

The role of the mast cell in the pathophysiology of asthma

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There is compelling evidence that human mast cells contribute to the pathophysiology of asthma. Mast cells, but not T cells or eosinophils, localize within the bronchial smooth muscle bundles in patients with asthma but not in normal subjects or those with eosinophilic bronchitis, a factor likely to be important in determining the asthmatic phenotype. The mechanism of mast cell recruitment by asthmatic airway smooth muscle involves the CXCL10/CXCR3 axis, and several mast cell mediators have profound effects on airway smooth muscle function. The autacoids are established as potent bronchoconstrictors, whereas the proteases tryptase and chymase are being demonstrated to have a range of actions consistent with key roles in inflammation, tissue remodeling, and bronchial hyperresponsiveness. IL-4 and IL-13, known mast cell products, also induce bronchial hyperresponsiveness in the mouse independent of the inflammatory response and enhance the magnitude of agonist-induced intracellular Ca^{2+} responses in cultured human airway smooth muscle. There are therefore many pathways by which the close approximation of mast cells with airway smooth muscle cells might lead to disordered airway smooth muscle function. Mast cells also infiltrate the airway mucous glands in subjects with asthma, showing features of degranulation, and a positive correlation with the degree of mucus obstructing the airway lumen, suggesting that mast cells play an important role in regulating mucous gland secretion. The development of potent and specific inhibitors of mast cell secretion, which remain active when administered long-term to asthmatic airways, should offer a novel approach to the treatment of asthma. (*J Allergy Clin Immunol* ■■■■;■■■:■■■-■■■.)

Key words: Mast cells, asthma, airway smooth muscle, bronchial mucous glands, chemokines, $\text{TNF-}\alpha$

Mast cells are resident in all normal tissues, where they are believed to play an important role in tissue homeostasis, wound healing, and host defense, particularly

Abbreviations used

ASM: Airway smooth muscle
BHR: Bronchial hyperresponsiveness
EB: Eosinophilic bronchitis
HLMC: Human lung mast cell
LT: Leukotriene
PAR: Protease-activated receptor
PG: Prostaglandin
SCF: Stem cell factor
SHIP: src homology 2-containing inositol phosphatase
TLR: Toll-like receptor

bacterial infection (see review¹). Chronic mast cell activation contributes to the pathophysiology of many diverse diseases through the synthesis and release of numerous proinflammatory mediators and cytokines, the pattern of which varies depending on the stimulus.² It is beyond our scope to review in detail all of the evidence implicating mast cells in the pathophysiology of asthma, and for further information, the reader is referred to references.^{3,4} We therefore focus on important recent advances in this field.

MAST CELLS IN THE PATHOPHYSIOLOGY OF ASTHMA: A HISTORICAL PERSPECTIVE

Mast cells secrete the autacoid mediators histamine, prostaglandin (PG) D_2 , and leukotriene (LT) C_4 , which are capable of inducing bronchoconstriction, mucus secretion, and mucosal edema, all features of asthma. This is particularly evident during experimental allergen challenge, in which blockade of these mediators attenuates the early fall in lung function (see review³). However, mast cells also synthesize and secrete a large number of proinflammatory cytokines (including IL-4, IL-5, and IL-13), which regulate both IgE synthesis and the development of eosinophilic inflammation, and several profibrogenic cytokines, including TGF- β and basic fibroblast growth factor (FGF₂).^{1,3} The serine proteases tryptase, chymase, and carboxy-peptidase are major secretory products of human mast cells that can interact with various cell types via protease activated receptors (PARs) and by other processes to alter their behavior profoundly.³ Importantly, bronchial mucosal mast cells in subjects with asthma

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FIG 1. An electron micrograph of an activated mast cell in the airway mucosa of a subject with asthma. There is variable loss of granule contents (*arrowheads*) although granule membranes (*arrows*) remain intact, a process known as *piecemeal degranulation*. Magnification $\times 6000$. Picture courtesy of Dr Susan Wilson.

exhibit features of chronic ongoing activation³ (Fig 1). Although most of these studies have been performed in atopic individuals, similar evidence has been obtained to support a role for ongoing mast cell secretory activity in both nonatopic and occupational asthma.³

MAST CELLS IN THE PATHOPHYSIOLOGY OF ASTHMA: RECENT ADVANCES

Although, in general, total mast cell numbers appear not to be increased in the bronchial mucosa of subjects with asthma compared with normal subjects, this inadequately describes the complexity, because it is evident that they localize to 3 key sites: the airway smooth muscle (ASM), the airway mucous glands, and the bronchial epithelium.

Mast cell microlocalization within asthmatic ASM

It is a widely held view that the disordered airway physiology and airway wall remodeling characteristic of asthma are consequences of the inflammatory process, but there are examples where this relationship is weak. This is most evident in patients with eosinophilic bronchitis (EB), a condition that accounts for about 15% of cases of cough referred to respiratory specialists. It is characterized by corticosteroid responsive cough and the presence of a sputum eosinophilia occurring in the absence of variable airflow obstruction or bronchial hyperresponsiveness (BHR).⁵ However, despite differing functional effects on the airways, the immunopathology of asthma and EB is virtually identical.^{6,7} Thus, in bronchoalveolar lavage, induced sputum, and airway biopsies, the extent of T-cell and eosinophil infiltration and activation, mucosal mast cell numbers, IL-4 and IL-5 cytokine expression, epithelial integrity, subbasement membrane collagen deposition, and mediator concentrations including histamine and PGD₂ are almost identical. This leads us to the conclusion

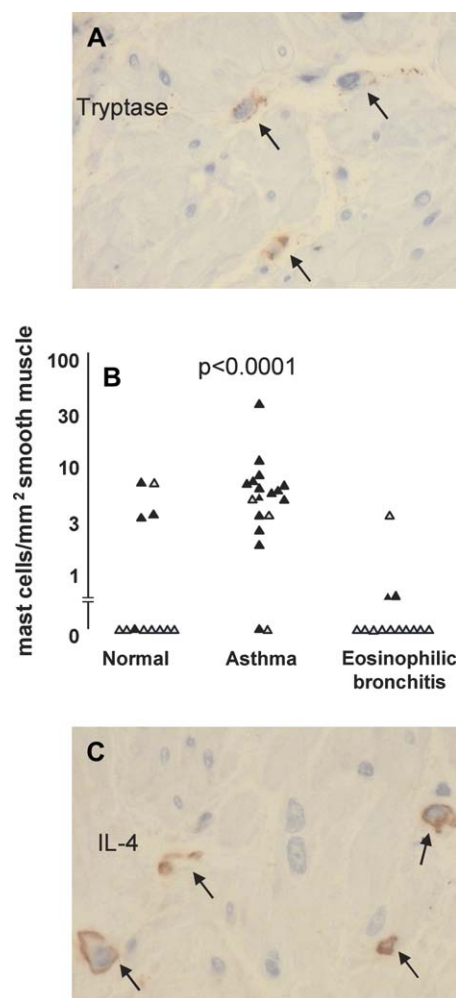


FIG 2. A, A 2- μ m section of bronchus from a patient with asthma demonstrating the presence of degranulated tryptase⁺ mast cells (*arrows*) within an ASM bundle. **B,** Numbers of mast cell within the bronchial smooth muscle bundles of normal subjects and patients with asthma and eosinophilic bronchitis. Copyright © 2002 Massachusetts Medical Society. All rights reserved. Reproduced with permission.⁹ **C,** IL-4⁺ mast cells (*arrows*) within an ASM bundle.

that many of the immunopathological features previously attributed to causing asthma may not, in fact, be fundamental to the development of airflow obstruction and BHR.

After a series of in-depth studies we have found only 2 key differences between the pathology of asthma and eosinophilic bronchitis. The first is that the concentration of IL-13 is elevated in the induced sputum of subjects with asthma but not subjects with EB.⁸ The numbers of IL-13⁺ cells in the airway mucosa in asthma is relatively low but increased when compared with EB, with most of the IL-13⁺ cells identified as eosinophils. However, the most striking difference between the pathology of asthma and EB is in the ASM. Abnormal ASM function is fundamentally important to the pathophysiology of asthma and yet, surprisingly, this compartment has not been the focus of

detailed immunopathological assessments. In subjects with asthma, we have observed that there are many mast cells between the ASM bundles, but virtually none in the ASM from patients with EB or normal subjects (Fig 2).⁹ This has been confirmed in a further independent study.¹⁰ The majority of mast cells in the ASM were of the MC_{TC} phenotype—that is, containing both tryptase and chymase—and also expressed IL-4 and IL-13, but interestingly, not IL-5 (Fig 2).¹¹ In contrast, in asthmatic biopsies there were almost no T cells or eosinophils in the ASM of any of the study groups. This indicates that ASM infiltration by mast cells may be one of the critical determinants of the asthmatic phenotype, and could explain the observed correlation between ASM mast cell numbers and BHR within the asthmatic group. These observations have further implications. For example, not only might it clarify why many atopic patients do not have asthma but also it could explain why the presence of asthma is such a strong risk factor for death from anaphylaxis and allergen desensitization.¹²

Putative mast cell–ASM interactions

In many instances, it is likely that cellular communication within the airways works across a distance of 1 to 2 μ m and that cell-cell contact is critical to influence function. The localization of mast cells within the ASM in asthma will facilitate specific interactions between these cells and ASM in terms of both localized mediator release and direct cell-cell contact. Therefore, it is entirely plausible that the presence of mast cells within the ASM could contribute to the development of ASM hypertrophy and hyperplasia, smooth muscle dysfunction expressed as BHR, and variable airflow obstruction.

It is reasonable to hypothesize that the primary stimulus for mast cell recruitment lies within the ASM involving the release of chemoattractants for mast cells or their progenitors. The ASM secretes many chemokines and growth factors that exhibit mast cell chemotactic activity. These include CCL11, CXCL8, and CXCL12. We have demonstrated that human lung mast cells (HLMCs) express CXCR3,¹³ and ASM secretes the 3 CXCR3 ligands CXCL9, CXCL10, and CXCL11.¹⁴ Of importance, cultured human ASM from subjects with asthma preferentially secrete CXCL10 after cytokine activation, and this accounts in large part for the greatly enhanced HLMC chemotaxis mediated by conditioned medium from asthmatic compared with normal ASM.¹⁴ The relevance of this to mast cell recruitment by the asthmatic ASM *in vivo* is further demonstrated by the increased expression of CXCL10 by the ASM in bronchial biopsies from subjects with asthma compared with normal subjects, and by the enrichment of CXCR3⁺ mast cells within the ASM bundles compared with the surrounding airway mucosa.¹⁴ Other chemoattractants may also contribute to the ASM mast cell myositis. Stem cell factor (SCF; c-kit ligand) is produced by both ASM and mast cells and is both a chemoattractant and an essential survival factor for mast cells. In addition, TGF- β is another mast cell chemoattractant released by ASM after exposure to tryptase,

providing a mechanism through which mast cells might contribute to further mast cell recruitment¹⁵ via autocrine pathways.

Once present in the ASM bundle, adhesion of mast cells to ASM cells is likely to be important for the retention of mast cells and the functional interaction between the 2 cell types. This hypothesis is supported by the observation that unlike T cells and eosinophils, which adhere poorly to ASM, HLMCs adhere readily.¹⁶ Interestingly, this is mediated in part via a molecule known as tumor suppressor in lung cancer 1 (TSLC-1; also known as SgIGSF, IGSF4, RA175, Necl2, and SynCAM). Tumor suppressor in lung cancer 1 is highly expressed by HLMC and mediates HLMC adhesion to ASM through a heterophilic Ca²⁺-independent mechanism. The classic mast cell autacoid mediators histamine, PGD₂, and LTC₄ are all potent agonists for ASM contraction. Exogenously administered tryptase induces bronchoconstriction and BHR in dogs and sheep,¹⁷ and *in vitro*, tryptase can potentiate the contractile response of sensitized bronchi to histamine.¹⁸ Tryptase-induced bronchoconstriction in animal models is likely to be mediated by mast cell activation because it may be blocked by pretreatment with antihistamines. Human tryptase can act as a stimulus for histamine release from mast cells from several tissues including those of the lung,¹⁹ and consistent with this, inhibitors of this protease can be effective as mast cell stabilizing agents. In addition to its ability to stimulate cytokine release from ASM, tryptase can act as a potent mitogen *in vitro*.^{20,21} The precise mechanism whereby tryptase may interact with these cells is unclear. Several studies have suggested the need for an intact catalytic site,²⁰ although there is a report that nonproteolytic actions may be involved in mitogenesis.²¹ ASMs abundantly express the G-protein-coupled receptor PAR2, peptide agonists of which can stimulate mitogenesis and cytokine release similar to that seen with tryptase.²⁰

IL-4 and IL-13 are also believed to be key in the development of BHR. This is supported by an *in vivo* study in mice in which instillation of T_H2 cell conditioned medium to the airways of naive mice induced BHR within 6 hours. This required expression of the IL-4 receptor α subunit and signal transducer and activator of transcription 6, suggesting a critical role for IL-4 and/or IL-13, and both of these cytokines produced similar effects when administered individually.²² IL-4 and IL-13 and also enhance the magnitude of agonist-induced intracellular Ca²⁺ responses in cultured human ASM. In asthma, there are few T cells within the ASM, but mast cells at this site do express both IL-4 (Fig 2) and IL-13,¹¹ revealing a further pathway through which mast cells could contribute to the development of BHR.

Chymase is a chymotryptic protease of mast cells whose roles in asthma have been less extensively studied than tryptase, but it is expressed by those mast cells infiltrating the ASM in asthma.⁹ Interestingly, chymase degrades human ASM pericellular matrix and inhibits T-cell adhesion to ASM, which might explain the paucity of T cells within this structure in asthma.²³ Consistent with

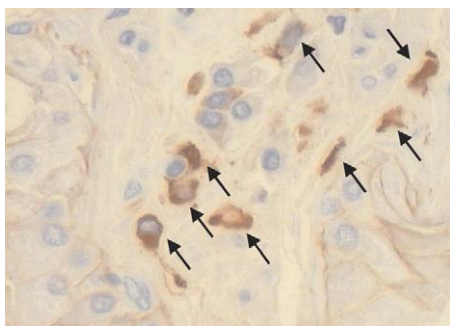


FIG 3. Photomicrograph of tryptase⁺ mast cells (arrows) within the airway mucous gland stroma in a patient with mild asthma.

roles in tissue remodeling are the ability of chymase to process type 1 procollagen to initiate the formation of collagen fibrils, to activate matrix metalloprotease 1, and to stimulate the release of extracellular TGF- β_1 (see review²⁴). Further roles in controlling the bioavailability of cytokines and growth factors are suggested by the findings that chymase can convert IL-1 β into the active form, degrade IL-4, and induce the release of membrane-bound SCF. Although characterized as a potent stimulus for mucus secretion, relatively little is known of the direct actions of chymase on cells, and apart from being able to inactivate PAR1, it does not seem to act on any of the PARs. The effects of combined secretion of chymase, tryptase, and other mast cell mediators *in vivo* remains unclear and will perhaps be best delineated in coculture experiments using intact human lung mast cells and primary cultures of asthmatic ASM cells.

Mast cell microlocalization within airway epithelium and submucosal glands

Mast cells infiltrate the bronchial epithelium in asthma.³ This is likely to be of importance in disease pathogenesis for 2 reasons. First, mast cells are placed at the portal of entry of noxious stimuli such as aeroallergens, which would facilitate an effector role in the ongoing immunologic response (antigen presentation, T_H2 cell differentiation, IgE synthesis). Second, there are likely to be important consequences of mast cell degranulation on epithelial function. For example, mast cells adhere avidly to bronchial epithelial cells,²⁵ and tryptase stimulates airway epithelial IL-8 release and can upregulate intercellular adhesion molecule 1 expression.²⁶ At this site, mast cells could also more readily respond to other stimuli, such as hyperosmolar or inhaled adenosine.

Severe mucus plugging is a well known feature of severe fatal asthma but is also recognized as a feature of milder disease, and results from mucus hypersecretion by hyperplastic submucosal glands and epithelial goblet cells. Carroll et al²⁷ performed a detailed analysis of cartilaginous airways in postmortem lung specimens from patients with fatal asthma, patients with asthma who died from other causes (nonfatal asthma), and subjects without asthma who died of nonpulmonary causes.

Immunohistochemistry for mast cell tryptase revealed a significant increase in the number of mast cells within the mucosal gland stroma in nonfatal asthma (Fig 3) and a marked increase in the number of degranulated mast cells in both fatal asthma and nonfatal asthma compared with normal controls. They established significant correlations between the density of both intact and degranulated mast cells in the within mucous glands with the degree of mucus obstruction in the airways. Taken together, these data provide some support for a role of mast cells in the development of mucous gland hyperplasia and the mucus gland secretion characteristic of asthma. Numerous mast cell products are likely to contribute to these features of asthma including histamine, PGD₂, LTC₄, IL-6, IL-13, TNF- α , tryptase, and chymase.

A further molecule of interest and of relevance to the epithelium and mucosal glands is amphiregulin, a member of the epidermal growth factor family. Amphiregulin expression is induced in human progenitor-derived mast cells after activation through Fc ϵ RI,^{28,29} an effect that is not suppressed by dexamethasone. Amphiregulin is expressed at increased levels by mast cells in the asthmatic bronchial mucosa, and *in vitro*, mast cell-derived amphiregulin increases mucin gene expression in the NCI-H292 epithelial cell line. These observations focus attention on mast cell-derived amphiregulin as contributing to epithelial goblet cell metaplasia and mucus hypersecretion in asthma, which is refractory to corticosteroids. In addition, recombinant amphiregulin induces the proliferation of human airway fibroblasts but not ASM cells, suggesting a further mechanism whereby mast cells can contribute to subepithelial fibrosis (Fig 4).

Mast cell-derived TNF- α as a pivotal cytokine in asthma

TNF- α is a proinflammatory cytokine strongly implicated in the pathogenesis of asthma.³⁰ When administered by inhalation to animals and human beings, it induces both BHR and sputum neutrophilia and exacerbates BHR in patients with asthma.^{31,32} TNF- α immunoreactivity is increased in the airways of patients with mild asthma, largely because of increased expression by mucosal mast cells.³⁰ Two studies have recently shown that TNF- α expression is increased markedly in severe asthma, as shown by increased protein in bronchoalveolar lavage, increased protein expression on PBMCs, and both increased protein and a 30-fold increase in mRNA in the bronchial mucosa,^{33,34} despite patients receiving high-dose inhaled and oral corticosteroid therapy. Interestingly, the increased protein expression in endobronchial biopsies was accounted for by increased numbers of TNF- α ⁺ mast cells.³⁴ An uncontrolled proof-of-concept study demonstrated that administration of the soluble TNF- α P75 receptor IgG₁Fc fusion protein Etanercept (Enbrel; Wyeth Laboratories, Berkshire, United Kingdom) for 12 weeks significantly improved quality of life, lung function, and BHR by 2.5 doubling dilutions of methacholine.³⁴ Interestingly, there was no change in sputum inflammatory

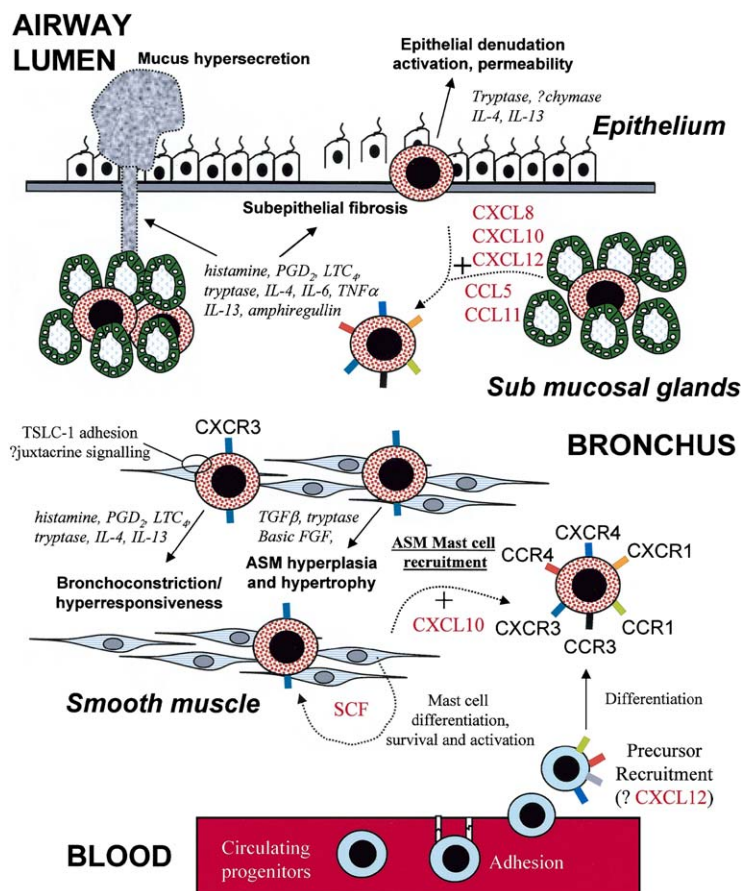


FIG 4. An overview of some potentially important interactions between mast cells and structural airway cells in the pathogenesis of asthma.

cells. A further small, double-blind, placebo-controlled cross-over study of Etanercept for 10 weeks in patients with severe refractory asthma³³ has confirmed marked improvements in quality of life, lung function, and BHR (an increase of 3.5 doubling dilutions of methacholine). Again, there were no significant changes in sputum inflammatory cells but a marked reduction in sputum histamine concentration. This latter observation suggests that Etanercept did result in inhibition of primary mast cell activation. *In vitro*, TNF- α induces mast cell mediator release, and after IgE-dependent activation, the release of preformed mast cell-associated TNF- α has been shown to serve as a positive autocrine feedback signal to augment nuclear factor- κ B activation and further production of TNF- α and other cytokines, including GM-CSF and IL-8.³⁵ Taken together, these studies provide strong evidence that mast cell-derived TNF- α plays a major role in asthma pathophysiology, especially in patients with severe disease.

Possible mechanisms of ongoing mast cell activation in asthma

The mechanisms of chronic mast cell activation in asthma are not known. It is often assumed that allergen is the dominant factor resulting in cross-linking of Fc ϵ RI,

but although allergen exposure is strongly linked to asthma once established, allergen avoidance usually has a minor effect on the state of established disease that appears to become self-perpetuating. This is typified in cases of occupational asthma, in which asthma and accompanying mast cell activation will persist on removal of the sensitizing agent if exposure is not prevented early in the course of the disease. Mast cells can be activated by several diverse stimuli, including monomeric IgE alone, proteases (including tryptase), cytokines (eg, stem cell factor, TNF- α , IFN- γ), complement, adenosine, Toll-like receptor (TLR) ligands, neuropeptides, and hyperosmolality. Therefore, the mechanism of activation of mast cells in the asthmatic bronchial mucosa is undoubtedly complex.

Monomeric IgE induces mouse mast cell mediator release and prolongs their survival through the autocrine release of cytokines.³⁶ In HLMC, monomeric IgE induces the release of histamine, LTC₄, and IL-8.³⁷ Importantly, when free IgE is present, intracellular signaling continues, suggesting that these findings are physiologically relevant. This is particularly interesting because in human beings, there is a reproducible correlation between total serum IgE concentration, BHR, and asthma. Thus, it is attractive to hypothesize that in asthma, heightened mast cell

activation arises, at least in part, from the increased binding of IgE to FcεRI. This is supported to some extent by the observation that anti-IgE therapy markedly reduces both airway inflammation and mast cell activation, as manifest by reduced IL-4 expression.³⁸ However, anti-IgE therapy has a minimal effect on BHR, suggesting that monomeric IgE-dependent activation of mast cells in asthmatic ASM does not account for this aspect of airway dysfunction.

Stem cell factor primes mast cells for mediator release and, at higher concentrations, directly induces degranulation. Concentrations of SCF are elevated in asthmatic airways³⁹ and could therefore make an important contribution to ongoing mast cell activation. The C5a receptor CD88 was not thought to be expressed on HLMC, but recent work demonstrates that it is in fact expressed on the MC_{TC} subset of HLMC.⁴⁰ Elevated C5a concentrations have been identified in the induced sputum of subjects with asthma,⁴¹ thus providing a further potential means of mast cell activation, and of particular relevance to those mast cells (MC_{TC}) within the ASM bundles.

Human progenitor-derived mast cells and mouse mast cells express TLRs 1 to 7 and 9.⁴²⁻⁴⁴ These play an important role in the innate host response to pathogens, activating diverse programs of gene expression depending on the stimulus. For example, in mouse mast cells, functional responses to TLR2 (the receptor for bacterial peptidoglycan) result in production of TNF-α, IL-4, IL-5, IL-6, IL-13, and IL-1β, whereas activation of TLR4 (the receptor for LPS) induces production of TNF-α, IL-1β, IL-6, and IL-13 but not IL-4 or IL-5. In addition, activation of TLR2 but not TLR4 induces Ca²⁺ mobilization, degranulation, and LTC₄ production.⁴²⁻⁴⁴ Examination of the gene expression profile from human cord blood-derived mast cells using high density oligonucleotide probe arrays after activation with LPS compared with anti-IgE demonstrates that both induce a core response, plus an LPS or anti-IgE specific program of gene expression.² Perhaps of more relevance to asthma is mast cell activation via TLR3, the ligand for which is double-stranded viral RNA.⁴³ Polyinosinic-polycytidylic acid (Poly:IC), a synthetic activator of TLR3, induces the specific release of IFN-α, as does exposure to respiratory syncytial virus and influenza virus. Because viruses are a common cause of asthma exacerbations, the mast cell antiviral response may be an important contributor to the deteriorating airway physiology.

A further interesting area of study is the role of immunoglobulin free light chains. These are present in serum in normal subjects, and their production is augmented in inflammatory diseases such as rheumatoid disease. In mice, immunoglobulin free light chains can confer mast cell-dependent hypersensitivity through an unknown mechanism, and antigen-specific light chains can mediate mast cell-dependent bronchoconstriction after antigen challenge.⁴⁵ Concentrations of immunoglobulin free light chains are elevated in the sera of subjects with asthma compared with normal subjects, suggesting they may be relevant to the pathophysiology of human asthma.⁴⁵

G-protein-coupled receptors on mast cells may also be independently regulated to increase (eg, adenosine A_{2B}) or decrease (eg, adenosine A_{2A}) releasability of mast cells to a wide range of stimuli.⁴⁶ Because in mouse models, adenosine interacts with both inflammatory and remodeling responses, it provides another potential therapeutic target in this disease.

Finally, there are likely genetic factors that lower the mast cell threshold for activation in asthma. For example, an important negative regulator of mast cell activation is src homology 2-containing inositol phosphatase (SHIP).⁴⁷ SHIP-deficient mast cells exhibit markedly enhanced secretory responses, and with respect to human basophils at least, cells that are hyperreleasable demonstrate a relative deficiency of this molecule.⁴⁸

CONCLUSION

The mast cell has emerged as a pivotal cell in the pathogenesis of asthma. The poor efficacy of several so-called “mast cell stabilizing drugs” to improve asthma control is a result of the fact that these compounds are ineffective at inhibiting mast cell activation in chronic asthma. For example, disodium cromoglycate is only a weak inhibitor of IgE-dependent HLMC secretion, with maximal inhibition of histamine release *in vitro* of 10% to 20% when used in the high micromolar range,⁴⁹ and it also exhibits rapid tachyphylaxis. β₂-Adrenoceptor agonists such as salbutamol are more potent inhibitors of HLMC mediator release *in vitro*,⁴⁹ but again there is rapid tachyphylaxis, so that with chronic administration, the clinical evidence is that they do not attenuate mast cell secretion in the asthmatic airway and may even enhance it.⁵⁰ The mechanism of chronic ongoing mast cell degranulation in asthma is likely to occur piecemeal (Fig 1), the mechanism of which is unknown, and may not be susceptible to inhibition by drugs that attenuate classic IgE-dependent anaphylactic degranulation. Nevertheless, the clear clinical efficacy of the antihuman IgE omalizumab in severe allergic asthma and its ability to reduce airway inflammation markedly point to the importance of this pathway in ongoing disease that is refractory to corticosteroids. However, what is needed are novel and potent drugs that inhibit pathological mast cell mediator release in asthmatic airways. These will help determine the exact role of mast cells in the development and propagation of asthma, and may offer truly new and safe approaches to the management of this common disease.

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