agents. In some instances, the occurrence and extent, and biological significance, of mast cell activation through the listed receptors *in vivo* is not yet clear.

exposed to the environment, including the skin, airways, and gastrointestinal tract (7, 12–14, 27). It has long been known that mast cells arise from hematopoietic progenitor cells and that mature mast cells ordinarily do not circulate in the blood; instead, mast cells acquire their mature phenotype locally in the tissues where they ultimately will reside (27). Two reports

recently attempted to clarify the hematopoietic developmental pathway that leads to the generation of committed mast cell progenitors in adult mice, but these reports arrived at rather different conclusions (28, 29). While it will be important to resolve the differences between the results and conclusions presented in those two reports, several lines of evidence indicate

that changes in mast cell numbers as well as local changes in the cells' tissue distribution and/or phenotypic characteristics can be finely controlled by growth factors such as stem cell factor, the main survival and developmental factor for mast cells, interleukin (IL)-3, and the T-helper type-2 (Th2) cell-associated cytokines IL-4 and IL-9, and probably many other factors (7, 12–14, 27, 30, 31). The 'heterogeneity' of mast cells, which refers to the ability of this lineage to generate individual mast cell populations that exhibit differences in biochemical and functional properties (7, 27, 32–34), probably contributes to the flexibility and diversity of the ways that mast cells can be activated in and contribute to the many physiological, immunological, inflammatory, or other biological responses in which this cell participates.

Mast cell activation and secreted products

Mast cells have the potential to secrete diverse biologically active products upon exposure to a variety of immunological or non-immunological stimuli. Depending on the particular mechanism of activation and the strength of the stimulus, mast cells may release either a very large array of products or distinct subsets of mediators and/or cytokines (12, 13, 22, 35).

The best studied mechanism of mast cell activation is that induced by the cross-linking of FcεRI, the high affinity receptor for immunoglobulin E (IgE), e.g. with IgE and specific bi- or multivalent antigen (Table 1). Although IgE can also bind to the IgG receptors FcγRII and FcγRIII and to galectin-3, which are expressed on some mast cell populations, IgE is thought to influence mast cell functions mainly through its interaction with FcεRI (12, 13, 35, 36). In mice, mast cells can also be activated to degranulate and release mediators in response to antigen- and IgG₁ (i.e. immune complex)-dependent signaling through FcγRIII (12, 37–39).

Activation through Fc receptors is only one of many potential mechanisms of mast cell activation (Table 1). For example, mast cells can be activated directly by pathogens, including bacteria, viruses, and parasites, as well as by many soluble products derived from these pathogens. Microbial products can activate mast cells directly by Toll-like receptors (TLRs) or receptors for bacterial toxins or indirectly, e.g. by activating the complement system that then can generate products that interact with appropriate receptors expressed by mast cells (22, 40, 41). Furthermore, mast cells can be activated by a variety of endogenous and exogenous peptides, cytokines, and other inflammatory mediators (12, 13, 23, 42, 43).

After undergoing activation, mast cells can release a diverse array of potent biologically active products (which sometimes also are referred to collectively as 'mediators'). These mediators

can be summarized as falling into three classes: (i) preformed mediators that are stored in the cells' prominent cytoplasmic granules; these include histamine (and in rodents, serotonin), proteoglycans, and proteases such as chymase, tryptase, and carboxypeptidase A; (ii) *de novo* synthesized lipid mediators, e.g. metabolites of arachidonic acid through either the cyclooxygenase (e.g. prostaglandin D₂) or lipoxygenase (e.g. leukotriene C₄) pathways; and (iii) a vast array of cytokines, chemokines, and growth factors (7, 13, 14, 21, 24, 25).

Many of these products are considered to be proinflammatory, in that they can elicit, individually or in concert, the features of different patterns of inflammation that are observed in settings of acute and/or chronic inflammation, e.g. vasodilation, plasma extravasation, and the recruitment and activation of granulocytes (i.e. neutrophils, eosinophils, and basophils), T cells, B cells, dendritic cells (DCs), and monocytes (44–48). Mast cell products also can promote changes in the tissues that are affected by such inflammatory responses, including the local degradation or remodeling of structural elements of the tissues, which can be associated with significant changes in the function of the affected organs (49–51).

In addition to the difficulty of predicting exactly how mast cell activation will influence chronic inflammation or other types of biological responses is the fact that some of the mast cell-associated products, which might be released in these settings, can have anti-inflammatory effects. For example, IL-10 and TGF-β, which are both known to have several 'anti-inflammatory' properties, are expressed by mast cells and can be released upon their appropriate activation (13, 52–54).

c-kit mutant mast cell-deficient and 'mast cell knockin' mice

In vitro studies of mouse bone marrow-derived cultured mast cells (BMCMCs) or human mast cell populations can be very useful for investigating mechanisms by which mast cells might influence adaptive immunity or other biological responses (55–60). However, the *in vivo* relevance and biological importance of such *in vitro* observations should also be assessed. *c-kit* mutant mice that virtually lack mast cell populations can be used to assess the regulation by mast cells of the expression of biological responses *in vivo*.

The most commonly used model for such studies has been the (WB/ReJ × C57BL/6)F₁-Kit^{W/W^{-v}}, or WBB6F₁-Kit^{W/W^{-v}}, mouse (61–63) (Table 2). However, C57BL/6-Kit^{W^{-sh}/W^{-sh}} mice are gaining popularity as an alternative model (64–66). While each of these *c-kit* mutant mice is profoundly mast cell deficient and also virtually lacks melanocytes and interstitial cells of Cajal,

Table 2. Comparison of phenotypic characteristics of c-kit mutant C57BL/6-Kit^{W-sh/W-sh} and WBB6F₁-Kit^{W/W-v} mice

Phenotypic characteristics of naive mice (at baseline)	C57BL/6-Kit ^{W-sh/W-sh} mice	WBB6F ₁ -Kit ^{W/W-v} mice
Markedly reduced KIT signaling	Yes	Yes
Sterile	No	Yes
Anemic	No	Yes
Virtually lack melanocytes*	Yes	Yes
Lack interstitial cells of Cajal†	Yes	Yes
Virtually lack mast cells in all tissues‡	Yes	Yes
Develop mast cell populations after transfer of congenic Kit ^{+/+} BMCMCs	Yes	Yes
Unfractionated bone marrow cells‡	No	Yes
Normal levels of neutrophils and macrophages in bone marrow, spleen, and peritoneal cavity†	Yes	Yes
Normal levels of basophils in bone marrow and spleen†	Yes	Yes
Normal levels of CD11c ⁺ DCs and natural killer cells in spleen†	Yes	Yes
Normal levels of CD19 ⁺ B cells in bone marrow and spleen†	Yes	Yes
Normal levels of CD8 ⁺ and CD4 ⁺ T cells in thymus and spleen†	Yes	Yes
Deficiency in number of TCRγδ T cells in the small intestine†	No	Yes
Exhibit bile reflux	Yes	Yes
Develop stomach papillomas and ulcers	No	Yes
Develop idiopathic dermatitis	No	Yes

Text in bold indicates characteristics where differences have been observed.

*Kit^{W-sh/W-sh} mice can have some melanocytes in the ear pinnae. This can complicate interpretation of the results in certain experimental models, such as those involving exposure of the skin to ultraviolet irradiation.

†In adult mice.

‡Mast cells develop in Kit^{W-sh/W-sh} mice that have received whole bone marrow cells i.v. after 950 rad irradiation; however, this does not produce selective mast cell engraftment, as multiple hematopoietic lineages of donor origin develop in such mice. Mast cells do not develop after i.v. injection of whole bone marrow cells into non-irradiated Kit^{W-sh/W-sh} mice.

the double dose of *W^{sh}* mutations in C57BL6-Kit^{W-sh/W-sh} mice results in fewer, or less severe, phenotypic abnormalities than are observed in Kit^{W/W-v} mice. For example, unlike WBB6F₁-Kit^{W/W-v} mice, C57BL/6-Kit^{W-sh/W-sh} mast cell-deficient mice are not anemic and are fertile, and therefore can easily be crossed with mice carrying other defined genetic abnormalities of interest (12, 64–66).

As in WBB6F₁-Kit^{W/W-v} mice, the mast cell deficiency of C57BL/6-Kit^{W-sh/W-sh} mice can be selectively ‘repaired’ by the adoptive transfer of genetically compatible, *in vitro* derived mast cells (65, 66) (Fig. 2). Such mast cells can be derived from the BMCMCs of the congenic wildtype mice or various transgenic or mutant mice (62), from BMCMCs that are transduced with short hairpin RNA (shRNA) to reduce expression of proteins of interest (43), from other sources of hematopoietic cells (such as fetal liver), or directly from embryonic stem cells (63). These *in vitro*-derived mast cells can be administered by intravenous, intraperitoneal, or intradermal injection (27, 62, 65) or by direct injection into the anterior wall of the stomach (67) to create the so-called ‘mast cell knockin mice’ (Fig. 2).

Using mast cell knockin mice, one can assess the extent to which differences in the expression of biological responses observed in mast cell-deficient and wildtype mice reflect the absence of mast cells or the reduction or absence of specific proteins expressed by mast cells. However, in conducting

studies of this type, it is important to recognize that the phenotypic characteristics and the anatomical distribution and numbers of adoptively transferred mast cells change in the interval after the injection of the mast cell populations into the recipient c-kit mutant mice. Therefore, decisions about how to design and interpret the results of experiments using such mice should take these facts into consideration.

In those anatomic sites that have been investigated, the phenotypic characteristics of the *in vitro*-derived mast cell populations that have been adoptively transferred into c-kit mutant genetically mast cell-deficient mice gradually come to resemble those of the corresponding native mast cell populations in wildtype mice (62, 65, 66, 68, 69). However, it probably is impossible to prove that such adoptively transferred mast cells are literally ‘identical’ to the native mast cell populations at the corresponding anatomical site in wildtype mice (this would require knowing fully the entire patterns of gene expression in cells in the two populations, the amounts of all expressed products, etc.). Furthermore, the rate at which mast cells appear in various anatomical sites after adoptive transfer of whole bone marrow cells (61) or *in vitro*-derived mast cells (65, 66, 70, 71) into c-kit mutant recipient mice varies according to the anatomic site. Notably, 4 weeks appears to be a suitable time interval to wait after intraperitoneal transfer of mast cells, as that time is sufficient for the mast cells to enter the

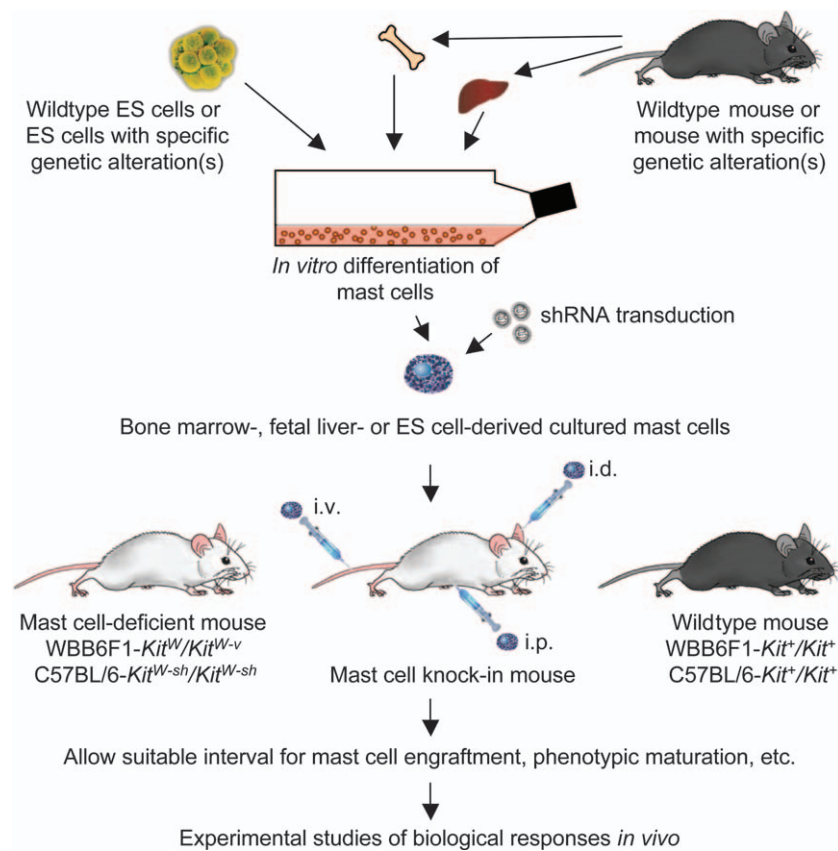


Fig. 2. Mast cell knockin mouse model for *in vivo* analyses of mast cell function. Mast cells are generated from bone marrow cells (or other hematopoietic cells; e.g. those in the fetal liver) from wildtype mice or from mice with specific genetic alterations. Alternatively, embryonic stem (ES) cell-derived cultured mast cells can be generated from wildtype or genetically altered ES cells or mast cells can be transduced *in vitro* with shRNA to silence specific genes of interest. These bone marrow-, ES cell-, or fetal liver-derived cultured mast cells can then be transplanted intravenously (i.v.), intraperitoneally (i.p.), or intradermally (i.d.) into mast cell-deficient *c-kit* mutant mice, such as WBB6F1-Kit^W/Kit^{W-v} or C57BL/6-Kit^{W-sh}/Kit^{W-sh} mice, to produce mast cell knockin mice. A suitable interval is then allowed for engraftment and phenotypic maturation of the adoptively transferred mast cells (the

length of this interval can vary based on the route of mast cell transfer, the anatomical site of interest, etc.). The importance of mast cell function(s) in biological responses can be analyzed by comparison of the responses in wildtype mice, *c-kit* mutant mast cell-deficient mice, and selectively mast cell-engrafted *c-kit* mutant mice (mast cell knockin mice). The contributions of specific mast cell products (surface structures, signaling molecules, secreted products, and so on) to such biological responses are analyzed by comparison of the features of the responses of interest in mast cell knockin mice engrafted with wildtype mast cells versus mast cells derived from mice or ES cells that lack or express genetically altered forms of such products or that have been transduced with shRNA to silence the specific genes that encode these products.

mesentery and to achieve normal numbers, as assessed by evaluation of peritoneal lavage fluids (43, 65, 72, 73). This time interval is also sufficient for the transferred mast cells to acquire phenotypic characteristics that more closely resemble those of native peritoneal mast cells than do those of the *in vitro*-derived mast cells, as assessed prior to intraperitoneal transfer (62, 65, 66, 68, 69).

Accordingly, many groups studying the functions of mast cells in biological responses elicited in the peritoneal cavity now use an interval of about 4 weeks after adoptive intraperitoneal transfer of mast cells before initiating the experiments to assess mast cell function in these responses (42, 43, 66, 74, 75). A similar interval (4–8 weeks) often is used in studies of mast cell

function in the skin of *c-kit* mutant mice that have undergone adoptive transfer of *in vitro*-derived mast cells by the intradermal route (76–84). However, at least for certain phenotypic features, including appearance by transmission electron microscopy (62) and content of histamine (62) and heparin (62, 68), extending the interval after intraperitoneal transfer for longer than 4 weeks will permit the phenotype of the adoptively transferred mast cells to resemble even more closely that of the corresponding native population of mast cells in wildtype mice. The same is likely to be true in the case of intradermal mast cell transfer.

Depending on the anatomical site examined, the anatomical distribution of the adoptively transferred mast cells can either be

very similar to that of the native mast cell population in the corresponding wildtype mice or exhibit differences from the native distribution, which should be considered when interpreting the results of experiments using such animals. For example, intravenous transfer of mast cells results in a distribution of mast cells in the gastrointestinal tract of the *c-kit* mutant mice that differs from that in wildtype mice. Specifically, more mast cells typically are present in the submucosa and muscularis propria of the stomach and small intestine of the mast cell knock-in mice than normally occurs in the corresponding sites in age-matched wildtype mice (65).

Intravenous transfer of mast cells to *c-kit* mutant mice also results in an abnormal distribution of mast cells in the airways and lungs. Compared with the corresponding wildtype mice, such mast cell knockin mice have, at baseline, fewer mast cells in the trachea and more mast cells in the lung parenchyma (65, 66, 85). Remarkably, despite this difference, we have found that the magnitude of many features of immune responses affecting the airways and lung, which are thought to have important mast cell-dependent components, is very similar in wildtype mice and mast cell knockin mice (85–87). This observation may reflect, at least in part, the fact that depending on the specific feature of the biological response that is being measured, fully sufficient mast cell-dependent function can be provided by a relatively small amount of mast cell activation at that site (88).

The decision about how long to wait after adoptive transfer of mast cells before performing experiments to assess mast cell function in mast cell knockin mice therefore can be influenced by a number of factors, including the increased cost of using very extended intervals between adoptive transfer of mast cells and studies of mast cell function and the fact that many experimental protocols have been designed for use in relatively young adult mice. However, in interpreting the results of experiments using mast cell knockin mice, it is best to keep in mind how the findings may have been influenced by differences in the numbers, anatomical distribution, phenotype, and/or maturity of the mast cell population in the wildtype and various mast cell knockin mice used in such work.

In studies using *c-kit* mutant mast cell-deficient mice, one must always directly assess whether the chronic inflammatory response (or other biological response) under investigation might itself alter the mast cell-deficient status of the mice in which that inflammatory response is being induced. In certain instances, the induction of chronic inflammatory responses in *c-kit* mutant mast cell-deficient mice has resulted in the development of mast cell populations at the affected site (89–94). While the numbers of mast cells that develop in such settings have been reported to be significantly less than those

present in the identically treated wildtype mice, one clearly ought to take into account that the *c-kit* mutant mice were less mast cell deficient at the end of the experiment than they were at baseline (89–94).

Additional genetic models to investigate mast cell function *in vivo*

Several groups are attempting to produce and characterize ‘mast cell-specific Cre’ mice, which then can be mated with mice in which genes for different proteins of interest have been ‘floxed’ to produce mice in which only mast cells lack the protein of interest. Cre recombinase recognizes the loxP consensus sequence and catalyzes the recombination of DNA sequences flanked (i.e. floxed) by two loxP sites. When a gene sequence is flanked by loxP sites, Cre recombinase can then excise that specific segment from the gene sequence (95–99).

To delete a gene of interest specifically in mast cells *in vivo*, transgenic mice expressing the Cre recombinase gene under the control of a mast cell-specific promoter are crossed with floxed mice in which an essential element of the gene of interest is flanked by loxP sites. In the offspring of such mice, Cre is expressed only in mast cells, resulting in the deletion of the targeted gene specifically in mast cells.

In principle, this will be a powerful for investigating the roles of specific mast cell-associated products in health and disease. However, among the issues to address in evaluating such work will be the following: to what extent can it be shown that the deletion of the gene of interest is in fact ‘mast cell-specific’? Indeed, efforts of this type will be interesting in part because they may help to settle some long-standing issues about which, if any, of the mast cell-associated products whose promoters were selected to drive Cre production in mast cells are truly unique to the mast cell. If the selected putative mast cell-specific promoter actually drives sufficient levels of Cre expression in cell types other than the mast cell, then the floxed product will be deleted in those cell types as well.

Similarly, it is known that genetically deleting certain mast cell-associated products [e.g. certain proteoglycans (100, 101) or proteases (102)] can have effects on mast cells that result in significant additional phenotypic abnormalities beyond those attributable to the loss of the best studied of the biological functions of the targeted mediator. For example, genetic deletion of the mouse mast cell carboxypeptidase A (mMC-CPA) results not only in the loss of that protease’s enzymatic activity but also in abnormalities in the mast cell’s content of other mediators that are packed in the cytoplasmic granules in association with mMC-CPA (102, 103). This general

consideration applies to all genetic approaches ‘targeting’ the mast cell and therefore should be kept in mind when evaluating the results of experiments using the Cre–lox approach in these cells; both ‘expected’ and ‘unexpected’ results may occur, reflecting consequences of the loss of the targeted product on known and perhaps as yet to be described functions of that product in the regulation of mast cell phenotype and/or function.

One must also carefully consider the possible differences in phenotypes that may be produced based on when in the development of the mast cell, or in the development or adult life span of the mouse, the particular targeted product is deleted. This in turn will depend on when the gene whose promoter is driving the Cre expression is expressed in that mast cell population. The deleted product may or may not have important roles in the mast cell development or maturation, and/or it may be more important as a mast cell product during some stages of the mouse’s life span than in others.

Mice in which the Cre–lox approach can be used to delete, in mast cells, specific products that either only can be produced by mast cells (assuming that such truly mast cell-specific products exist) or can be produced by mast cells and other cell types will be powerful tools for studying mast cell biology. However, it will be important to exercise care both in characterizing the phenotypic effects of such approaches, in mast cells and possibly in other cell types, and in interpreting the results of experiments using such mice.

Functions of mast cells in innate responses

In hosts that survive their first encounter with a pathogen or other harmful agent, such as a toxin, the initial phase of the innate response to such agents (that, by definition, occurs independently of a pre-existing acquired B-cell or T-cell-dependent immune response to that agent) can be followed by the development of chronic inflammation, in some cases associated with the development of an acquired immune response to the inciting agent. Current interest therefore focuses on two aspects of the mast cell’s potential roles in the long-term consequences of such exposures. (i) Can the mast cell regulate (i.e. promote and/or suppress) the chronic inflammation and tissue remodeling that follows the initial response to the agent? (ii) Can the mast cell regulate (i.e. promote or suppress) the subsequent development of an acquired immune response to that agent?

Roles in innate immune responses to pathogens

It has now been shown in several experimental model systems that mast cells can enhance the effectiveness of the host response

to certain bacterial infections (72, 104–108). *In vitro* and/or *in vivo* studies indicate that mast cells can enhance host defense through direct effects on pathogens, by initiating and modulating the inflammation associated with innate immune responses and possibly also by initiating adaptive immune responses to pathogens.

In vitro studies suggest that mast cells might be able to control bacterial infections by direct bactericidal activity, e.g. by phagocytosis of bacteria (109) or by secretion of anti-microbial peptides that are able to eliminate pathogens (110). Whether these mechanisms can improve the clinical course of specific infections *in vivo* remains to be determined. However, many studies have established that mast cells can have a critical role in the development of the inflammation associated with innate immune responses. Using mast cell knockin mice, Echtenacher and Malaviya and their respective coworkers (72, 104) provided clear evidence that mast cells can represent key effector cells for the orchestration of optimal innate host responses and for survival in mice during septic peritonitis and other bacterial infections.

Several lines of evidence indicate that an important way in which mast cells contribute to such responses is by initiating and regulating the magnitude of the leukocyte recruitment and perhaps other aspects of inflammation at the site of infection, thereby enhancing clearance of the pathogens. Experiments using mast cell-deficient mice showed that both tumor necrosis factor (TNF) and leukotrienes can be important contributors to the mast cell-dependent recruitment of neutrophils and the associated improvement of clinical symptoms in infected mice (72, 106, 107).

Studies in other experimental model systems indicate that the release of TNF (111), leukotrienes (46), and possibly other mediators, cytokines, and chemokines during inflammatory processes (whether from mast cells or other cellular sources) can promote the subsequent recruitment not only of neutrophils, a characteristic feature of acute inflammation, but also of other inflammatory cells including T cells and macrophages, which are common components of chronic inflammation. Such recruited leukocytes in turn can initiate and maintain various features of chronic inflammatory responses (16–18).

TNF and other products released in such settings also can promote chronic inflammation through effects on cell types that are ordinarily resident in the affected tissues. For example, TNF has procoagulant and perhaps other activities that can promote the formation of fibrinous adhesions in the peritoneal cavity of mice after induction of septic peritonitis (112). While the development of peritoneal adhesions in this model is thought to

improve survival by helping to contain the bacterial infection anatomically (112), other consequences of such chronic changes in the peritoneum, e.g. intestinal obstruction or persistent pain, can be detrimental (113). It remains to be investigated to what extent mast cells (as opposed to other sources of TNF) can contribute to these TNF-dependent chronic inflammatory changes and whether the release of other mast cell products might also be involved in regulating the formation and/or resolution of peritoneal adhesions.

Another function of mast cells during innate responses to bacterial infection is to limit the toxicity of certain endogenous products that are generated by the host in such settings and which have significant adverse effects when present in high concentrations. For example, we found that mast cells can limit the toxicity of endothelin-1 (ET-1) (42), a peptide known to be involved in sepsis in humans and mice (42, 114). The morbidity and mortality induced by the administration of ET-1 to mice was dramatically reduced by the activation of mast cells through endothelin receptors expressed on their surface, and pharmacological evidence indicated that mast cells reduced ET-1 concentrations by the release of proteases stored in the cells' granules (42).

Although initial pharmacological evidence implicated chymase as a key protease in this setting (42), subsequent work using shRNA knockdown approaches indicated that mMC-CPA is likely to be more important than chymase in degrading ET-1 (43). It will be of interest to examine this issue using mMC-CPA^{-/-} mice (102) as well.

It is likely that mast cell- and mast cell protease-dependent mechanisms can reduce the concentrations of other endogenously produced, potentially toxic mediators associated with inflammation. However, it is conceivable that the release of proteases from mast cells in the context of innate immune responses may also lead to chronic inflammatory changes, at least in some settings, because mast cell proteases (e.g. tryptase and chymase) have been implicated in inflammation-associated tissue remodeling in the skin, lung, and heart (51, 115–117).

Work using mast cell knockin mice has so far identified certain beneficial functions of mast cells in various models of innate immunity. However, a wide range of different mast cell products can be released when this cell is stimulated by different mechanisms, and some of these released products, including TNF (72), can have either beneficial or harmful effects, depending on the concentration of product present at key stages of the process under investigation. Therefore, we would not be surprised if there are situations in which mast cells either have a combination of beneficial and detrimental functions that 'balance' to a neutral role in individual innate responses or even

have net effects that promote pathology during certain examples of such responses.

Roles in innate responses to venoms or other exogenous toxins

Mast cells can limit the toxicity of the endogenous peptide ET-1 (42). The amino acid sequence of ET-1 exhibits striking similarity to that of sarafotoxin 6b (118), the most toxic component of the venom of the Israeli mole viper (*Atractaspis engaddensis*). This finding raised the possibility that mast cells might be able to enhance resistance in mice to envenomation with *A. engaddensis* venom.

Mast cell-deficient mice were approximately 10 times more susceptible than wildtype mice to the deadly effects of the venom, and both pharmacological and shRNA knockdown experiments implicated mMC-CPA as an important contributor to the mast cell's ability to 'detoxify' either sarafotoxin 6b or the whole venom (43). Mast cells also enhanced resistance in mice to the venom of two species of pit vipers (*Crotalinae*) and the honeybee (*Apis mellifera*); in these settings as well, the ability of mast cells to enhance resistance appeared to reflect, at least in part, the ability of these cells to release proteases (43).

Animal venoms can contain toxins that act by a variety of mechanisms, and most venoms represent complex mixtures of toxins (119, 120). Accordingly, we think it very unlikely that mast cells can enhance resistance to all venoms. However, we think that it will be of great interest to investigate the extent to which mast cells might be able to modulate host responses to the diverse toxins found in nature, including those from microbes, plants, and animals. Because many different potentially toxic products of microbial organisms, plants, and animals can induce mast cell degranulation (77, 121–123), it will be of interest to determine, in those hosts that survive an initial exposure to such agents, the extent to which mast cells modulate the chronic inflammation and tissue remodeling that can represent the sequelae of such exposures (124).

In examples of experimental inflammatory responses induced by phorbol esters, which are produced by a number of plant species whose oils can promote inflammation and other pathology (125), work in wildtype, mast cell deficient, and mast cell knockin mice showed that inflammatory reactions could be induced in *c-kit* mutant mast cell-deficient mice (77, 91, 126). However, the acute inflammation induced by phorbol esters was greater in the *c-kit* mutant mast cell-deficient mice than in the congenic control mice (77, 126). By contrast, repeated application of phorbol 12-myristate 13-acetate (PMA) induced very similar levels of persistent tissue swelling in

genetically mast cell-deficient mice and the congenic normal mice (91).

In settings such as these, mast cells may promote pathology by enhancing inflammation. Moreover, in one model in which PMA was applied repetitively to the skin of mice, the chronic inflammatory response induced in WBB6F₁-Kit^{W/W-v} mice resulted in the development of significant populations of mast cells at the affected site (91), despite the defect in c-Kit signaling in such cells (88). This finding emphasizes the importance, in studies using c-kit mutant mast cell-deficient mice, of directly assessing whether the chronic inflammatory response under investigation might itself alter the mast cell-deficient status of the mice in which that inflammatory response is being induced (89–94).

Positive or negative effects on the transition from innate to acquired immune responses

Mast cells can provide many signals with the potential to enhance or suppress aspects of the sensitization phase of adaptive immune responses (13, 22). Accordingly, there is considerable interest in understanding the extent to which mast cells might contribute to or retard the transition from innate immunity to the development of acquired immune responses to the inciting agent (13, 22, 41). For example, recent work has implicated TNF and other mediators derived from mast cells in the development of acquired immune responses to pathogens. McLachlan et al. (111), investigating responses to the injection of *Escherichia coli* into the footpads of wildtype, mast cell-deficient, and mast cell knockin mice, showed that the recruitment of T cells to draining lymph nodes and the degree of lymph node hypertrophy are substantially increased by mast cell-derived TNF. Similarly, Jawdat and colleagues (127) recently reported an important role for mast cell-derived TNF in promoting the migration of Langerhans cells from the skin to the draining lymph nodes in response to bacterial peptidoglycan. They also provided evidence for a mast cell- and complement-dependent but TNF-independent effect of peptidoglycan on lymph node hypertrophy in that setting (127).

Whether any of the mast cell-dependent differences observed in these studies also result in significant differences in the clinical outcome (e.g. morbidity and mortality related to the bacterial infection) remains to be determined. However, it is likely that there is considerable redundancy in the mechanisms that promote the development of many if not all acquired immune responses and that, in many of these settings, the roles of the mast cells may not be critical for the ultimate outcome of the response. For example, studies of humoral immune

responses in mast cell-deficient mice have for the most part not detected marked abnormalities in such features of these responses as titers of antigen-specific antibodies (128, 129).

In models of fluorescein isothiocyanate (FITC) challenge in the skin (epicutaneously) or airways (intranasally), c-kit mutant mast cell-deficient mice exhibited significantly reduced levels of DC migration to draining lymph nodes in the first 24 h after hapten administration, an abnormality that was abolished in c-kit mutant mice engrafted with wildtype but not TNF^{-/-} mast cells (130). However, even in the c-kit mutant mast cell-deficient mice, no defect in DC migration to draining lymph nodes was detectable by 48 h after hapten challenge, indicating the existence of mechanisms that eventually compensated for the lack of mast cells and mast cell-derived TNF.

The delay in DC recruitment in the contact hypersensitivity (CHS) model was not associated with an obvious abnormality in the sensitization phase of the response to FITC. We did detect a reduced expression of CHS at sites of hapten challenge in Kit^{W-sh/W-sh} mice, and this defect was fully repaired by prior local transfer of wildtype BMCMCs to sites later challenged with hapten but only partially repaired by transfer of TNF^{-/-} BMCMCs (130). However, the mast cell-associated defect in CHS to FITC in this setting appeared to be because of the impairment of effector phase of the response, not of the sensitization phase.

There is at least one example in which mast cells are required for the optimal development of an acquired immune response (131). Remarkably, the mast cell's role in enhancing the development of this acquired immune response to the newly encountered antigen appears to require that the mast cell be 'primed' for this role by having bound to its surface an important product of a past acquired immune response, namely, IgE. However, in this instance, the key effects of IgE appear to be independent of the antibody's antigen specificity.

Bryce et al. (131) reported that both mast cells and physiological concentrations of antigen-non-specific IgE are required for optimal sensitization in a model of CHS to oxazolone (Ox) and can enhance the emigration of skin Langerhans cells from the epidermis of naive mice in response to epicutaneous application of Ox in ethanol. The binding of IgE to FcεRI on dermal mast cells, even in the absence of antigen known to be recognized by that IgE, enhanced levels of mRNA for certain mast cell-associated products that were detected in the skin 1 h after the first epicutaneous application of the hapten. In addition, several products that might be secreted by such mast cells (TNF, IL-6, and MCP-1) can influence the biology of DCs. These findings are the strongest evidence currently available that the effects of IgE on mast cells that can be

detected in the absence of specific antigen known to be recognized by such IgE (30, 132) can be important in modulating the mast cell function *in vivo*.

Evidence also has been reported that mast cell activation by TLR4 can enhance the sensitization phase of the immune response to ovalbumin (OVA) in mice that have been exposed to that antigen by the airways (133). Specifically, Nigo et al. (133) showed that OVA-induced eosinophil infiltration into lung tissues was significantly increased by administration of lipopolysaccharide in wildtype mice, whereas this effect was not observed in mast cell-deficient mice or TLR4^{-/-} mice. Adoptive transfer to mast cell-deficient mice of BMCMCs derived from wildtype mice but not TLR4^{-/-} BMCMCs permitted such mice to express levels of eosinophil infiltration after OVA challenge that were similar to those in wildtype mice.

These findings are quite interesting. However, immunization with OVA was begun only 2 days after adoptive transfer of the BMCMCs, which is too short an interval for the cells to occupy their ultimate anatomical locations and provides little time for the cells to acquire a more mature, tissue-appropriate phenotype. Therefore, the transferred BMCMCs may still retain properties more characteristic of BMCMCs maintained *in vitro* than of the native lung mast cell populations that would participate in immune response in the normal mouse *in vivo*. Accordingly, it will be important to investigate whether similar findings can be obtained in mast cell-deficient mice that had undergone mast cell engraftment at longer intervals (e.g. 8–12 weeks) before immunization.

Evidence recently has been reported that, in certain circumstances, mast cells can have effects that suppress the development of adaptive immune responses. It has been known for some time that several mast cell products can have actions that might interfere with antigen sensitization (47, 134–140). Depinay et al. (141) recently showed that the bites of *Anopheles* mosquitoes can have effects that impair the development of antigen-specific T-cell responses in a model of delayed type hypersensitivity to OVA in mice and that this phenomenon requires the presence of mast cells in the skin at the site of the bite.

The mechanism of mast cell-dependent immunomodulation in this setting and the extent to which the critical mast cell function(s) might be influenced by the effects on mast cells of antigen-non-specific IgE remain to be determined. However, it is speculated that mast cell-derived histamine may be one key mediator. Notably, mast cells and histamine had previously been shown to be required for the optimal expression of ultraviolet B-induced immune suppression of CHS responses to 2,4,6-trinitro-1-chlorobenzene (TNCB) in mice (142), and

histamine was shown to be able to influence T-cell- and antibody-dependent responses by actions on H1 and H2 receptors (45, 48).

Mast cells in the acute, late-phase, and chronic inflammation associated with IgE-associated immune responses

IgE-dependent aggregation of FcεRI on the mast cell surface is the mechanism of mast cell activation that has been most extensively studied *in vitro*, and mast cell knockin mice have been used to investigate the roles of mast cells in the expression of several different models of IgE-associated immune responses *in vivo*. It has long been accepted that mast cells contribute significantly to the features of acute responses to antigen in hosts that bear IgE antibodies that are reactive with that antigen, and work in mast cell knockin mice confirms the importance of the mast cell in such acute IgE-dependent responses. However, it only recently has become clear, through work with mast cell knockin mice, that mast cells also can contribute significantly to the chronic inflammation and long-term tissue changes that are associated with repetitive antigen challenge in mice that have been sensitized to contain IgE antibodies that recognize that antigen.

The 'mast cell/basophil autonomous' or 'immediate hypersensitivity' phase of IgE-associated immune responses. The immediate phase of an IgE-associated allergic response generally occurs within the first 2 h after antigen exposure (Fig. 3B). In appropriately sensitized hosts, encounter with specific antigen by IgE on the mast cell surface rapidly induces aggregation of FcεRI, initiating a signaling response that results, within minutes, in the secretion of diverse mediators; as noted above, mouse mast cells also can undergo activation by IgG₁- and FcγRIII-dependent mechanisms, e.g. in response to immune complexes of antigen and IgG₁ antibodies (7, 12, 13, 35, 36, 39).

Studies using *c-kit* mutant mice have proven that such mast cell activation can contribute to the pathology of IgE-associated acute allergic disorders, including anaphylaxis, in actively or passively sensitized animals (143–145). Moreover, work in mast cell knockin mice showed that mast cells are essential for virtually all of the augmented vascular permeability and tissue swelling associated with IgE-dependent passive cutaneous anaphylaxis reactions (12, 76, 146).

However, mast cells are not required for all effects of IgE that can be observed *in vivo*. For example, IgE can result in the development of a chronic inflammatory response in the skin in

A Sensitization

Effects on migration, maturation, and function

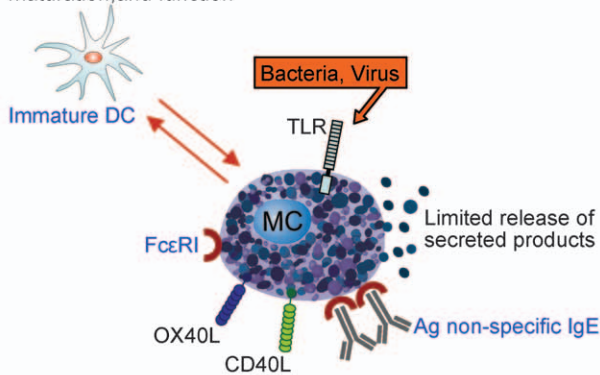
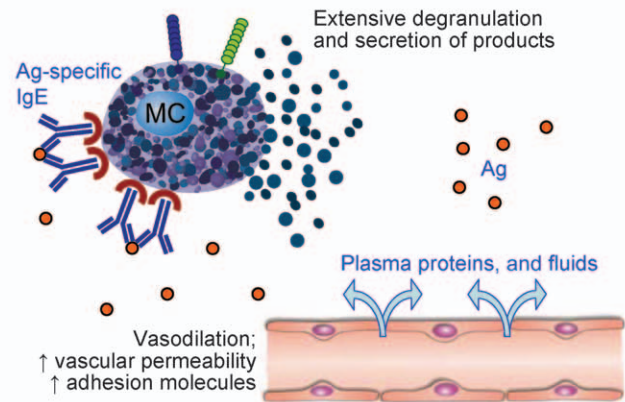
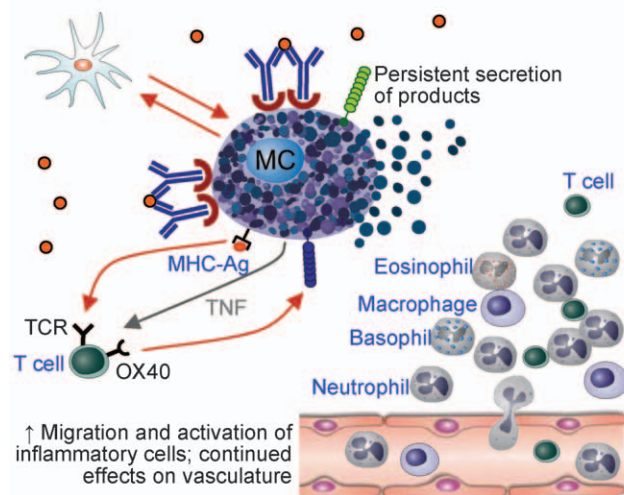
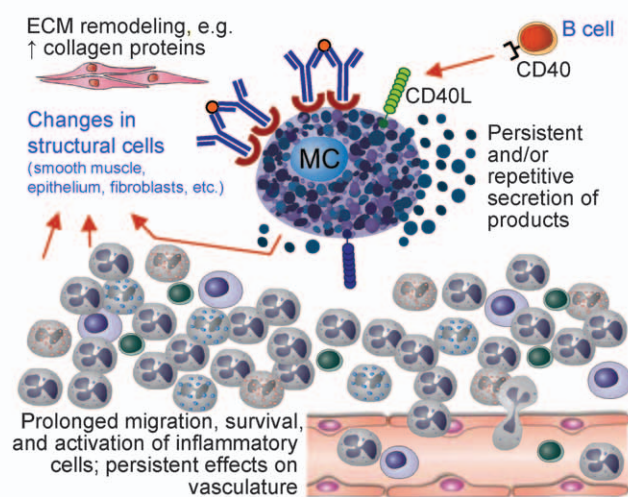
**B Immediate hypersensitivity reaction (up to approximately 2 h)****C Late-phase response (approximately 2–24 h)****D Chronic allergic inflammation (>24 h)**

Fig. 3. Mechanisms by which mast cells might promote the transition from innate to acquired immune responses or contribute to the development of immediate hypersensitivity reactions, LPRs, and chronic allergic inflammation. (A) Mast cells (MCs) can respond to local signals (e.g. certain haptenated non-microbial proteins or other antigens, or the products of pathogens). Such responses can include secretion of mediators or expression of other functions that can enhance the sensitization phase of acquired immune responses through effects on DC migration, maturation, and/or function. For example, exposure to antigen-non-specific IgE might 'prime' MCs to express higher levels of mRNA for some crucial mediators or cytokines. (B) In hosts sensitized to express an IgE-associated immune response, secondary exposure to bi- or multivalent antigen initiates IgE + antigen-dependent MC activation. (C) Sometimes, IgE-associated immediate reactions are followed, hours later, by LPRs. MCs can contribute to such reactions through effects of secreted mediators that promote the recruitment and/or functional activation of

circulating leukocytes, as well as by persistent effects on cells resident at the site. IgE-dependent MC activation can also promote DC migration from sites of antigen challenge. Direct activation of effector T cells by antigen and perhaps bidirectional interactions between MCs and T cells also can contribute to LPRs. (D) At sites of prolonged or repetitive exposure to specific antigen, MC activation can be prolonged and/or recurrent, MC numbers can increase, and MC phenotype and/or anatomical distribution can change. In such settings, MCs can contribute significantly to many of the features of chronic allergic inflammation, including functional changes in the affected organ (e.g. airway hyperresponsiveness to immunologically non-specific agonists of bronchoconstriction in the asthmatic lung). MCs also might have effects on the immune response itself (e.g. mediated through interactions of MC CD40 ligand with B-cell CD40). However, in some circumstances, certain MC products or functions can serve to limit or to resolve aspects of these inflammatory responses.

the absence of mast cells (146), and one example has been reported in which genetically mast cell-deficient mice can exhibit an acute allergic response (anaphylaxis) that may be IgE dependent but is mast cell independent (147). However, both mouse studies and clinical observations in humans support the conclusion that mast cells are either fully sufficient for or

importantly contribute to the development of the great majority of (if not virtually all) IgE-dependent immediate hypersensitivity reactions (7, 12–14, 36, 145). Basophils may also contribute significantly to some acute reactions of this type, particularly those that have a prominent systemic component, such as anaphylaxis (7, 12–14, 36, 145).

Contributions of mast cells to the development of the inflammatory cell influx during IgE-associated 'late-phase responses'

Many of the clinically significant consequences of IgE-associated, antigen-dependent reactions, in the respiratory tract, skin, and other sites are now thought to depend critically on the actions of leukocytes recruited to these sites during late-phase responses (LPRs) rather than on the direct effects of the mediators released by mast cells at early intervals after antigen challenge (148–150). LPRs can be defined as the recurrence, at a few to several hours after initial antigen challenge in sensitized hosts, of signs and symptoms of inflammation at the site of antigen challenge. In many but not all cases, such LPRs are preceded by evidence of an acute allergic reaction at the same site (148, 149).

It is difficult to define exactly when the immediate phase of an IgE-associated response transitions into the LPR (Fig. 3C). However, it seems reasonable to propose that the appearance of significant numbers of recruited leukocytes at the reaction site should be one of the defining criteria. Moreover, several lines of evidence, drawn from human experiments and mouse studies (including work in mast cell knockin mice), indicate that IgE-dependent activation of mast cells can contribute to the development of LPRs (7, 12, 14, 79, 146, 148).

In humans, experimental LPRs also can be induced by challenge with allergen-derived peptides that can activate T cells but that are not able to induce IgE-dependent activation of mast cells or basophils (149). Such work indicates that some clinically significant LPRs in naturally sensitized hosts may occur by T-cell-dependent but mast cell-independent mechanisms.

While each of a diverse set of mast cell-derived mediators has the potential to influence the leukocyte recruitment and other features of IgE-associated LPRs (7, 12, 14, 148), TNF is the mast cell-derived product that has been most clearly implicated in this setting based on work with mast cell knockin mice (79, 151). However, it is likely that many other mast cell-derived products also importantly influence the features of certain IgE-associated LPRs.

In addition to contributing to the features of LPRs, the ongoing release of cytokines, chemokines, and other mediators by mast cells that have been activated by IgE-dependent mechanisms at sites of antigen challenge can have additional effects. These include actions that have the potential to influence further the development of acquired immune responses. For example, histamine derived from IgE- and antigen-stimulated skin mast cells can promote the migration of Langerhans cells to draining lymph nodes by actions on H2 receptors (81). This

finding raises the possibility that, in individuals who already have developed IgE specific for particular epitopes of an environmental antigen, subsequent challenge with that antigen (alone, or in the presence of additional antigens) may result in IgE- and mast cell-dependent effects on local DCs, which in turn may function to increase the spectrum of epitopes of the original antigen (or of the additional antigens encountered at the same time) against which that individual develops sensitivity. As suggested by Jawdat et al. (81), should this mechanism actually occur in naturally acquired allergic disorders, it would have the potential to increase the severity of that individual's chronic allergic disease.

Roles of mast cells in the chronic allergic inflammation and the associated tissue remodeling

Mast cells can provide mediators and cellular functions with the potential to influence multiple features of the pathology observed at sites of 'chronic allergic inflammation' – the inflammation that can persist for very long periods of time at sites of disease in subjects with IgE-associated allergic disorders such as asthma, allergic rhinitis, and atopic dermatitis (7, 12–15, 22, 55, 58, 60, 148). At the affected sites, chronic allergic inflammation can be associated with dramatic changes in the extracellular matrix, as well as with important alterations in the numbers, structure, and function of cells that are normally resident there, including epithelial cells, vascular endothelial cells, vascular and airway smooth muscle cells, fibroblasts, and nerves (Fig. 3D).

In humans, it can be quite difficult to assess the relative importance of the mast cell's contributions to individual examples of chronic allergic inflammation, as opposed to those of the many other cell types that participate in these complex inflammatory disorders. By contrast, work in mice has shown that mast cells can contribute significantly to the development of many of the characteristic features of such disorders. Most studies of 'asthma models' in mice have used relatively short-term protocols for antigen sensitization and challenge, which can replicate some but not all of the features of chronic asthma in humans; mast cells have been shown to contribute to several features of such models (86, 152).

Yu et al. (87) recently reported, however, that mice that had been sensitized to OVA without using an artificial adjuvant and then were exposed to long-term, repetitive, intranasal antigen challenge developed many of the features of chronic asthma. Furthermore, based on studies in mast cell-deficient ($\text{Kit}^{\text{W-sh}/\text{W-sh}}$ or $\text{Kit}^{\text{W}/\text{W-v}}$) mice engrafted with $\text{Fc}\gamma^{\text{+/+}}$ (wildtype) or $\text{Fc}\gamma^{\text{-/-}}$ mast cells, optimal expression of all of these features of the disorder was shown to be mast cell dependent (87). Notably,

while many features of the response were markedly reduced in mice whose mast cells lacked Fc ϵ R γ and therefore could not be activated by IgE/Fc ϵ RI or IgG $_1$ /Fc γ RIII-dependent mechanisms, optimal development of other features of the response appeared to require mast cells but not FcR γ . Thus, mechanisms of eliciting mast cell function independently of Fc ϵ RI- or IgG $_1$ /Fc γ RIII-dependent signaling must also contribute to the development of those features of this model. While the alternative mechanisms of mast cell activation in this setting have not yet been identified, work in other models has implicated certain chemokines (153) or products of complement activation (154, 155) as potentially important modulators of mast cell function in allergic inflammation.

In addition to influencing certain features of chronic inflammation directly, e.g. by the effects of their secreted products on local resident cells such as vascular endothelial cells, epithelial cells, smooth muscle cells, and fibroblasts, mast cells also have the potential to modulate certain features of these disorders by effects on the recruitment, phenotype, and/or function of lymphocytes and other cells which contribute to these responses (7, 12–15, 22, 55, 57, 58, 60, 130). TNF is one but not the only mediator derived from mast cells stimulated by the Fc ϵ RI that has the potential to enhance the proliferation and cytokine production of multiple T-cell subsets *in vitro* and to enhance the expression of T-cell-dependent inflammation *in vivo* (58, 60). Certain costimulatory molecules expressed by mast cells, including the OX40 ligand, also have the potential to enhance T-cell function in these settings (13, 22, 58, 60).

While pathological IgE-associated immune responses can account for significant human morbidity and mortality, it is thought that IgE-associated acquired immune responses can confer benefit in the context of infection with certain parasites (7, 12, 14, 94, 156, 157). The broad group of 'IgE-associated immune responses', like the broad spectrum of 'innate immune responses', appears to represent a context in which, depending on the individual circumstances, the role(s) of mast cells can either benefit or harm the host.

Roles of mast cells in chronic inflammatory responses in which IgE is thought to have little or no effector role

Acquired immune responses

Work in mast cell knockin mice as well as evidence from human studies indicate that mast cells can contribute to the pathology observed in some autoimmune disorders (150), including multiple sclerosis (158), bullous pemphigoid (159), and rheumatoid arthritis (160) in humans, and in models of experimental autoimmune encephalomyelitis (56, 161, 162),

bullous pemphigoid (80), and IgG $_1$ antibody-dependent autoimmune arthritis (163) in mice. Moreover, mast cells may express effector and/or immunoregulatory functions in such disorders (56, 59, 161). In addition, under some (but not all) experimental conditions, mast cells can be required for optimal elicitation of the inflammation associated with mouse models of hapten-induced CHS (130, 131, 164–166), including the elongation of cutaneous nerves that can accompany such responses (82), as well as for optimal development of mouse models of hapten-induced non-atopic asthma (167) or inflammatory bowel disease (168).

Mast cells have been implicated as potential effector cells in mouse models of fibrosis associated with chronic immunological responses. For example, scleroderma, or sclerosis of the skin, is a manifestation of systemic sclerosis, an autoimmune disorder characterized by extensive fibrosis of the affected organs (169). Patients with scleroderma exhibit increased collagen synthesis and accumulation in the skin, as well as in various internal organs such as the lungs, heart, kidneys, and gastrointestinal tract (169). Claman and associates reported a model of chronic graft versus host disease (CGVHD) across minor histocompatibility barriers in which the affected mice develop a syndrome similar to that of idiopathic scleroderma. CGVHD was induced by injecting spleen cells from B10.D2 mice into irradiated BALB/c recipients. Claman *et al.* (170) noticed that mast cells in the affected skin become depleted of their granules (they termed these cells 'phantom mast cells'), leading them to hypothesize that mast cell activation may contribute to the fibrosis that represents a major feature of both CGVHD and scleroderma.

Other 'models' of scleroderma include the local injection of bleomycin into the skin of mice (171). Testing that model in wildtype and congenic Kit^W/Kit^{W-v} mast cell-deficient mice, Yamamoto *et al.* (172) found that after 1 week of daily bleomycin injections, dermal sclerosis was induced only in the wildtype mice. However, after 4 weeks, dermal sclerosis developed equally in both the mast cell-deficient mice and the identically treated wildtype littermates. These observations indicate that mast cells are not necessary for the development of fibrosis of the skin, but they might accelerate the development of such fibrosis in some settings (172).

Work in mice has implicated mast cells as potentially important participants in a variety of models of allergic (i.e. CHS) or autoimmune diseases in which IgE is thought to have little or no role. However, defining the mechanisms by which mast cells can enhance (or suppress) features of the sensitization or elicitation phases of such acquired immune responses and identifying the signals which can elicit mast cell functions in

these settings represent important goals for future research. For example, in an experimental autoimmune encephalitis model, mast cell-derived IL-4 appears to be critical to the mast cell's ability to alter the phenotype and perhaps the function of T cells that then mediate disease in the central nervous system (59). Notably, such mast cell-dependent 'conditioning' of T cells in this model appears to occur in the peripheral lymphoid tissue, not in the central nervous system itself (59). By contrast, in models of hapten-induced CHS in mouse skin, mast cell-derived TNF appears to be required locally to promote leukocyte infiltration (82, 166) and to enhance the elongation of cutaneous nerves that occurs during the elicitation phase of the CHS response (82). The extent to which the important effects of mast cell-derived TNF on the elongation of cutaneous nerves reflect simply the cytokine's ability to elicit a local inflammatory response, as opposed to its ability to influence directly resident cells such as keratinocytes or the nerves themselves, is not yet clear. Nevertheless, the studies of Gregory *et al.* (59), Biedermann *et al.* (166), and Kakurai *et al.* (82) clearly indicate that some important effects of mast cells in the chronic inflammation associated with acquired immune responses may reflect the local actions of mast cells at sites of disease (82, 166), whereas others may reflect the mast cells' ability to act systemically by altering the phenotype, migration, or function of other effector or immunoregulatory cell populations (59).

Mast cell:T-cell interactions

Emerging evidence suggests that interactions between mast cells and T cells may be especially important in regulating the expression of acquired immune responses, including those that appear not to involve an important role for IgE (12, 55, 173). For example, *in vitro* studies show that human T cells can induce mast cells to secrete histamine, TNF, and metalloproteinase 9 by heterotypic adhesion to activated T cells (173). Mouse mast cells can promote T-cell receptor-dependent T-cell proliferation and cytokine production independently of IgE *in vitro* (58), and products from activated T cells (lymphotoxin and LIGHT) can induce cytokine and chemokine production by mouse mast cells without enhancing degranulation or FcεR aggregation (174). These observations suggest that cytokines derived from activated T cells can promote IgE/antigen-FcεR-independent mast cell activation, which, in turn, can result in the mast cell-dependent augmentation of T-cell function, e.g. by TNF.

We recently found that IL-17 represents another T-cell product that may influence inflammation in part through mast cell-dependent but FcεR-independent mechanisms. IL-17-producing Th17 cells are a Th cell subset that is distinct from classical Th1 cells and Th2 cells (175), and IL-17 and Th17 cells

are thought to be involved in the development of diverse allergic and autoimmune diseases (175–180). We found that IL-17 can enhance TNF production by mouse mast cells *in vitro* independently of FcεR aggregation (181).

Using OTII mice that transgenically express a TCR specific for OVA, we developed a mouse model of antigen-induced airway inflammation that is predominantly associated with neutrophils rather than eosinophils and that is mediated by Th17 cells, rather than Th1 or Th2 cells (181). We crossed C57BL/6-OTII and C57BL/6-Kit^{W-sh/W-sh} mice to produce C57BL/6-OTII expressing Kit^{W-sh/W-sh} mice. In studies after the engraftment of wildtype versus TNF^{-/-} mast cell populations into these animals, we showed that mast cells and mast cell-derived TNF, but not IgE/antigen-FcεR signals are required for the Th17-mediated airway neutrophilia observed in this model. Moreover, it appears that the mast cell-derived TNF functions downstream of Th17-derived IL-17 in this setting (181).

It remains to be determined to what extent pathogenetic mechanisms linking IL-17, mast cells, and mast cell-derived TNF are relevant to human asthma and other diseases. In 'allergic' or 'atopic' asthma, both human studies and work in mouse models support a role for Th2-cell-dominant inflammation (182). In such patients, the airway inflammation is characterized by the involvement of IgE and mast cells and by the development of inflammatory infiltrates that include large numbers of eosinophils (182–184). However, certain patients with the so-called 'non-atopic' asthma, who lack evidence for an antigen- and IgE-dependent component, exhibit increases in the numbers of airway mast cells that are similar to those in subjects with atopic asthma (185). Airway inflammation featuring a prominent neutrophil component has been reported both in some subjects with non-atopic asthma and in patients with severe atopic asthma (185–191).

The possibility that TNF represents a therapeutic target in some patients with asthma is currently of great interest. TNF can induce lung neutrophil infiltration and increase airway hyperreactivity to methacholine in normal (non-asthmatic) human subjects (192); some patients with asthma exhibit elevated serum levels of TNF (193), and TNF levels in sputum from non-eosinophilic (neutrophilic) asthmatic patients are higher than those in eosinophilic patients (194). TNF immunoreactive cells (most of them mast cells) were increased in number in the airway submucosa in biopsies of asthma patients versus normal controls (195, 196), and anti-TNF therapy reduced the severity of certain features of the disease in patients with severe asthma (including small reductions in both eosinophils and neutrophils in the sputum) (196–198) but not

the pulmonary eosinophilia in patients with mild and moderate asthma (199). Finally, IL-17 can be increased in the sera, sputum, and/or bronchoalveolar lavage (BAL) fluids from patients with asthma (200–202).

Much additional work is required to understand the importance of IL-17 or TNF in various subsets of patients with asthma and to define more clearly the roles of mast cells (as opposed to other potential sources of TNF) in these settings. Nevertheless, the *in vitro* and *in vivo* findings in mice suggest that not only do interactions between mast cells (and mast cell-derived TNF) and T cells have the potential to influence pathology in patients with atopic asthma but that interactions between mast cells and mast cell-derived TNF and Th17 cells may be important in non-atopic, IgE-independent asthma associated with inflammation, including a prominent component of neutrophils.

Non-immunological responses

Mast cells have been implicated in the development of several chronic inflammatory disorders in which immunological mechanisms are thought to have little or no role. We focus here on those examples in which a role for mast cells has been supported based on work in mast cell knock-in mice.

Pulmonary disorders

Exposure to silica particles can induce a chronic inflammatory response that is followed by fibrosis and, in some subjects, cancer (203). Silicosis can diminish lung function and increase susceptibility to respiratory infection (204). Subjects exposed to silica dust have increased numbers of mast cells in their lungs (205).

After 2 weeks of exposure to silica, wildtype mice but not mast cell-deficient $\text{Kit}^W/\text{Kit}^{W-v}$ mice developed pulmonary inflammation, and wildtype mice developed increased numbers of neutrophils and mast cells in BAL fluid (206). Mast cells also have been implicated in the development of a chronic model of silicosis in mice (207). Chronic exposure to silica induced wildtype mice but not $\text{Kit}^{W-sh}/\text{Kit}^{W-sh}$ mast cell-deficient mice to develop pulmonary inflammation and collagen deposition. Furthermore, *in vitro* evidence indicated, like macrophages, that mouse mast cells can be activated by scavenger receptors to generate reactive oxygen species, TNF, IL-13, MIP-1, and proteolytic activity (207).

Large numbers of peribronchiolar mast cells can be observed in patients with idiopathic pulmonary fibrosis, a progressive and fatal fibrotic lung disease of unclear etiology (208), and mast cell numbers also are increased in the lungs of patients

exhibiting pulmonary toxicity to bleomycin (209, 210). Mouse models of bleomycin-induced inflammation have been widely used to analyze factors contributing to chronic inflammation and fibrosis of the lung (211). Moreover, several mast cell-associated mediators such as chymase, histamine, and cysteinyl leukotrienes have been implicated in the development of bleomycin-induced lung fibrosis (212–214).

However, $\text{Kit}^W/\text{Kit}^{W-v}$ mast cell-deficient mice develop lung fibrosis after treatment with bleomycin (215, 216). Similar results were obtained by Okazaki *et al.* (212) using *c-kit* mutant, mast cell-deficient *Ws/Ws* rats. These findings suggest that mast cells may not contribute significantly to bleomycin-induced lung fibrosis in mice and rats.

By contrast, O'Brien-Ladner *et al.* (219) found that *mi/mi* mice, which are partially mast cell deficient as a result of their mutations in the gene encoding the microphthalmia transcription factor (73, 217, 218), do not develop as much bleomycin-induced fibrosis as do the littermate wildtype mice (219). Whether the reduced susceptibility of *mi/mi* mice to bleomycin-induced lung fibrosis is related to their mast cell deficiency, as opposed to other consequences of their *mi* mutations, has not been determined.

Pulmonary hypertension is another chronic lung disorder (or group of disorders) in which mast cells have been implicated (220, 221). Heath and Yacoub (220) reported an increase in tryptase-positive mast cells in the lungs of patients with pulmonary hypertension. More recently, Mitani *et al.* (221) found chymase-positive cells in the areas of the lung affected by interstitial fibrosis and neovascularization, which was interpreted as evidence that mast cells might contribute to the tissue remodeling observed in pulmonary hypertension.

Studies in animal models of pulmonary hypertension have not yet produced a clear picture of what role (if any) mast cells have in this disorder. For example, Kay *et al.* (222) reported that numbers of lung mast cells were increased in Wistar albino rats with hypoxic pulmonary hypertension, whereas Zhu *et al.* (223) reported that they detected no differences in the pulmonary vascular response to hypoxia in $\text{Kit}^W/\text{Kit}^{W-v}$ mast cell-deficient mice and the congenic wildtype mice. Investigating a model of pulmonary hypertension that is produced by pneumonectomy followed by treatment with monocrotaline, Vaszar *et al.* (224) found significant changes in the levels of mRNA for mast cell proteases during the development of vascular obliteration in rats. Taken together, these findings indicate that some models of pulmonary hypertension are associated with changes in mast cell numbers and/or phenotype, but the importance of mast cells in these models remains to be determined.

Cardiovascular disorders

Mast cells are found in the heart (225) and, in humans, around coronary arteries and within complex atherosclerotic plaques (226). Mast cells also have been implicated in the development of chronic cardiovascular diseases such as idiopathic and ischemic cardiomyopathy, in which mast cell numbers are increased (227), and in atherosclerosis or its complications (228, 229). However, relatively few studies have attempted to investigate the roles of mast cells in cardiovascular disorders using mast cell-deficient mice. One impediment to work in this area is that intravenous transfer of BMCMCs to adult *c-kit* mutant mast cell-deficient mice does not result in the engraftment of mast cells in the heart of the recipient animals (65, 230). However, Hara et al. (230) showed that, compared with wildtype mice, *Kit^W/Kit^{W-v}* mast cell-deficient mice exhibited less myocardial fibrosis and systolic pressure overload-induced myocardial hypertrophy after banding of the abdominal aorta, as well as reduced development of subsequent ventricular dilatation and cardiac failure.

Angiotensin II (Ang II), a component of the renin-angiotensin system that regulates blood pressure and kidney function, is locally produced in the heart, where it is thought to exert hypertrophic and profibrotic actions on cardiomyocytes (231). Urata et al. (232) identified chymase, not angiotensin converting enzyme, as the major Ang II-forming enzyme in the human heart, and Li et al. (233) recently showed that mast cell-derived chymase participates in an alternative pathway for Ang II formation that may contribute to blood pressure regulation in wildtype but not in *Kit^W/Kit^{W-v}* mast cell-deficient mice.

The role of mast cell-derived chymase in the regulation of Ang II generation in normal animals is not yet clear. Chymase as well as mMC-CPA participates not only in the formation but also in the degradation of Ang II (234). Moreover, Jin et al. (235), using a pharmacological approach, showed that angiotensin-converting enzyme but not chymase is responsible for most of the Ang II generated in the renal artery clipping model of hamster hypertension.

One interesting area for future work is to assess the possible roles of mast cells in promoting or regulating the consequences of the local activation of the renin-angiotensin system in particular disease settings. Several groups have proposed that chymase-dependent pathologies can be chronic and that disease progression may reflect complex processes that are both localized to the affected tissues and associated with increased numbers of mast cells in the affected sites (236). For example, Matsumoto et al. (237) observed that the chymase inhibitor SUNC8257 prevents the formation of profibrogenic factors in

the heart, including Ang II and the mature form of TGF- β , which can contribute to the heart failure induced by rapid right ventricular pacing (270 b/min, 22 days) in dogs. In this model, the number of chymase-positive mast cells increased in the left ventricle of dogs that had developed tachycardia-induced heart failure compared with corresponding values in the normal control group (237).

An additional potential contribution of cardiac mast cells to the local renin-angiotensin system is their ability to produce renin, which, upon mast cell degranulation, can generate Ang I from angiotensinogen (238, 239). By using the *ex vivo* model of Langendorff-perfused mouse hearts, Mackins et al. (239) showed that mast cell renin can play a key role in the generation of reperfusion-induced cardiac arrhythmias. Whether this process might also contribute to chronic pathology in the heart remains to be determined.

The possible link between mast cells and atherogenesis probably was first reported in 1953 by Constantinides (240). Since then, several groups have proposed that mast cells may contribute to the pathogenesis of atherogenesis (229, 241, 242). However, the importance of the mast cell in atherogenesis or its complications, such as plaque hemorrhage in coronary arteries (228) or the development of abdominal aortic aneurysms (243), has not yet been shown *in vivo*.

Evidence that mast cells can limit the extent or duration of chronic inflammation

Examination of the known biological effects of the many mediators that can be produced by mast cells shows that several of these products, including TGF- β 1 (244), IL-4 (245), IL-10 (246), and histamine (247), can have effects that are anti-inflammatory and/or that might negatively regulate aspects of certain biological responses. However, very few studies have shown the ability of mast cells to contribute to anti-inflammatory activities using genetic approaches *in vivo*.

Hart et al. (142) provided the first evidence in mast cell knockin mice, specifically BMCMC-engrafted (C57BL/6 $\times$ DBA/2 F₁)-*Kit^{W-I/W-I}* mice, of an immunosuppressive function for mast cells. They showed that mast cells, through effects mediated at least in part by histamine, can contribute to the immunosuppressive actions of ultraviolet-B irradiation on the expression of CHS responses to TNCB in mice. As noted above, Depinay et al. (141) recently showed that the bites of *Anopheles* mosquitoes can have effects that impair the development of antigen-specific T-cell responses in a model of delayed type hypersensitivity to OVA in mice and that this phenomenon requires the presence of mast cells in the skin at the site of the

bite. However, the mechanism(s) by which mast cells exert this interesting effect remains to be determined.

Another example of the ability of mast cells to downregulate aspects of an acquired immune response was recently reported by Lu et al. (31), who showed that mast cells are critical for the optimal expression of peripheral tolerance to skin allografts. Although the mechanism by which mast cells promoted immunosuppression in this setting was not identified, elevated levels of IL-10 mRNA were observed at the site of tolerant skin grafts (31).

These studies show that mast cells can have effects that suppress the development or magnitude of adaptive immune responses, at least in certain settings. Defining the mechanisms by which mast cells can suppress, rather than enhance, features of the sensitization or elicitation phases of acquired immune responses and identifying the signals that can elicit such distinct mast cell functions are important goals for future research. It will also be of interest to assess whether and under what circumstances mast cells might be able to suppress chronic inflammation that occurs by non-immunological mechanisms.

Conclusions and therapeutic implications

Based on rather indirect lines of evidence, mast cells have been implicated in chronic inflammatory responses and in many diseases characterized by chronic inflammation. Many of these conditions are associated with changes in the numbers or the phenotype of mast cell populations at the affected organ or anatomical site, and, in many of these settings, there is clear morphological evidence of various forms of 'mast cell

activation'. Moreover, many mast cell-derived products have effects that can significantly influence the survival, proliferation, phenotype, or function of many other cell types that participate in chronic inflammation and tissue remodeling (Fig. 4). Thus, there is a 'smoking gun' (to use a term that probably is used too often in the USA). However, is the 'weapon' (the mast cell) harming or protecting the host? How can we answer that question?

We have argued that genetic approaches that permit the roles of mast cells to be investigated in wildtype mice, *c-kit* mutant mast cell-deficient mice, and mast cell-engrafted *c-kit* mutant mice (mast cell knockin mice) can provide useful direct evidence for or against roles of mast cells and some of their specific products in experimental models of adaptive host responses or diseases. Work in such mouse models has shown that mast cells can promote many features of chronic inflammation, e.g. in certain models of chronic allergic inflammation (86, 87), experimental autoimmune encephalomyelitis (161), antibody-induced arthritis (163), or silicosis (206), whereas, in other models, mast cells can have immunosuppressive or anti-inflammatory effects (31, 141, 142).

In humans, it is much more difficult to be sure of the roles of mast cells in chronic inflammatory disorders or other biological responses. Not only do mouse and human mast cells differ in some aspects of phenotype and function, but, so far, no 'experiment of nature' that has resulted in a selective defect in human mast cells has been reported. Thus, in attempting to decide on the potential importance of mast cells in biological responses in humans, one must use a combination of several

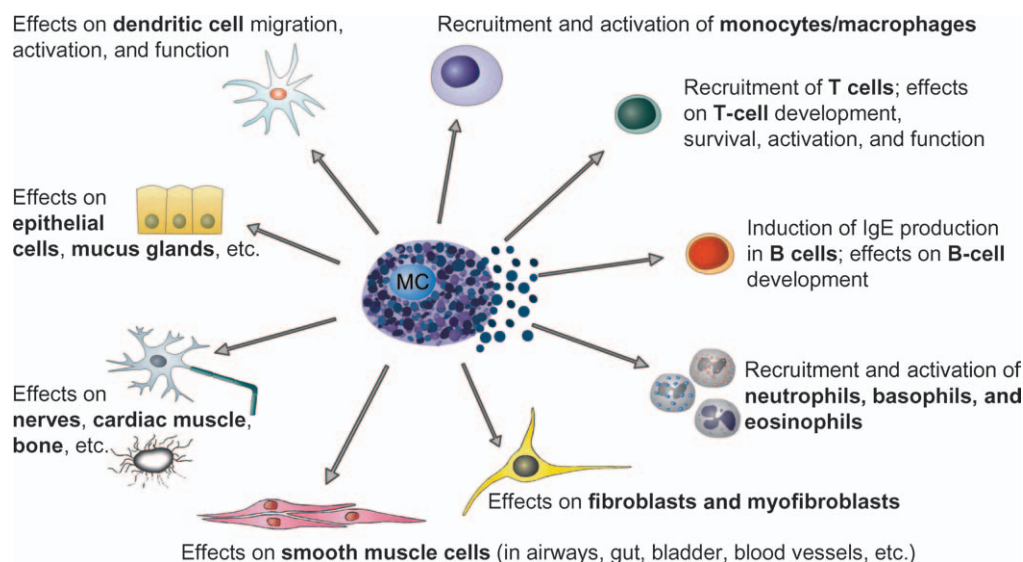


Fig. 4. Mast cells can modulate the development, intensity, local and systemic consequences, and persistence or resolution of chronic inflammation by influencing the function of many different cell types.

lines of more indirect evidence: (i) inference from mouse and other animal models of disease, (ii) observational studies in humans (e.g. using biopsies of affected sites and measurements of mast cell-associated mediators in sites of disease or in the blood or urine), and (iii) the clinical results of interventions that 'target' particular mast cell-associated mediators (many of which can be produced by other cell types as well) or that attempt to influence mast cell numbers and/or activation.

It may be possible eventually to evaluate the results of clinical trials of agents that have been designed to interfere specifically with mast cell activation or that even can ablate this cell selectively. If these agents actually are specific in suppressing mast cell function or eliminating mast cells in humans, then it may be possible to know in which settings of chronic disease

this cell is a friend or a foe, or, in more complex situations, at what stages of the disorder mast cells work for us or against us. Until then, one can try to make educated guesses. Our guess is that mast cells will be found to contribute to many, but not all, clinically problematic features of certain medically important disorders associated with chronic inflammation. We think that this list will include at least some forms of asthma, as well as allergic rhinitis, and is likely to include rheumatoid arthritis. We would not be surprised if mast cells also can promote pathology in several other disorders associated with chronic inflammation. However, in other settings of host defense or pathology in which chronic inflammation is a feature, mast cells may have net effects that are favorable to the host. Time will tell whether these guesses are correct.

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