

Molecular mechanisms in allergy and clinical immunology

(Supported by a grant from Merck & Co, Inc, West Point, Pa)

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Overall Purpose/Goal: To provide excellent reviews on key aspects of allergic disease to those who research, treat, or manage allergic disease.

Target Audience: Physicians and researchers within the field of allergic disease.

Activity Objectives

- (a) To understand the role of mast cells in innate immunity and tissue repair.
- (b) To understand the potential for T_H2 cytokines to modify mast cell functions in the setting of disease.
- (c) To understand the potential importance of non-IgE-dependent mast cell activation mechanisms in health and disease.

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Mast cells: Beyond IgE

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Mast cells, historically known for their involvement in type I hypersensitivity, also serve critical protective and homeostatic functions. They directly recognize the products of bacterial infection through several surface receptor proteins, releasing proteases, cytokines, and eicosanoid mediators that recruit neutrophils, limit the spread of bacterial infection, and facilitate subsequent tissue repair. In vitro studies suggest that the spectrum of microbes capable of initiating mast cell activation is broad and extends to common respiratory viruses,

mycoplasma, and even products of tissue injury, such as nucleotides. T_H2 -polarized inflammation elicits a reactive hyperplasia of mast cells at the involved mucosal surfaces in both mice and human subjects. Several recombinant T_H2 cytokines (IL-3, IL-4, IL-5, and IL-9) act synergistically with stem cell factor to facilitate proliferation of nontransformed human mast cells in vitro. IL-4 induces the expression of critical inflammation-associated genes by human mast cells, such as those encoding leukotriene C_4 synthase, $Fc\epsilon RI$, and several cytokines. Consequently, priming with IL-4 not only amplifies classical $Fc\epsilon RI$ -dependent mast cell activation but also dramatically alters the product profile of mast cells activated by innate signals and by chemical mediators of inflammation. Strikingly, IL-4 induces an activation response by mast cells to cysteinyl leukotrienes, which act through a receptor shared with uridine diphosphate to induce cytokine generation without exocytosis. It is possible that alterations in mast cell phenotype by the T_H2 milieu of allergy permits otherwise trivial infections or homeostatic chemical signals to initiate harmful inflammatory cascades and sustain tissue pathology. Drug development must take these nonclassical mast cell activation pathways into account without compromising the beneficial and protective functions of mast cells. (*J Allergy Clin Immunol* 2003;111:24-32.)

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Supported by National Institutes of Health grants AI31599, HL36110, AI48802, and AI52353 and by grants from the Charles Dana Foundation and the Vinik Family Fund for Research in Allergic Diseases in Children. Received for publication October 7, 2002; accepted for publication October 16, 2002.

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0091-6749/2003 \$30.00 + 0

doi:10.1067/mai.2003.60

Abbreviations used

AA: Arachidonic acid
CysLT: Cysteinyl leukotriene
FLAP: 5-Lipoxygenase activating protein
GPCR: G protein-coupled receptor
5-LO: 5-Lipoxygenase
LT: Leukotriene
LTC₄S: Leukotriene C₄ synthase
PG: Prostaglandin
TLR: Toll-like receptor
UDP: Uridine diphosphate

Key words: Mast cells, IL-4, cysteinyl leukotrienes, cysteinyl leukotriene 1 receptor, uridine diphosphate

Mast cells are distributed widely throughout mammalian tissues, especially the perivascular spaces and connective tissues of the skin, gut, and respiratory and gastrointestinal tracts.¹ Cross-linkage of FcεRI elicits the release of preformed inflammatory mediators from mast cell secretory granules (including histamine, neutral proteases, preformed cytokines, and proteoglycans).² The same stimulus triggers rapid synthesis and release of lipid mediators that are the products of endogenous arachidonic acid (AA) metabolism, such as prostaglandin (PG) D₂, leukotriene (LT) B₄, and LTC₄, the parent molecule of the cysteinyl LTs (cysLTs) LTC₄, LTD₄, and LTE₄.³⁻⁷ Activated mast cells also synthesize and secrete several proinflammatory, chemoattractive, and immunomodulatory cytokines over a period of several hours.⁸⁻¹⁰ The diverse profile of chemical mediators generated and released by activated mast cells leads to plasma extravasation, tissue edema, bronchoconstriction, leukocyte recruitment, and mucosal inflammation, which result from allergen exposure in sensitized hosts. IgE-dependent mast cell activation is a prominent mechanism in a substantial number of diseases treated by allergists, including anaphylaxis, urticaria, angioedema, and acute exacerbations of asthma and rhinoconjunctivitis.

The firmly established role of mast cells and their products in allergic (type I) hypersensitivity contrasts with the comparatively sparse knowledge, until recently, of their physiologic functions and their role in nonallergic diseases. The prominence of lesional mast cells in multiple sclerosis,¹¹ cardiomyopathy,¹² rheumatoid arthritis,^{13,14} and a number of other chronic inflammatory and fibrotic diseases in human subjects¹⁵⁻¹⁷ suggested mast cell involvement. The critical importance of mast cells in many of these diseases has recently been validated by animal models (Table I).^{11,13,14,16-27} It is now clear that mast cells receive and integrate a wide array of non-IgE-driven chemical signals from their environment, permitting mobilization of their effector properties. Moreover, mast cells use these same activation mechanisms and effector properties to serve protective and homeostatic functions. The extent of mast cell involvement in both allergic and nonallergic disease, as well as host defense, is likely far greater than would be predicted strictly on the basis of studies of classical IgE-dependent immune responses.

TABLE I. Physiologic and pathophysiologic roles of mast cells

Physiologic	Pathophysiologic
Antibacterial ^{21,22}	Allergy, asthma ²⁷
Antihelminthic ^{25,26}	Fibrosis ^{16,17}
Wound healing ²⁴	Rheumatoid arthritis ^{13,14,18}
Angiogenesis ²³	Multiple sclerosis ^{11,20}
	Congestive heart failure ¹⁹

Involvement in both normal host defense and in the indicated diseases are strongly inferred by animal models, by human studies, or both.

This article will review recent progress in mast cell biology, as well as the potential implications for disease pathophysiology and therapy.

DIVERGENT PATHWAYS OF MAST CELL DEVELOPMENT: THE CONCEPT OF T CELL-DEPENDENT AND T CELL-INDEPENDENT POPULATIONS BELONGING TO A SINGLE LINEAGE

Mast cells are unique among granulated hematopoietic cells for their exclusive and normal residence in all vascularized tissues. Unlike other such cells, mast cells exit the bone marrow as committed progenitors with sparse secretory granules.²⁸⁻³⁰ The subsequent development of these progenitors into mature mast cells requires input from constitutive signals that dictate their trafficking from the circulation, their survival, and their maturation. The basal homing of these progenitors to the gut, but not to other tissues, is profoundly deficient in mice lacking the β7 integrin subunit,³¹ which pairs with the α4 integrin subunit to form a heterodimer that interacts with both mucosal addressin cell adhesion molecule 1 and vascular cell adhesion molecule 1 (Fig 1). The most critical viability and differentiation signal for mast cells is provided by the interaction between the membrane-associated receptor tyrosine kinase c-kit,³² which is strongly expressed by mast cells throughout their life cycle, and its ligand, stem cell factor (SCF),³³ a growth factor that is expressed constitutively by endothelial cells, fibroblasts, and other stromal cells. Tissue mast cells frequently juxtapose with the stromal cells that produce SCF, likely reflecting the SCF/c-kit interaction in vivo. Under experimental circumstances, membrane-bound SCF, its soluble isoform, or both are chemotactic for mast cells and their progenitors,³⁴ elicit their adhesion,³⁵ facilitate their proliferation,³⁶ and sustain their survival, differentiation, and maturation.^{37,38} The critical requirement for the SCF/c-kit interaction in normal mast cell development is confirmed by the mast cell deficiencies manifested by mouse strains lacking the normal function of either c-kit (W/W^v strain)³⁹ or the membrane-bound isoform of SCF (SI/Sld strain).⁴⁰ Thus the widespread constitutive expression of SCF and the presence of the c-kit receptor at all stages of mast cell development ensure the presence of mast cells in tissues under normal conditions.

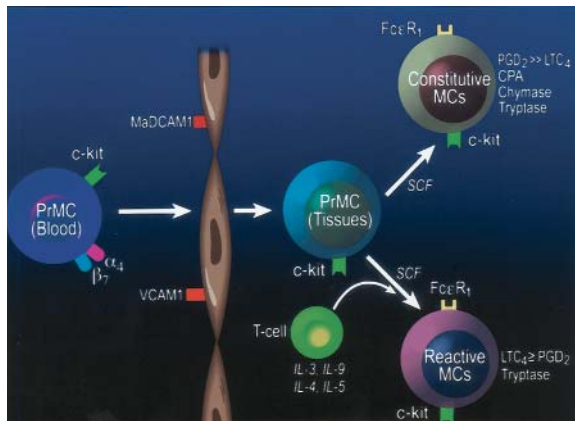


FIG 1. Constitutive versus reactive tissue mast cell development on the basis of the prototype of the intestine. Basal homing of blood-borne committed mast cell progenitors (PrMC) depends on the intact function of the $\beta 7$ integrin subunit forming a heterodimer with the $\alpha 4$ integrin subunit that interacts with mucosal addressin cell adhesion molecule 1 (MadCAM1) and vascular cell adhesion molecule 1 (VCAM1). Differentiation of all mast cells requires the input of SCF and its receptor, c-kit. Development of the reactive subtype, observed in the epithelial compartment in mucosal inflammation, requires additional cytokines derived from activated T cells. The subtypes differ in protease content and effector profile. CPA, Carboxypeptidase A.

What is the evolutionary purpose of constitutive populations of mast cells? Mast cells likely normally serve both protective and homeostatic functions under basal conditions. Compared with normal congenic control mice, mast cell-deficient W/Wv mice exhibit poor survival rates when experimentally subjected to septic peritonitis or gram-negative pneumonia because they lack the capacity to efficiently recruit neutrophils to the infected tissue.^{21,22} Both survival and neutrophil recruitment are restored to normal levels by means of transplantation of histocompatible mast cells into the W/Wv mice. These observations prompted studies revealing that mast cells express several receptors (toll-like receptor [TLR] 4, TLR2, CD48, and complement receptor 1)⁴¹⁻⁴⁴ that recognize microbes and their products through direct pattern recognition. Such receptors can activate mast cells without requirements for antibody or immunologic education (ie, innate immunity). In innate immune responses mast cells generate several mediators (TNF- α , tryptases, and LTB₄) that recruit neutrophils by means of distinct mechanisms.^{21,22,41,42,45-47} Recently, mouse mast cells were activated in vitro by the *Staphylococcus aureus* peptidoglycan in a TLR2-dependent manner,²² as well as by *Mycoplasma pneumoniae*⁴⁸ and the cutaneous parasites *Leishmania major* or *Leishmania infantum*,⁴⁹ for which the respective responsible receptor or receptors on mast cells are not yet identified. It is noteworthy that β tryptases, which are among the mast cell products likely involved in neutrophil recruitment,⁴⁵ also stimulate angiogenesis,²³ epithelial cell proliferation,⁴⁷ type I collagen production by fibroblasts,⁵⁰ and bronchial smooth muscle proliferation.⁵¹ These proper-

ties might relate to a role for mast cells in wound healing, as implicated in some experimental models,^{24,52} and in fibrosis and tissue remodeling in various diseases. Thus constitutive mast cells are specialized to receive danger signals through pattern recognition and to generate mediators involved both in the protective neutrophil response and in subsequent repair and healing. Such functions are teleologically consistent with the apparent strategic localization of mast cells in the skin and the gastrointestinal, respiratory, and genitourinary tracts, which are subject to both frequent trauma and potential infectious insults.

Although the SCF/c-kit interaction maintains relatively stable numbers of mast cells within connective tissue matrices, mast cell numbers in gastrointestinal, genitourinary, and respiratory mucosal epithelial surfaces (commonly referred to as mucosal or reactive mast cells, Fig 1) can fluctuate widely. Under normal conditions, there are few mast cells in these locations, but their numbers increase markedly in mucosal inflammation. Intestinal helminthic infections, which elicit a prototypical T_H2-polarized adaptive immune response, give rise to a profound intraepithelial mastocytosis.⁵³ This mastocytosis is critical to the elimination of most helminthic worms and resolves after worm clearance. As with constitutive mast cell development, reactive mast cell hyperplasia at mucosal surfaces requires the normal interaction of c-kit and SCF.³⁹ However, reactive intraepithelial mast cells differ from their constitutive counterparts in their additional requirement for T-lymphocyte function.²⁵ Athymic nude mice, which lack functional T cells, lack the capacity to mount a reactive intraepithelial mast cell hyperplasia in response to helminth infections.²⁵ Similarly, human subjects with profound lymphopenia caused by HIV infection lack mast cells in the intestinal epithelium.⁵⁴ In both human subjects and mice, T-cell deficiency has no effect on constitutive and connective tissue-associated mast cells. Thus mast cell development, at least in the intestine, can be viewed as a dichotomous process that occurs in T cell-dependent (reactive) and T cell-independent (constitutive) compartments (Fig 1). On the basis of selective antibody blockade or gene knockout strategies, IL-3 and IL-4 are especially critical in supporting helminth-induced reactive mast cell hyperplasia in the mouse intestine.^{26,55} Another T_H2 cytokine, IL-9, induces profound intraepithelial mast cell hyperplasia when overexpressed in the lungs or guts of mice.⁵⁶ Thus reactive intraepithelial mast cell hyperplasia, which is required for the elimination of helminthic parasites, reflects T cell-dependent modulation of mast cell development through several cytokines. The fact that the reactive mast cells arising during helminth infection exhibit striking alterations in their granule ultrastructure and protease content⁵⁷ suggests that T-cell cytokines induce alterations in the expression of genes that are involved in the mast cell-dependent elimination of helminths.

Is the paradigm of constitutive versus T cell-dependent reactive mast cell development pertinent to human allergic diseases? Immune responses to helminth infection

share mechanistic and histologic features in common with human allergic diseases, including prominent expression of T_H2 cytokines, IgE synthesis, and local eosinophilia. Asthma, allergic rhinitis, and allergic esophagitis are each also associated with increased numbers of mast cells within the involved mucosal epithelial surfaces.⁵⁸⁻⁶¹ The histopathology of asthma is also characterized by an additional increase in the numbers of mast cells within the bronchial smooth muscle layer.²⁷ The numbers of mast cells in this layer correlate with indices of airway hyper-responsiveness and distinguish the histopathology of asthma from that of eosinophilic bronchitis, a disease process with an otherwise similar pattern of mucosal inflammation. Several human T_H2 cytokines (IL-3, IL-4, IL-5, IL-6, and IL-9) can support the survival of human mast cells in vitro,⁶² synergistically stimulate their proliferation when provided in combination with SCF,⁶³⁻⁶⁵ or both. Conversely, the T_H1 cytokine IFN- γ inhibits SCF-dependent proliferation of cultured human mast cells,^{63,66} while inducing their expression of the high-affinity receptor for IgG, Fc γ RI.⁶⁷ These observations support the hypothesis that in human allergic mucosal inflammation, mast cell numbers (and very likely key aspects of their function) are under T-cell control, just as they are in the mouse intestine during helminth infection.

AA METABOLISM: A MARKER OF MAST CELL PHENOTYPE AND T-CELL MODULATION?

The cysLTs and PGD₂ secreted by mast cells act through specific 7 transmembrane-spanning G protein-coupled receptors (GPCRs)⁶⁸⁻⁷¹ to elicit powerful vasoactive and bronchoactive effects. CysLTs are especially powerful bronchoconstrictors^{72,73} and elicit bronchial eosinophilia,⁷⁴ edema,⁷⁵ and mucous hypersecretion⁷⁶ when instilled into the human lung in vivo. Recent experimental evidence from a mouse model of allergic airway disease implicates the cysLTs in the development of bronchial smooth muscle hyperplasia and submucosal collagen deposition,⁷⁷ 2 features of airway remodeling in asthma. CysLTs act through at least 2 GPCRs, termed the CysLT1 and CysLT2 receptors, respectively.^{70,71} Both the 5-lipoxygenase (5-LO) inhibitor zileuton, which interferes with cysLT synthesis,⁷⁸ and CysLT1 receptor antagonists⁷⁹ improve lung function in asthmatic subjects compared with placebo. CysLT1 receptor antagonists also attenuate both early- and late-phase pulmonary responses to inhaled allergen in asthmatic subjects.⁸⁰ These clinical studies confirm the involvement of cysLTs and at least one of their receptors in human asthma.

Although mast cells from all human tissues studied generate abundant PGD₂ after Fc ϵ RI cross-linkage, there is striking tissue-to-tissue heterogeneity in the quantity of cysLTs generated. For example, mast cells isolated from normal human skin (which are immunohistochemically similar to the constitutive mast cell subset in intestine and lung) generate an average of 3.5 ng of

cysLTs/10⁶ cells when stimulated by means of IgE receptor cross-linking,⁸¹ whereas those isolated from the human lung, intestine, or uterus generate roughly 10 times more.⁸²⁻⁸⁵ These observations suggest that the cysLT-producing ability of human mast cells is regulated by tissue-specific factors. Perturbations in these regulatory processes could underlie the observation that endobronchial allergen challenge results in 10- to 20-fold higher levels of cysLTs from subjects with asthma than from subjects with allergic rhinitis alone⁸⁶ and could be a key determinant of the development and severity of asthma and other diseases.

CysLT generation by mast cells and other immune cell types depends on the intact function of the 5-LO/LTC₄ synthase (LTC₄S) metabolic pathway. Calcium influx subsequent to cell activation permits phospholipase A₂ to liberate AA from cell membrane phospholipids.⁸⁷ AA is then converted sequentially to 5-hydroperoxyicosatetraenoic acid and then to LTA₄ by 5-LO, which is reversibly translocated to the perinuclear envelope⁸⁸ and requires the additional presence of 5-LO activating protein (FLAP)⁸⁹ for its catalytic function. LTA₄ is then either converted through an LTA₄ hydrolase to LTB₄⁹⁰ (as occurs dominantly in neutrophils) or is conjugated to reduced glutathione by LTC₄S,⁹¹ an integral membrane protein with homology to FLAP, forming LTC₄ (as occurs dominantly in mast cells, basophils, and eosinophils). LTC₄, the parent compound of the cysLTs, is then exported to the extracellular space by a distinct, carrier-mediated step⁹² and is sequentially converted to the receptor-active metabolites LTD₄ and LTE₄ by extracellular enzymes.⁹³ The gene encoding the terminal enzyme, *LTC4S*, is located on chromosome 5q35,⁹⁴ telomeric to the sites containing the T_H2 cytokine gene cluster, the β_2 adrenergic receptor, and the glucocorticoid receptor. A commonly occurring single-nucleotide polymorphism consisting of an A to C substitution at base -444 in the 5' untranslated region of the gene encoding LTC₄S gives rise to a consensus binding sequence for the histone H4 transcription factor.⁹⁵ Heterozygosity for this polymorphism is overrepresented among individuals with aspirin-sensitive asthma in some,^{95,96} but not all,⁹⁷ ethnic populations. In one study peripheral blood eosinophils from asthmatic subjects who were heterozygous for the polymorphic *LTC4S* allele exhibited greater ionophore-induced cysLT synthesis than did eosinophils from subjects who were homozygous for the wild-type allele.⁹⁸ In this study the presence of the polymorphic *LTC4S* allele also predicted a more favorable clinical response to CysLT1 receptor antagonists.

Could the heterogeneity of cysLT-generating capacity in mast cells reflect T-cell control of the 5-LO/LTC₄S pathway? Such control is suggested by studies of primary cultured human mast cells derived in vitro from umbilical cord blood mononuclear cells in medium supplemented with SCF, IL-6, and IL-10. When stimulated by means of Fc ϵ RI cross-linkage, such mast cells generate few cysLTs but produce nanogram quantities of

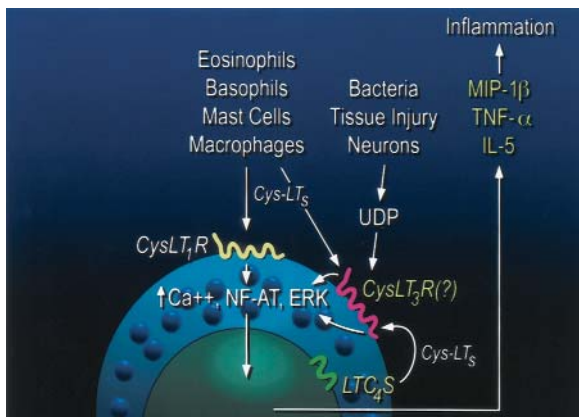


FIG 2. Regulatory effects of IL-4 on biosynthesis and reception of cystLTs by mast cells. Human mast cells express the CysLT₁ receptor constitutively, which transduces a calcium flux to both cystLTs and UDP. With IL-4 priming, both LTC₄ and UDP elicit mast cell activation at 3 log-fold lower doses and elicit cytokine generation through a nuclear factor of activated T cells- and extracellular regulated kinase-dependent pathway, without exocytosis. The priming effect occurs without changes in CysLT₁ receptor expression. IL-4 priming also upregulates FcεRI-dependent generation of cystLTs by inducing LTC₄S expression. Autocrine actions of cystLTs are indicated by partial antagonism of FcεRI-dependent cytokine generation by the CysLT₁ receptor selective antagonist MK571 and by MK886, a biosynthetic inhibitor. MIP, Macrophage inflammatory protein.

PGD₂. Exposure of these mast cells for 5 days to IL-4 resulted in a 27-fold increase in cystLT production, with only a 2-fold increase in PGD₂ generation. The selective enhancement of cystLT-producing ability likely reflected 2 events. First, IL-4 priming upregulates the expression of FcεRI on the surface of cord blood-derived mast cells,⁹⁹ enhancing the strength of the activation signal in response to FcεRI cross-linkage. Second, priming with IL-4 profoundly and rapidly (within hours) induces the expression of LTC₄S mRNA and protein by human mast cells,¹⁰⁰ thus increasing the available activity of the terminal enzyme necessary for the conversion of LTA₄ to LTC₄. Additional cytokine control of the 5-LO/LTC₄S pathway is introduced by priming with either IL-3 or IL-5, which each induce a nuclear import of pre-existing cytosolic 5-LO stores.¹⁰⁰ The combination of either IL-3 or IL-5 with IL-4 markedly augments FcεRI-mediated cystLT generation by cord blood-derived mast cells (to approximately 30 ng/10⁶ cells), without a corresponding effect on PGD₂ generation. Thus T_H2 cytokines act nonredundantly at proximal (5-LO) and distal (LTC₄S) control points of the 5-LO/LTC₄S pathway to amplify the capacity of mast cells to generate cystLTs in response to FcεRI cross-linkage. IL-4 and IL-5 both also prime mast cells for enhanced FcεRI-dependent cytokine generation.¹⁰¹ It thus seems likely that cytokines prominently

expressed in asthma not only control mast cell numbers but can also alter several mast cell effector properties, which in turn could regulate the magnitude of IgE-dependent early and late asthmatic responses and the clinical severity of asthma.

CysLTs, NUCLEOTIDES, AND IL-4: CONVERGENCE AT THE MAST CELL AND A NOVEL ACTIVATION MECHANISM

The CysLT₁ and CysLT₂ receptors map to different chromosomes (X and 13 in human subjects, respectively), bear essentially no nucleotide sequence homology, and have only 37% amino acid identity. These 2 receptors also differ in their respective ligand-binding preferences. The CysLT₁ receptor binds LTD₄ approximately 10-fold more avidly than it binds LTC₄, whereas the CysLT₂ receptor has equal affinity for both ligands.^{70,71} LTE₄, the least potent cystLT in vivo, binds weakly to both cystLT receptors. On the basis of ex vivo pharmacologic profiling of guinea pig lung tissues, the existence of a third, LTC₄-preferring cystLT receptor has been proposed,^{102,103} although such a receptor has not yet been isolated or cloned. Both of the known cystLT receptors are loosely homologous (24%-32% amino acid sequence identity) to the purinergic (P2Y) receptors, a family of widely expressed GPCRs that bind extracellular nucleotides.¹⁰⁴ Nucleotide ligands are released from cells as a result of cellular injury and microbial invasion and could thus serve as innate danger signals. Proved functions for P2Y receptors consistent with their roles in responses to tissue injury include ADP-induced platelet activation (through the P2Y₂ and P2Y₁₂ receptors),¹⁰⁵ ATP-dependent amplification of pain perception to tissue trauma (through the P2Y₁ receptor),¹⁰⁶ and uridine diphosphate (UDP)-induced macrophage activation (through the P2Y₆ receptor).¹⁰⁷ The UDP-selective P2Y₆ receptor shares several regions of sequence identity with the CysLT₁ receptor, including 8 of 10 identical amino acids in their respective first extracellular domains.¹⁰⁸ Like the cystLT receptors, the distribution of P2Y₆ includes smooth muscle.¹⁰⁹ Evolutionary and functional relationships between the cystLT and P2Y receptor classes is supported by the surprising observation that the mouse CysLT₁ receptor, like the P2Y₆ receptor, binds and transduces a calcium flux in response to UDP when expressed in a heterologous cell line. This UDP response was blocked by the competitive CysLT₁ receptor antagonist MK571, which does not interfere with the P2Y₆ receptor.¹⁰⁸ UDP-induced activation of both immune and nonimmune cells bearing CysLT₁ or P2Y₆ receptors could thus provide part of the cellular danger-sensing system operative in circumstances of infection or tissue injury.

Although pharmacologic studies of the cystLT receptors have traditionally focused on smooth muscle, the fact that several blood-borne cells^{71,110} also express these receptors suggests potential immune functions. Cultured cord blood-derived human mast cells express CysLT₁

receptor mRNA and the corresponding membrane protein and respond to both LTD₄ and LTC₄ with the anticipated dose-dependent calcium response (with a median effective concentration of approximately 10⁻⁹ and 10⁻⁸ for LTD₄ and LTC₄, respectively).¹⁰⁸ Priming of these mast cells with IL-4 shifted the dose-response curve for LTD₄ by 10-fold but enhanced the sensitivity to both LTC₄ and UDP by 1000-fold; the latter 2 agonists exhibited complete cross-desensitization in a calcium flux assay, confirming their use by a shared receptor.¹⁰⁸ Additionally, LTC₄, LTD₄, and UDP each induced TNF- α , macrophage inflammatory protein 1 β , and IL-5 production by IL-4-primed mast cells through a mechanism dependent on nuclear factor of activated T cells transcription factors and extracellular regulated kinase (Fig 2).¹¹¹ Importantly, cysLT- and UDP-induced cytokine generation occurred without morphologic or biochemical evidence of degranulation. Pretreatment of the primed mast cells with either MK571 or MK886 (an inhibitor of FLAP and LTC₄S) also attenuated the generation of IL-5 and TNF- α in response to Fc ϵ RI cross-linkage by approximately 30%, indicating an autocrine function for cysLTs in classical IgE-dependent mast cell activation. Despite its striking priming effect for cysLT-mediated mast cell activation, IL-4 did not alter the levels of CysLT1 receptor protein or mRNA; moreover, the complete inhibition by the selective CysLT1 receptor antagonist MK571 indicated no involvement of the CysLT2 receptor. Whether IL-4 induces expression of a novel, MK571-sensitive third CysLT receptor ("CysLT3" receptor, Fig 2) or works by an alternate mechanism to enhance the sensitivity of mast cells to cysLTs and UDP is not presently known. The overlapping purinergic-cysLT receptor system is thus an example of a T_H2-modulated pathway for mast cell activation. The fact that IL-4 priming permits human intestinal mast cells to generate IL-5 in response to stimulation by *Escherichia coli*¹¹² suggests that the T_H2 milieu alters mast cell function downstream of additional innate activation mechanisms. Cytokine-dependent amplification of innate receptor-mediated mast cell activation pathways would thus facilitate tissue inflammation during infections or tissue injury. In such a context mast cells could elicit these tissue changes with no morphologic evidence of degranulation. Indeed, given their capacity to secrete cytokines without undergoing exocytosis, it is possible that traditional light microscopy underestimates the true contribution of mast cells in tissue pathology.

IMPLICATIONS OF MAST CELLS FOR HEALTH AND DISEASE

The unique anatomic distribution of mast cells, their diverse effector repertoire, their ability to multiply and undergo functional changes in response to T cell-derived signals, and the expanding list of microbes and natural agonists for their activation presents a therapeutically relevant interface between innate and adaptive immunity. The constitutive SCF/c-kit axis provides basal popula-

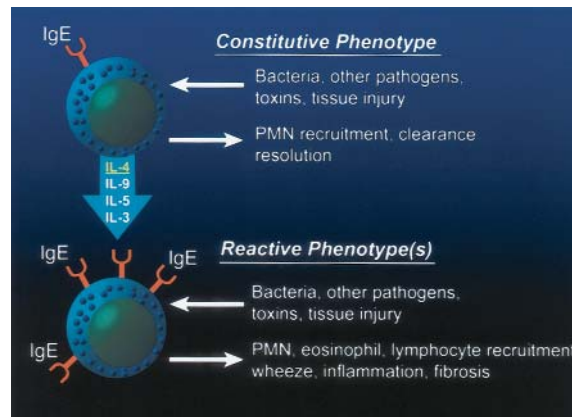


FIG 3. Hypothetic consequences of non-IgE-dependent activation of constitutive and reactive mast cell phenotypes. Under baseline conditions, mast cell activation by microbes, toxins, and humoral factors elicits neutrophil recruitment, contains invasive pathogens, and facilitates tissue healing. Under conditions of T-cell priming (particularly IL-4) in asthma and allergic diseases, the same activating stimuli produce an amplified mast cell response, resulting in sustained inflammation, tissue dysfunction, and remodeling.

tions of mature mast cells in connective tissues, as well as an ongoing supply of committed mast cell progenitors (Fig 1). The normal function of mast cells as sensors of infection and injury through innate recognition receptors and their response to these signals both limits the potential spread of infection (by recruiting neutrophils) and facilitates wound healing (through actions on fibroblasts and other connective tissue elements, Fig 3). In the context of T_H2-polarized mucosa inflammation, cytokines transduce growth and survival signals to mast cells and their progenitors and alter their profile of gene expression. These alterations both expand the repertoire of mast cell effector mediators (cytokines and cysLTs) and lower their threshold for activation by both IgE-dependent and non-IgE-dependent mechanisms. These mechanisms for phenotypic modification of mast cells might have evolved as a result of pressures exerted by helminth infection. They very likely contribute to functional alterations in mast cells in the tissues of allergic individuals. It is possible that these alterations render mast cells capable of triggering sustained or deleterious inflammatory responses in response to otherwise innocuous stimuli. Indeed, the potential for microbes to elicit or exacerbate allergic mucosal inflammation directly through direct activation of reactive phenotypically altered mast cells is a fascinating possibility that requires further investigation. Under such circumstances, the mast cell effector systems normally specialized to protect against pathogens and heal tissue could lead instead to perpetuation of inflammation, fibrosis, angiogenesis, and tissue remodeling (Fig 3). The potential to modify mast cell phenotypes and attenuate their disease-related activation

without compromising their beneficial protective and housekeeping functions would appear to be one of the challenges in future drug development.

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Joshua Avram Boyce receives grant support from Millennium Pharmaceuticals and is on the speaker's bureau for Merck and GlaxoSmithKline.