

CHAPTER 13

MAST CELLS IN LUNG INFLAMMATION

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Abstract: Mast cells play an important role in the lung in both health and disease. Their primary role is to initiate an appropriate program of inflammation and repair in response to tissue damage initiated by a variety of diverse stimuli. They are important for host immunity against bacterial infection and potentially in the host immune response to non small cell lung cancer. In situations of ongoing tissue damage, the sustained release of numerous pro-inflammatory mediators, proteases and cytokines, contributes to the pathophysiology of lung diseases such as asthma and interstitial lung disease. A key goal is the development of treatments which attenuate adverse mast cell function when administered chronically to humans in vivo. Such therapies may offer a novel approach to the treatment of many life-threatening diseases.

INTRODUCTION

In this chapter, we will examine the role of mast cells (MCs) in the initiation and maintenance of airway and parenchymal inflammation and fibrosis. MCs play an important role in host defense against pathogens, particularly bacterial infection and in tissue repair through the synthesis and release of numerous pro-inflammatory mediators, proteases and cytokines, the pattern of which varies depending on the stimulus. In healthy human lung, MCs are ubiquitous in the airways and parenchyma, but redistribute to key tissue structures in disease. Their location at the interface between the external environment and lung tissue places them in the ideal location to respond rapidly to perceived tissue insults. MCs can respond to many diverse stimuli (Fig. 1) and it is our view that their primary role is to initiate an appropriate program of inflammation and repair in response to tissue damage, but that if this stimulus becomes chronic, then tissue dysfunction and fibrosis ensue. Human lung mast cells (HLMCs) achieve their biological effects through cell-cell interactions and the release

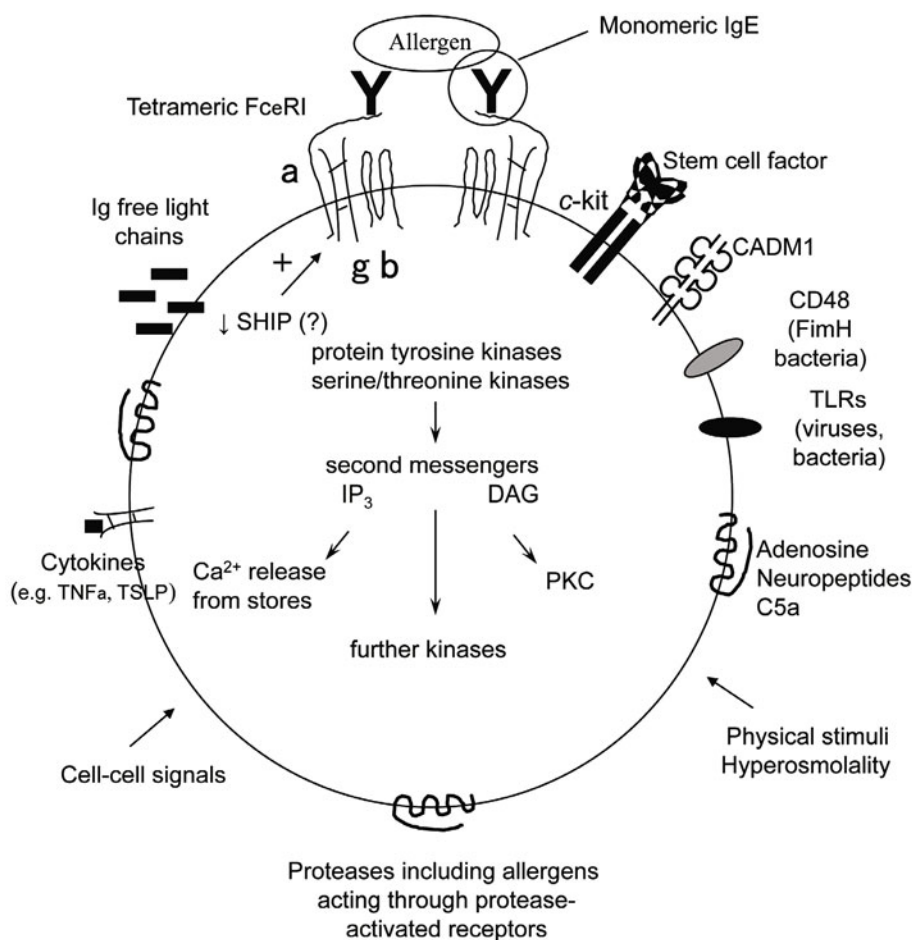


Figure 1. Potential mechanisms of human lung mast cell activation.

of numerous autacoid mediators (histamine, prostaglandin [PG]D₂, leukotriene [LT]C₄), proteases and multifunctional cytokines and chemokines. The biological activities of these mediators are summarized in Tables 1-3. The release of these mediators is not all-or-none, but highly regulated and stimulus-specific. This allows the MCs to interact with structural cells, as well as cells of both the innate and acquired immune systems.

MAST CELL RECRUITMENT TO THE LUNG

The mechanisms of MC homing to human lung are poorly understood and the primary signals are unknown. However, MC expansion in sensitized murine lung is induced by Type 2 NKT cells and IL-9 after allergen challenge and PGE₂, produced by nasal mucosa.^{1,2} In addition, systemically available sphingosine 1-phosphate (S1P) increases

Table 1. The classical human mast cell mediators and their biological effects

Mediator	Activity
Histamine (stored)	Bronchoconstriction; tissue edema; ↑ vascular permeability; ↑ mucus secretion; ↑ fibroblast proliferation; ↑ collagen synthesis; ↑ endothelial cell proliferation, dendritic cell activation
Heparin (stored)	Anticoagulant; storage matrix for mediators; sequestering of growth factors from degradation; fibroblast activation; endothelial cell migration
Tryptase (stored)	Degrades respiratory allergens and cross-linked IgE; generates C3a and bradykinin; degrades neuropeptides; increases BHR and ASM contractility; indirectly activates collagenase; ↑ fibroblast proliferation and collagen synthesis; epithelial activation, potentiates mast cell histamine release; neutrophil recruitment; TGF-beta activation
Chymase (stored)	↑ mucus secretion; ECM degradation, Type I procollagen processing, angiotensin II synthesis, ↓ T-cell adhesion to airway smooth muscle, activates IL-1b, degrades IL-4, releases membrane-bound SCF
PGD ₂ (synthesized)	Bronchoconstriction; tissue edema; ↑ mucus secretion; dendritic cell activation; chemotaxis of eosinophils, Th2 T cells and basophils via the CRTH2 receptor
LTC ₄ /D ₄ (synthesized)	Bronchoconstriction; tissue edema; ↑ mucus secretion; enhances IL-13-dependent airway smooth muscle proliferation; dendritic cell maturation and recruitment; eosinophil IL-4 secretion, mast cell homing, mast cell IL-5, IL-8 and TNFα secretion; tissue fibrosis

MC number in bronchoalveolar lavage (BAL) fluid in mice.³ Mediators from mature MCs may also recruit further immature MC progenitors⁴ (see Tables 1 and 2). In addition, the lung MC population may expand in pathological conditions such as fibrosis via several stimuli, including stem cell factor (SCF), S1P and other growth factors. There is evidence that several factors may lead to the redistribution of MCs in human lung. For example, HLMCs express CXCR3, particularly on the cells within the ASM bundles in asthma, which in turn preferentially express the chemotactic ligand CXCL10.⁵

MAST CELLS IN HEALTHY LUNG

HLMCs show a marked heterogeneity within the tissue with variability in their size and shape, protease and cytokine content and response to various stimuli (reviewed in ref. 6). In comparison with MCs from other sources, HLMCs display distinct differences in phenotype distribution, biomarker expression patterns, chemokine receptor profiles and released mediators.⁷⁻¹²

HLMCs belong to either a predominant tryptase+ (MC_T) or tryptase/chymase+ (MC_{TC}) subtype. The rare chymase-only (MC_C) subtype has also been identified within the lung in several studies.^{7,9,13} Isolated lung MC_T are smaller and produce lower amounts of the autacoid mediators histamine and PGD₂ compared with MC_{TC} cells.^{10,14,15} In situ,

Table 2. Biological activity of human mast cell-derived chemokines in vitro

Chemokine	Target Cells	Biological Effects
CCL1	T cells	T-cell recruitment
CCL2	Mast cells	Chemotaxis
	Epithelial cells	Proliferation, chemotaxis
	Fibrocytes	Chemotaxis
	T cells	→Th2 phenotype
	Eosinophils	Chemotaxis
	Monocytes	Chemotaxis
	Basophils	Activation, mediator release
CCL3	Mast cells	Activation, mediator release
	T cells	→Th1 phenotype and chemotaxis (Th1-selective)
	Macrophages	Differentiation
	Neutrophils	Chemotaxis (in vivo) and
	Eosinophils	cytotoxicity
	Monocytes	Chemotaxis
	Basophils	Chemotaxis
		Activation, mediator release
CCL4	T cells	→Th1 phenotype and chemotaxis (Th1-selective)
	Eosinophils	Chemotaxis
	Neutrophils	Chemotaxis (in vivo)
CCL5	Mast cells	Chemotaxis?
	T cells	→Th1 phenotype and chemotaxis (Th1-selective)
	Eosinophils	Chemotaxis
	Monocytes	Chemotaxis
CCL7	Eosinophils	Chemotaxis
	Monocytes	Chemotaxis
	Basophils	Activation, mediator release
CCL12	Fibrocytes	Chemotaxis
	Monocytes	Chemotaxis
	Eosinophils	Chemotaxis
	Lymphocytes	Chemotaxis
CCL17	T cells	Chemotaxis (Th2-selective)
CCL19	Airway smooth muscle	Chemotaxis
CCL20	Dendritic cells	Chemotaxis
	T cells	Chemotaxis
CCL22	T cells	Chemotaxis (Th2-selective)
CXCL5	Neutrophils	Chemotaxis
CXCL8	Mast cells	↓ chemotaxis, ↓ mediator release
	Endothelial cells	Proliferation, survival, chemotaxis, angiogenesis
	Neutrophils	Chemotaxis
	Eosinophils	Chemotaxis after priming with IL-3, IL-5 or GM-CSF

Table 3. Biological activity of human mast cell-derived cytokines in vitro

Cytokine	Target Cells	Biological Effects
IL-4	B cells	proliferation; ↑ MHC class II, ↑ CD40, ↑ CD25; ↑ IgE, ↑ IL-6
	T cells	Proliferation, →Th2 cell phenotype
	Monocyte/macrophages	↓ H ₂ O ₂ and O ₂ ⁻ , ↓ parasite killing, ↓ tumoricidal activity; monocyte → macrophage differentiation; ↓ IL-1, IL-6, IL-8 and TNF ↑ MHC class II and CD23; 15-lipoxygenase expression
	Eosinophils	Transendothelial migration
	Fibroblasts	Proliferation, chemotaxis, ↑ ECM; ↑ ICAM-1
	Endothelial cells	Proliferation, ↑ VCAM-1, ↓ ICAM-1
IL-3, IL-5, GM-CSF	Mast cells	↑ FcεRI, ↑ ICAM-1
	Eosinophils	Growth, adhesion, transendothelial migration, chemotaxis, activation and prolonged survival
IL-6	B cells	Ig secretion including ↑ IgE
	T cells	Growth, differentiation
	Mast cells	Survival
IL-13	B cells	IgE synthesis
	Monocyte/macrophages	As for IL-4
	Eosinophils	Activation, survival
	Endothelial cells	↑ VCAM-1
TNFα	Monocyte/macrophages	↑ cytotoxicity, chemotaxis, prolonged survival
		Class II antigen and IL-2R expression, proliferation
	T cells	↑ cytotoxicity, chemotaxis
	Neutrophils	↑ cytotoxicity, oxidant production
	Eosinophils	↑ E-selectin, ↑ ICAM-1, ↑ VCAM-1;
	Endothelial cells	↑ intravasation of most leucocytes
		Growth, chemotaxis, ↓ collagen, ↑ collagenase, ↑ IL-6, ↑ IL-8
SCF	Fibroblasts	Histamine and tryptase secretion
	Mast cells	
NGF	Mast cells	Growth, differentiation, survival, chemotaxis
	B cells	Differentiation, proliferation, ↑ Ig
	T cells	Differentiation, proliferation
	Eosinophils	Proliferation
	Basophils	Activation, mediator release
	Neutrophils	Chemotaxis, survival, mediator release
	Monocyte/macrophages	Proliferation, mediator release
	Fibroblasts	Migration, contraction
	Smooth muscle cells	Migration, contraction, proliferation

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Table 3. Continued

Cytokine	Target Cells	Biological Effects
	Mast cells	Differentiation, survival, activation, mediator release
TGFβ	Smooth muscle cells	Differentiation, activation
	Epithelial cells	Inhibition of proliferation
	Endothelial cells	↑ angiogenesis
FGF-2	Fibroblasts	Proliferation
	Endothelial cells	↑ angiogenesis
IFNα	NK cells	↑ cytotoxicity
	Macrophages	Development, maturation
	Dendritic cells	Activation, maturation, ↑ IFNγ
	T cells	↑ survival of activated T cells, ↑ Th1 phenotype
TSLP	Dendritic cells	induction of Th2 immunity
	Mast cells	↑ IL-13 expression

the size of lung MC_T and MC_{TC} cells varies according to the location.¹⁶ In addition, MC_{TC} cells accumulate other peptidases, express CD88 and a distinct set of cytokines and chemokine receptors.^{8-10,17,18} Their properties are summarized in Table 4. The MC_T phenotype is typically found in the lung parenchyma, bronchial epithelium and the bronchial lamina propria, whereas the MC_{TC} phenotype localizes around blood vessel walls.^{8,9,16,19} Andersson et al identified several site-specific populations within conventional MC_T and MC_{TC} types on the basis of expression patterns for the IgE receptor, IL-9 receptor, renin, histidine decarboxylase, VEGF, basic FGF (bFGF, FGF2), 5-lipoxygenase and leukotriene C₄ synthase. Interestingly, renin expression is observed predominantly in the MC_T subtype and only in pulmonary vascular MC_{TC} cells. The relatively high expression of the high-affinity IgE receptor and histidine decarboxylase, present in central airway MC_T and MC_{TC} cells, with IgE receptor virtually absent in alveolar MCs, are likely to be of particular physiological importance.

A differential expression pattern of proteases among MCs and the varying anatomical location of these cells indicate that they will have distinct roles in normal lung physiology and disease, but these remain poorly understood. MCs subtypes develop in the presence of specific factors and show a high degree of plasticity—a rapid conversion of cultured MC_{TC} cells to MC_T has been reported in coculture with human airway epithelium.²⁰ Hence, it is perhaps not surprising that MCs adopt the MC_T subtype in close proximity to epithelial cells in the alveoli and bronchi. In contrast, coculture of MC_T cells with endothelial cells transforms them into the MC_{TC} subtype,²¹ which may explain the predominance of the MC_{TC} subtype around blood vessels.

Taken together, the existence of site-specific subpopulations of HLMCs is likely to be a response to local environmental cues affecting gene expression, resulting in appropriate physiological function.¹⁶ For example, renin expression in combination with chymase, converting AngI into AngII, in MC_{TC} cells close to pulmonary vasculature, as well as VEGF expression in MCs around pulmonary vessels, suggests involvement of these MCs in local regulation of vascular function.

Table 4. Phenotypes and localization of lung mast cells in normal lung and in disease

Mast Cells	Total Population	MCT Subtype	MCTC Subtype
Parameter	Normal Lung		
Location and percentage	MC _T , MC _{TC} and rare MC _C . ⁷ MC density is increased in small airways and alveolar parenchyma compared with central airways. ¹⁶	85-95% in the alveolar walls, bronchioles and bronchi. ^{8,9,16,19}	4-15% in alveolar attachments, small airway outer wall and in bronchi, ^{8,9,16,19} ~50% around pulmonary vessels. ¹⁶
Protease content	Decreased renin in central airways compared to other locations. ¹⁶	Tryptase. ⁸	cathepsin G, ¹⁷ carboxypeptidase A. ⁸
Specific biomarkers	Increased FcεpsilonRI and histidine decarboxylase, decreased 5-lipoxygenase in central airways compared to other locations. ¹⁶		CD88, ¹⁰ increased leukotriene C4 synthase in small airways and around blood vessels. ¹⁶
Cytokines, chemokines and receptors	CCR3 and CXCR1 in ~15% of MCs; CXCR3 and CXCR4 in ~30% MCs. ¹¹ Increased IL9R in small airways and around pulmonary vessels compared to central airways; increased VEGF around pulmonary vessels. ¹⁶	IL-4(low), IL-5, IL-6, IL-13 (low). ⁹	IL-4(high), IL-13 (high), ⁹ CCR3. ¹⁸
Responsiveness to stimuli		FcεpsilonRI, substance P. ¹⁰	FcεpsilonRI, C5a, compound 48/80, substance P. ¹⁰

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Table 4. Continued

Mast Cells	Total Population	MCT Subtype	MCTC Subtype
Asthma			
Location	Increased density and degranulation in epithelium, ^{87,88,91,93} in lamina propria, ⁹³ the airway mucous glands, ^{96,97} ASM. ⁹⁶⁻⁹⁸ Increased percentage in small airway outer and inner wall regions, alveolar attachments, peripheral alveolar tissue and large airway in severe asthma. ¹³ Increased numbers around small blood vessels in bronchi. ⁹⁹ Increased density in the adventitial layer of small pulmonary arteries in fatal asthma. ¹⁰⁰		Predominant subtype infiltrating ASM (83%). ⁹⁸ Higher numbers and the MCTC/MCT ratio in alveolar attachments in severe asthma. ¹³ Increased density around blood vessels in the lamina propria. ⁹⁹ Increased density in the adventitial layer of small pulmonary arteries in fatal asthma. ¹⁰⁰
Cytokines, chemokines and receptors	7-fold increased TNF-alpha expression. ⁸⁸ 55% of MCs within the ASM express IL-4 and 17% express IL-13. ¹⁰¹ Increased MC number correlates with CCR1 and CCR4-expression in asthma; CCR1 and CCR4 in 24% and 19% of MCs, respectively. ¹⁰² CXCR3 is expressed in 100% of MCs in ASM and 47% of MCs in the submucosa. ⁵		100% IL-4-positive vs 60% in control. ⁹
Specific biomarkers	Increased amphiregulin ¹⁰³ and thymic stromal lymphopoietin. ¹⁰⁴		

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Table 4. Continued

Mast Cells	Total Population	MCT Subtype	MCTC Subtype
Functional correlations	MC localization to the bronchial ASM is associated with airway hyperresponsiveness in asthma. ¹⁰⁵ The numbers of total MCs and degranulated MCs correlate with the lumen area occupied by mucus. ¹⁰⁶		There was a significant inverse correlation between the number infiltrating the bronchial ASM and the PC 20 for methacholine in the subjects with asthma. ⁹⁸ The MC _{TC} number and the MC _{TC} /MC _T ratio in the small airway outer wall and alveolar attachments positively correlate with lung function in severe asthma. ¹³ The density in the lamina propria is related to the vascular area and to the number of VEGF ⁺ MC in mild to moderate asthma, ⁹⁹ with the MC _{TC} /MC ratio correlated with the PD20 methacholine values. ⁹⁹
COPD			
Location	Reduced net MC density with increased luminal MC number. ¹⁰⁷ Reduced density in submucosa and ASM bundle, but increased in glands (did not reach statistical significance). ¹⁰⁸	Lower density in the subepithelial area of central airways vs. controls. ¹⁰⁹ Lower numbers in the subepithelial area of airways vs controls. ¹⁰⁷	Higher numbers in both subepithelial area and ASM area of peripheral airways, but not in controls. ¹⁰⁹ Increased density in walls of small airways and in alveolar parenchyma vs control; ¹⁰⁷ several-fold increase in small airways, small airway epithelium, pulmonary vessels and alveolar parenchyma in very severe COPD. ¹⁰⁷
Specific biomarkers		Increased expression of CD88. ¹⁰⁷	Increased expression of CD88 and TGF-beta and decreased rennin. ¹⁰⁷

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Table 4. Continued

Mast Cells	Total Population	MCT Subtype	MCTC Subtype
COPD (continued)			
Functional correlations		Cell numbers in the epithelium and subepithelial area of peripheral airways positively correlate with FEV1/VC lung function, the cell percentage in the total airway wall area of peripheral airways positively correlate with higher values of FEV1 and FEV1/VC. ¹⁰⁹	Cell numbers in ASM of peripheral airways positively correlate with FEV1% pred lung function. Higher numbers in the adventitia of peripheral airways tend to correlate positively with FEV1% pred. ¹⁰⁹
Fibrotic Lung Disorders			
Location	Increased MC number in the alveolar septa and within alveoli. ¹¹⁰ Increased MC density and activation in bronchi. ¹¹¹⁻¹¹³		Increased MC _{TC} counts in ASM cells. ¹¹³
Specific biomarkers	increased bFGF in lung interstitium, surrounded by collagen, elastic fibers and ASM/myofibroblast cells. ¹¹⁴⁻¹¹⁶		
Functional correlations	MC counts significantly correlate with the degree of fibrosis. ¹¹⁰		MC _{TC} numbers correlate with accumulation of ASM cells and myofibroblast proliferation. ¹¹³

MAST CELLS IN LUNG DEFENSE

Since the role of MCs in the immune response is addressed in other chapters, here we present only those data which are directly related to respiratory infection and lung inflammation.

Defense against Bacterial Infections

Experimental animal models suggest that MCs are indispensable for the clearing of pulmonary infections caused by respiratory pathogens such as *Klebsiella pneumoniae*, *Mycoplasma pneumoniae*, *Streptococcus pneumoniae* and *Mycobacterium tuberculosis*.

Direct contact of MCs with clinical isolates of *K. pneumoniae* CIII decreased bacterial viability after 60 min in vitro, indicating involvement of MCs in the innate immune response.²² In an acute lung infection model, mast-deficient Kit^{W/W^v} mice had a much higher burden of *K. pneumoniae* KP1415 in the lungs compared with wild type or Kit^{W/W^v} mice reconstituted with MCs.²³ Similarly, MC-deficient Kit^{W-sh}/Kit^{W-sh} mice infected with *K. pneumoniae* intranasally have 4-fold worse survival after 4 days compared to wild-type mice.²⁴ Kit^{W-sh}/Kit^{W-sh} mice are more likely to die from *M. pneumoniae* compared with wild-type mice.²⁵ The mycoplasma burden is greater in MC-deficient mice with a 162-fold difference 4 days following infection when compared to wild-type mice. At later time points, bacterial burden is similar in both mouse strains, but neutrophilic inflammation remains severe in MC-deficient mice.²⁵ This latter observation raises the possibility that MCs may also have an anti-inflammatory role in the context of resolving bacterial infection.

MCs have the ability to bind and engulf bacteria and they also release a number of inflammatory mediators including TNF α , IL-4, IL-6, IL-10 and leukotrienes in response to bacterial infection. Exposure of the rat MC line RBL-2H3 to *M. pneumoniae* induces production of mRNA for IL-4, IL-6 and TNF α within 2 hours.²⁶ Interestingly, expression of the Fc ϵ RI alpha chain expression is required for activation of MCs for the IL-4 production induced by *M. pneumoniae*.²⁷

Several MC mediators are crucial for a full antibacterial response against acute infection. TNF α released locally by MCs plays a central role by recruiting neutrophils to sites of infection. TNF α is induced in response to bacterial FimH binding to the CD48 receptor in MCs and contributes to host defense against *K. pneumoniae*.²³ Inhibition of TNF α activity within the lung results in markedly decreased both short- and long-term survival of mice challenged with *K. pneumoniae* intratracheally.²⁸ Likewise, susceptibility to pneumonia in some strains of mice, infected with *S. pneumoniae*, correlates with the number of MCs and the speed of TNF α release in the lung, which is required for recruitment of circulating leukocytes.²⁹ MC-deficient Kit^{W-sh}/Kit^{W-sh} mice selectively reconstituted with IL-6^{-/-} or IL-6^{+/-} bone marrow MCs show that IL-6 produced specifically by MCs regulates survival from *K. pneumoniae* infection (peritonitis) by activating neutrophils and enhancing killing of intracellular bacteria by neutrophils.²⁴ Recently a new protective role of IL-4 produced by MCs has been identified in an acute infection model with life-threatening *Francisella tularensis*.³⁰ In this model, MCs were the first to infiltrate the lungs of infected animals prior to macrophages, dendritic cells or neutrophils. MCs significantly inhibit bacterial uptake and growth within macrophages specifically via production of IL-4 in response to infection.³⁰ MC transfer results in a remarkable level of survival of infected Kit^{W/W^v} mice.³⁰

Further important MC secreted factors required for host defense are the MC-specific proteases. For example, mice lacking protease-6, similar to human tryptase beta 1, cannot clear *K. pneumoniae* efficiently after peritoneal inoculation.³¹ These mice have 8-fold lower survival after 3 days compared with that of wild-type mice. Increased lethality in these mice is associated with higher bacterial burden and lower neutrophil recruitment despite normal MC production of IL-6 and TNF α . Likewise, when MC-deficient Kit^{W/W(v)} mice were given enzymatically active human tryptase beta I or its inactive zymogen before pulmonary infection with *K. pneumoniae*, tryptase beta I-treated mice had ~5 fold fewer viable bacteria in their lungs 6 hours after inoculation relative to zymogen-treated mice.³² When enzymatically active tryptase beta I, but not tryptase alpha, was instilled into the lungs of mice, neutrophil numbers in BAL increased 100-fold, but methacholine-induced bronchoconstriction was not altered. These data suggest that mMCP-6/tryptase beta I is the primary preformed granule mediator that protects mice from acute bacterial infections. The mechanism of neutrophil recruitment by mMCP-6/tryptase beta I is not clear, but the experimental data suggest that it is not due to IL-8, IL-6 or TNF α ,^{31,32} but may involve neutrophil chemotactic factors, produced by other cell types stimulated by tryptase.³³

In summary, experimental models of acute respiratory bacterial infection show that MCs are critical in the early stages of infection and have a profound effect on survival for days after the onset of infection. Mouse models show that MCs contribute to innate defenses against respiratory pathogens.

Defense against Viral Infection

Respiratory viral infections cause accumulation of MCs, as well as T and B lymphocytes during the first days of a symptomatic naturally acquired infection in the nasal mucosa.³⁴ Exposure of human MCs to respiratory viruses, such as influenza virus (PR8), Type 1 reovirus and respiratory syncytial virus (RSV), induced substantial production of IFN α ; a similar response was observed upon stimulation of these cells with the TLR3 ligand, poly I:C.³⁵ Poly I:C-induced IFN α production involved activation of NF-kappaB, p38 and C-Jun NH2-terminal kinase and mitogen-activated protein kinase. Activation of TLR3 with poly I:C did not induce TNF α , IL-1 β , IL-5, or GM-CSF production, in contrast to activation of TLR-2, 4, 5 and 9 by peptidoglycan, LPS, flagellin or CpG, respectively.³⁵ In addition, treatment with dsRNA alone or with IgE did not lead to MC degranulation.³⁵ Similarly, degranulation does not occur by direct RSV infection into MCs, however, it was detected in MCs sensitized with specific IgE.^{36,37} Furthermore, coculture with RSV-infected lung cancer A549 cells with the MC line HMC-1 induced degranulation in HMC-1 cells which coincided with upregulation of TNF α secretion. In this case, degranulation is likely to be mediated by defensins released from infected epithelial cells.³⁸ These data suggest that MC degranulation may occur during respiratory viral infection in vivo. In keeping with this, RSV infection increases the release of leukotrienes from MCs in rat airways.³⁹

Infections with some viruses, such as RSV or influenza virus, induce virus-specific IgE in humans and experimental models.^{37,40,41} IgE, specific to respiratory viruses, is implicated in MC degranulation, resulting in asthmatic and allergic responses in mouse models of allergic diseases.^{41,42} Similar to viral infections, respiratory bacterial infections with *Chlamydia pneumoniae*, *Haemophilus influenzae*, *S. pneumoniae* and *M. pneumoniae* often lead to production of specific IgE in a large population of patients.⁴³⁻⁴⁵ Moreover, the prevalence of bacterial-specific IgE is common in both asthma and chronic bronchitis.^{44,45} It is therefore plausible that on reinfection, a simultaneous engagement of TLRs, other

bacterial-specific receptors and FcεRI causes a synergistic activation of MCs, further amplified by pro-inflammatory signaling from surrounding cells in lung tissue. This amplified MC response is likely to be involved in asthma exacerbations commonly occurring during viral and bacterial infections and leading to deterioration of airway physiology.

Mast Cell Activation by Pathogens—Mast Cell Toll-Like Receptors

The functional response of HLMCs to bacterial, viral and other ligands has not been studied in detail. HLMCs show distinctive TLR expression profiles compared with MCs from umbilical cord, peripheral blood or skin.^{35,46,47} They express relatively high levels of TLR-2, -3, -4 and -7, lower levels of TLR-1, -5 and -10, whereas expression of TLR-6, -8, or -9 is not observed in these cells.⁴⁷ In contrast to HLMCs, murine peribronchial MCs strongly express TLR-9.⁴⁸

Exposure of HLMCs to the TLR2 ligand, lipoteichoic acid, reduced both FcεRI expression and IgE-induced degranulation, but the TLR2/TLR6 ligand, peptidoglycan, did not have similar effects.⁴⁹ These data suggest that some bacterial TLR2 ligands may modify some aspects of allergic disease. In murine lung MCs, a synthetic ligand for TLR2/TLR1 heterodimers, tripalmitoyl Cys-Ser-(Lys)4, induced JE (murine equivalent to human chemokine CCL2) production via NF-kappaB activation, but did not induce Ca²⁺ mobilization or degranulation.⁵⁰ These responses observed in murine cells were in contrast to efficient Ca²⁺ mobilization and degranulation in the human LAD-2 MC line, stimulated with the same TLR2 agonist.⁵⁰ TLR2^{-/-} mice displayed increased bacterial burden upon infection with *M. tuberculosis* compared with wild-type mice, whereas reconstruction of the mice with TLR2^{+/+} MCs reduced the bacterial burden, indicating direct involvement of MC TLR2 in the control of lung infection.⁵¹ Adoptive transfer of TLR2^{+/+} MCs to TLR2^{-/-} mice resulted in regulation of the pulmonary levels of IL-1β, IL-6, TNFα, an enhanced Th1 response and activated CD8(+) T cell homing to the lungs.⁵¹

MAST CELLS IN CHRONIC LUNG DISEASE

Asthma

Asthma is an airway disease that is characterized by airway obstruction due to bronchoconstriction, mucus hypersecretion, airway inflammation and edema and remodeling of various airway elements. Chronic MC activation and the relocation of these cells within specific airway structures play a key role in asthma pathogenesis. Increased MCs activation contributes to both chronic inflammation and tissue remodeling (reviewed in ref. 52).

Mast Cells in the Pathophysiology of Asthma

There is compelling evidence, based largely on studies from humans, that MCs play a pivotal role to the pathogenesis of asthma. They secrete a host of pro-inflammatory mediators which are directly relevant to the pathophysiology of asthma (see Tables 1-3). For example, the autacoid mediators, histamine, PGD₂ and LTC₄, induce bronchoconstriction, mucus secretion and mucosal edema and thus contribute to the symptoms of asthma. MCs also synthesize and secrete a 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 bFGF. Moreover, the serine proteases tryptase and chymase, which are major secretory products of human MCs, can interact with various cell types and profoundly alter their behavior.

Mast Cells and Human Experimental Allergen-Induced Asthma

Approximately 90% of subjects with asthma under the age of 30 are atopic, with positive skin prick tests to common aeroallergens such as the house dust mite (reviewed in see ref. 53). The role allergen exposure plays in day-to-day chronic asthma is probably over-stated, but the ability of pollen to exacerbate asthma,^{54,55} and the ability of anti-IgE therapy to attenuate the disease in some patients indicates that allergy remains a relevant factor in asthma pathophysiology. Bearing this in mind, the technique of acute bronchial challenge with a relatively large dose of allergen in the laboratory has provided a useful model for studying asthma pathophysiology. Following bronchial allergen challenge, most atopic asthmatics and many atopic nonasthmatics experience a rapid fall in pulmonary function (e.g., forced expiratory volume in one second [FEV₁]) which peaks at 10-20 minutes and then gradually recovers over the following 2 hours. This is defined as the early asthmatic response (EAR). In about 50% of subjects, after 4-6 hours there is a further fall in FEV₁, termed the late asthmatic response (LAR). This may last up to 12 hours and in some individuals may be followed by recurring airway obstruction for several days or even weeks (reviewed in see ref. 53).

The EAR: Several vasoactive and spasmogenic mediators are released during the EAR, most of which originate from MCs resident in the airway mucosa. The relative rate of mediator release from HLMCS *in vitro* is histamine>PGD₂>LTC₄ with one-half maximal release occurring at 2, 5 and 10 minutes respectively.⁵⁶ This is reflected *in vivo* by the recovery of these mediators in BAL fluid within 5-10 minutes following local bronchial allergen challenge.⁵⁷⁻⁶³ It has been calculated that the concentrations of mediators generated are similar to those required to produce bronchoconstriction *in vitro*⁶² or when delivered by nebuliser.⁶⁰ Evidence for their role is cemented by studies using potent and selective receptor antagonists, which demonstrate that the EAR is significantly attenuated by antagonists of histamine, LTC₄ and to a lesser extent PGD₂.⁶⁴⁻⁶⁸

The MC as a source of these mediators is supported by four lines of evidence. Firstly, the kinetics of IgE-dependent mediator release *in vivo* parallels that of purified MCs *in vitro*. Secondly, the presence of MC activation during the EAR is confirmed by the rapid increase in concentrations of the preformed MC-specific protease, tryptase, recovered by BAL within minutes following local bronchial allergen challenge.^{58,63} Thirdly, β -agonists such as salbutamol, known inhibitors of MC degranulation when applied acutely *in vitro*, completely abolish the early reaction and the associated increase in plasma histamine levels.⁶⁹⁻⁷¹ Lastly, the EAR is markedly attenuated after pretreatment with anti-IgE,^{72,73} confirming that IgE-dependent signaling is critical.

The LAR: In contrast to the acute mediator-induced bronchoconstriction and mucosal edema characteristic of the EAR, the LAR is associated with the recruitment and activation of many cell types including eosinophils, activated CD4⁺ T cells and activated macrophages.⁷⁴⁻⁷⁹ Subsequent mediator release and tissue damage following these events is thought to account for the ensuing airway obstruction and associated increase in bronchial hyperresponsiveness (BHR) which accompanies the LAR. The role of the MC as a source of bronchospastic mediators during the LAR is more difficult to define than

during the EAR due to the recruitment and activation of many cell types, but the LAR is also attenuated markedly by anti-IgE therapy,⁷² indicating that MC activation during the EAR initiates events leading to the LAR. It is therefore likely that the secretion of MC mediators and cytokines orchestrate the development of the LAR.

Mast Cells are Chronically Activated in Asthmatic Airways

Of particular relevance to chronic everyday asthma, MCs in asthmatic airways exhibit features of chronic on-going activation (summarized in Table 4). There are increased numbers of MCs in BAL fluid from stable asthmatics compared to normal controls,^{57,80,81} together with increased concentrations of histamine and tryptase, providing evidence of on-going MC degranulation.^{57,58,82} MCs from symptomatic asthmatic subjects exhibit greater constitutive histamine release in BAL and the secretory response of asthmatic BAL MCs to IgE-dependent activation is altered, with reports of increased IgE-dependent histamine release from atopic asthmatic BAL MCs compared to non-atopic normal controls.^{81,82} Strong correlations have been observed between the severity of BHR and MC numbers, histamine concentrations and spontaneous histamine release in BAL fluid.^{57,81,83,84} There is morphological evidence of continuous degranulation as assessed by microscopy in the airway epithelium, submucosa and smooth muscle in asthma.⁸⁵⁻⁸⁷ Finally, there is increased protein expression and transcription for the cytokines IL-4 and IL-5 in asthmatic compared to normal bronchial mucosal MCs, again suggesting on-going activation.⁸⁸⁻⁹⁰ While most of the studies referred to above have been performed in atopic individuals, similar evidence supports a role for on-going MC activation in both non-atopic and occupational asthma.⁹⁰⁻⁹⁵

Mast Cell Relocation to Airway Structures is a Key Event in Asthma Pathogenesis

It has been a long held view that the disordered airway physiology and airway wall remodeling characteristic of asthma are consequences of eosinophilic airway inflammation. However, this view is no longer sustainable. Anti-IL-5 therapy depletes airway eosinophils by over 50% and while it reduces the rate of severe asthma exacerbations,¹¹⁷ it has no effect on day-to-day symptoms, BHR or lung function. There are also many examples where the relationship between eosinophilic inflammation and disordered airway physiology is weak. This is most evident in patients with eosinophilic bronchitis (EB), a condition which accounts for about 15% of cases of cough referred to respiratory specialists. It is characterized by a corticosteroid responsive cough and the presence of a sputum eosinophilia occurring in the absence of variable airflow obstruction or BHR.¹¹⁸ Interestingly the immunopathology of asthma and EB in the airway mucosa are virtually identical with respect to T-cell infiltration, eosinophil infiltration and activation, mucosal MC numbers, IL-4 and IL-5 cytokine expression, epithelial integrity, sub-basement membrane collagen deposition and concentrations of histamine and PGD₂.¹¹⁹⁻¹²¹ This suggests that many immunopathological features previously believed to contribute to asthma may not, in fact, be important in the development of airflow obstruction and BHR.¹¹⁹

MC numbers are not increased in the bronchial lamina propria of asthmatics compared to healthy subjects (Table 4). However, they infiltrate 3 key sites: the ASM⁹⁸ (Fig. 2), the airway mucous glands,⁹⁶ and the bronchial epithelium.⁸⁸ For the ASM and glands this is relatively MC-specific and places activated MCs within these dysfunctional airway elements. This is likely to have profound effects on airway physiology (see below).

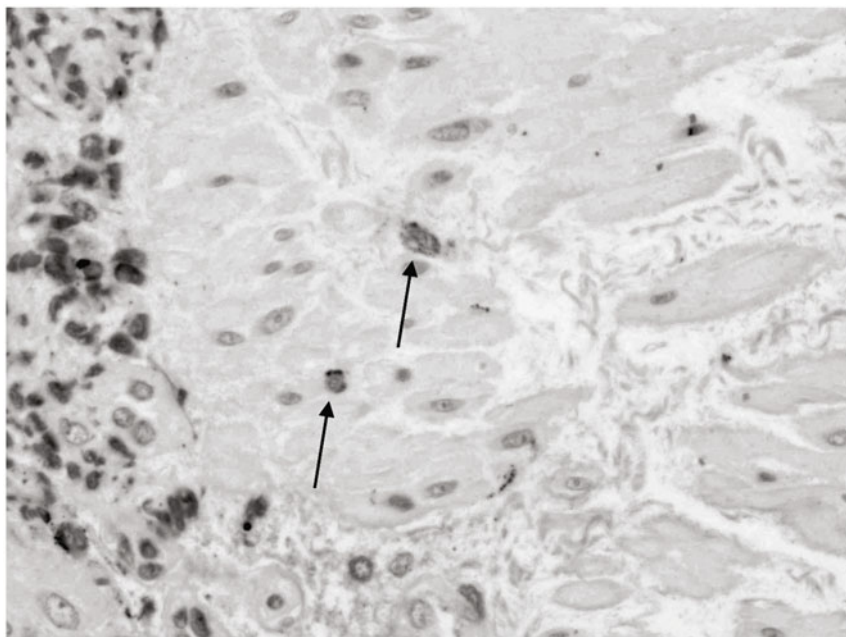


Figure 2. Mast cell infiltration of the airway smooth muscle in a patient with steroid-naïve asthma. Mast cells (arrowed) were immunostained for tryptase. Magnification x400.

Mast Cell Microlocalization within Asthmatic Airway Smooth Muscle

The striking difference between the pathology of asthma and EB lies in the ASM. Abnormal ASM function is critical in the pathophysiology of asthma and yet this structure had been largely neglected in previous immunopathological studies. In asthmatic subjects, there are numerous MCs within the ASM bundles, but virtually none in ASM from patients with EB or healthy subjects (Fig. 2).⁹⁸ This is evident across disease phenotypes such as eosinophilic and non-eosinophilic subjects and across the spectrum of asthma severity.^{105,122} These ASM cells are activated and there is a correlation between the number of MCs within the ASM and the severity of BHR,^{98,105,123} suggesting that this is functionally relevant. The majority of MCs within the ASM are of the MC_{TC} phenotype containing both tryptase and chymase and express IL-4 and IL-13 but not IL-5.^{98,101} In contrast we found that there were almost no T cells or eosinophils in the ASM in any of the study groups. A further interesting finding is that the number of degranulated MCs within the ASM is increased in fatal compared to nonfatal asthma,⁹⁶ implicating MCs in the pathogenesis of asthma death. Interestingly, both MC numbers in the ASM and disease duration (e.g., prolonged MC presence within ASM) seem to contribute to BHR in patients.¹⁰⁵ Taken together, these observations indicate that ASM infiltration by MCs is a critical determinant of the asthmatic phenotype and have thus created a major paradigm shift in our understanding of asthma pathophysiology. However, this also has further implications. For example, not only might it clarify why many atopic patients do not have asthma, it would also explain why the presence of asthma is such a strong risk factor for death from anaphylaxis and allergen desensitization.¹²⁴

Putative Mast Cell-Airway Smooth Muscle Interactions

MCs produce many mediators that adversely affect ASM function (Tables 1-3). In many instances, cellular communication within the airways is likely to work across a distance of one or two microns rather than the thousands of microns involved in the depth of the lamina propria. The microlocalization of MCs within the ASM would therefore undoubtedly facilitate specific interactions between MCs and ASM in terms of localized mediator release and direct cell-cell contact. It is therefore entirely plausible that the presence of MCs within the ASM in asthma contributes to the development of ASM hypertrophy and hyperplasia, BHR and variable airflow obstruction.

The ASM secretes many chemokines and growth factors which exhibit MC chemotactic activity, including CCL11, CXCL8 and CXCL12.¹¹ However, the dominant ASM-dependent chemotactic pathway for HLMCs involves the CXCR3-CXCL10 axis. HLMCs express CXCR3 and cultured ASM from asthmatic subjects preferentially secretes the CXCR3 ligand CXCL10.⁵ The relevance of this to MC recruitment by the asthmatic ASM *in vivo* is demonstrated by the increased expression of CXCL10 by the ASM in bronchial biopsies from asthmatic compared to normal subjects and by the enrichment of CXCR3+ MCs within the ASM bundles compared to the surrounding airway mucosa.⁵

The classical MC autacoid mediators histamine, PGD₂ and LTC₄ are all potent bronchoconstrictors. In mice, instillation of Th2 cell conditioned medium to the airways of naïve animals induced BHR within 6 hours.¹²⁵ This required expression of the IL-4 receptor alpha subunit and STAT6, suggesting a critical role for IL-4 and/or IL-13. We could not find any T cells within the ASM in asthma,⁹⁸ but ASM MCs do express both IL-4 and IL-13.¹⁰¹ Exogenously administered tryptase induces bronchoconstriction and BHR in dogs and sheep.^{126,127} *In vitro*, tryptase potentiates the contractile response of sensitized bronchi to histamine and induces proliferation of human ASM, but coculture of either resting or IgE/anti-IgE activated HLMCs with human ASM cells did not induce ASM proliferation.¹²⁸⁻¹³⁰ However, tryptase derived from HLMCs in coculture with human ASM induces the release of TGFβ from ASM cells.¹³¹ This in turn increases the expression of α-smooth muscle actin in ASM cells, leading to increased histamine-dependent ASM contractility in collagen gels. These studies thus highlight several mechanisms through which MCs could exacerbate BHR and promote bronchoconstriction.

Contribution of MCs to local production of renin/angiotensin (Ang) in lungs has been recognized recently. Not only can MCs produce all components of a nontraditional renin-Ang system, including angiotensinogen, renin (which cleaves angiotensinogen into Ang I), chymase (which further converts Ang I into Ang II) and Ang II, but according to the data published by Veerappan et al, MCs are essential for local production of Ang II, which is a critical factor governing bronchoconstriction.¹³² In isolated bronchial rings in animal models, local generation of Ang II by MC renin elicits ASM contraction mediated by Ang II Type 1 receptors.¹³² Whether the components of this system are altered in asthmatic MCs and particularly those located within ASM remains to be determined.

The majority of MCs (>80%) in the ASM compartment belong to MC_{TC} subtype.⁹⁸ Chymase degrades human ASM ECM and inhibits T-cell adhesion to ASM, which might explain the paucity of T cells within this structure in asthma.¹³³ Also of interest, a recent mouse model has suggested that mouse MC protease 4 (a homologue of human chymase) may actually offer protection against experimental antigen-induced airway inflammation.¹³⁴ In this model, MCs were found in close proximity to ASM and absence

of MCP-4 resulted in ASM thickening in response to the OVA sensitization and challenge, clearly indicating a positive role MCP-4 in ASM remodeling.

The communication between ASM cells and HLMCs is clearly bi-directional. In coculture HLMCs adhere to human ASM cells in part via cell adhesion molecule 1 (CADM1).¹³⁵ HLMCs in coculture with ASM not only survive in the absence of exogenous survival factors, but proliferate rapidly.¹³⁶ This is mediated through a co-operative interaction between CADM1, membrane-bound SCF expressed on ASM cells and soluble IL-6. Interestingly there is evidence for physical interaction in MCs between CADM1 and KIT, suggesting that CADM1 plays an important role in KIT function.¹³⁶ Our hypothesis is that the initial adhesion mediated through CADM1 facilitates the interaction of MC KIT with ASM membrane-bound SCF. Another feature of this interaction is that HLMCs exhibit increased constitutive histamine release but can still respond to IgE/anti-IgE activation.¹³⁶ This indicates the presence of an ASM-dependent mechanism for the activation of HLMCs and could explain the presence of chronically activated MCs within the ASM bundles in asthma.

In summary, the infiltration of the ASM by MCs in human asthma is a unique feature of asthma and likely to have a central role in the pathophysiology of ASM dysfunction. There is bi-directional cross-talk between these two cell types, with evidence to suggest the presence of a positive feedback loop between MC activation and disordered ASM physiology, leading to amplification of the airway inflammatory response and BHR (Fig. 3).

Mast Cell Microlocalization within Airway Epithelium and Submucosal Glands

MCs infiltrate the bronchial epithelium in asthma.^{87,88,137} This is likely to be of importance in disease pathogenesis for two reasons. Firstly, this places MCs at the portal of entry of noxious stimuli such as aeroallergens, which would facilitate an effector role in the on-going immunological response (antigen presentation, Th2 cell differentiation, IgE synthesis). Furthermore, since tryptase degrades respiratory allergens and MC IgE on FcεRI is cross-linked by allergen which may be followed by tryptase release, there may be a MC-dependent negative feedback mechanism controlling allergic inflammation.^{138,139} Secondly, there are likely to be important consequences of MC activation on epithelial function. For example, MCs adhere avidly to bronchial epithelial cells and tryptase stimulates airway epithelial IL-8 release and ICAM-1 expression.^{140,141} However, coculture of MCs with the BEAS-2B bronchial epithelial cell line actually inhibits IgE-dependent HLMC degranulation.¹⁴² Whether this extends to chemokine and cytokine release is not known and awaits further work. One explanation is that normal airway epithelium keeps MCs “in check”, but once the epithelium is injured, this inhibitory activity is lost and MCs are then “free” to respond to the tissue insult.

Severe mucus plugging is a well known feature of severe fatal asthma, but is also recognized as a feature of milder disease,¹⁴³ and occurs as a result of mucus hypersecretion by hyperplastic submucosal glands and epithelial goblet cells. Like the ASM, mucous glands have been largely ignored with respect to their infiltration by inflammatory cells. We had noted previously that IL-6⁺ MCs appeared abundant amongst airway mucosal glands.⁸⁸ However, the work of Carroll and coworkers has provided strong evidence that MCs make a significant contribution to glandular dysfunction in asthma. They performed a detailed analysis of cartilaginous airways in post mortem 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.¹⁰⁶ The number of MCs

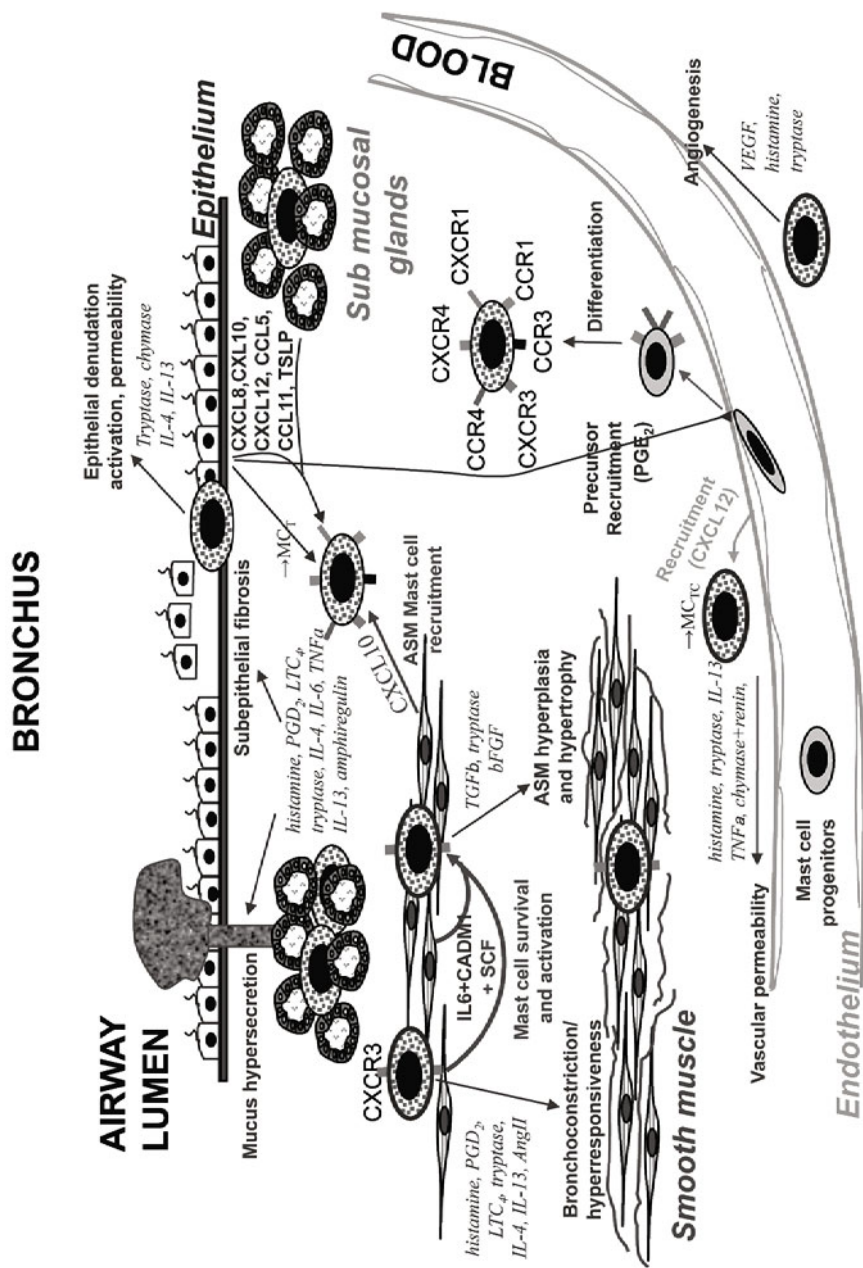


Figure 3. Summary of important interactions between mast cells and structural airway cells in asthma.

identified by tryptase immunostaining was increased significantly within the mucosal gland stroma in nonfatal asthma and a marked increase in the number of degranulated MCs was present in both fatal asthma and nonfatal asthma compared to normal controls. Importantly, the density of both intact and degranulated MCs within the mucous glands correlated strongly with the degree of mucus obstruction in the airways, again implicating MCs in the pathogenesis of asthma death. Numerous MC mediators affect glandular growth and secretion including histamine, PGD₂, LTC₄, IL-6, TNF α and chymase.⁵³

Amphiregulin, a member of the epidermal growth factor family, is a further MC-derived molecule of particular interest with respect to the airway epithelium and mucosal glands. Amphiregulin expression is upregulated in human MCs following activation via IgE/anti-IgE,^{103,144} and this is not inhibited by dexamethasone. MCs resident in the asthmatic bronchial mucosa demonstrate increased amphiregulin expression; and in vitro, MC-derived amphiregulin increases mucin gene expression in the NCI-H292 epithelial cell line.¹⁰³ Amphiregulin is present in increased concentrations in asthmatic sputum during asthma exacerbations; and it is proposed that this may aggravate airway wall remodeling.¹⁴⁵ These observations suggest that MC-derived amphiregulin may play an important role in goblet cell hyperplasia and mucus hypersecretion in asthma and that this is resistant to the effects of corticosteroids. In addition, recombinant amphiregulin induces the proliferation of human airway fibroblasts but not ASM cells, suggesting a further mechanism whereby MCs can contribute to subepithelial fibrosis. Figure 3 summarizes some potential interactions between MCs and structural airway cells in asthma.

Mechanisms of On-Going Mast Cell Activation in Asthma

The mechanisms of chronic MC activation in asthma are not fully understood. 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 the development of asthma, once established, allergen avoidance usually has a minor effect on the state of established disease which appears to become “self-perpetuating”. This is most evident in cases of occupational asthma, where asthma and accompanying MC activation will persist on removal of the sensitizing agent if exposure is not prevented early in the course of the disease. MCs can be activated by a number of diverse stimuli including monomeric IgE alone, proteases, cytokines (e.g., SCF, TNF α , IFN γ), complement, adenosine, TLR ligands, neuropeptides, hyperosmolality and cell contact (Fig. 1). It is likely that many of these pathways interact in the complex inflammatory milieu of the asthmatic airway.

Monomeric IgE induces rodent MC mediator release and prolongs MC survival through the autocrine release of cytokines.¹⁴⁶⁻¹⁴⁹ In HLMCs IgE alone induces the release of histamine, LTC₄ and IL-8 and prolongs MC survival after the withdrawal of SCF and IL-6 through autocrine secretion of IL-6.^{150,151} Importantly, while free IgE is present, intracellular signaling continues, suggesting that these findings are physiologically relevant. This is interesting because in humans there is a reproducible correlation between total serum IgE concentration, BHR and asthma.¹⁵²⁻¹⁵⁵ Thus, heightened MC activation may arise in part from the increased binding of IgE to Fc ϵ RI. This is supported to some extent by the observation that anti-IgE therapy markedly reduces both airway inflammation and MC activation as manifest by reduced IL-4 expression.¹⁵⁶ However, anti-IgE therapy has a minimal effect on BHR, suggesting that monomeric IgE-dependent activation of MCs in asthmatic ASM does not account for this aspect of airway dysfunction.

SCF primes MCs for mediator release and at higher concentrations, directly induces degranulation.¹⁵⁷ Concentrations of SCF are elevated in asthmatic airways and could therefore make an important contribution to ongoing MC activation.^{158,159} Interestingly, SCF and IgE have an important effect on the efficacy of β_2 -adrenoceptor agonists, used as reliever medication for asthma, with respect to HLMC function. When applied to HLMCs acutely *in vitro* in the absence of SCF, they inhibit IgE/anti-IgE-dependent degranulation.⁶⁹ However, after sustained exposure to the drug, rapid desensitization occurs with loss of efficacy.¹⁶⁰ Similarly, *in vivo*, acute administration of salbutamol and the long-acting β_2 -adrenoceptor agonist salmeterol to asthmatic subjects inhibit the airway response to allergen challenge. However, if these drugs are administered regularly for 1 week, not only is the protection against the EAR lost, the magnitude of the EAR and the accompanying MC mediator release are enhanced.^{161,162} Furthermore, regular administration of short-acting β_2 -adrenoceptor agonists to asthmatic subjects has been associated with loss of asthma control.^{163,164} When HLMCs are incubated with SCF, their ability to respond to β_2 -adrenoceptor agonists is lost within 2 minutes.¹⁶⁵ This is caused by the rapid uncoupling of the β_2 -adrenoceptor in HLMCs due to phosphorylation of Tyr350 on this receptor and subsequent internalization. Furthermore, in the presence of both SCF and IgE, salbutamol produced a dose-dependent increase in histamine release. This mechanism might explain the above clinical findings. If this applies *in vivo*, then targeting SCF may enhance the efficacy of β_2 -adrenoceptor agonists and improve asthma control. Further evidence that this might occur *in vivo* comes from a study of asthma death, which demonstrates that the extent of MC degranulation correlates with blood salbutamol concentrations.¹⁶⁶

The C5a receptor CD88 is expressed on the MC_{TC} subset of HLMCs.¹⁰ Elevated C5a concentrations have been identified in the induced sputum of asthmatic subjects,¹⁶⁷ thus providing a further potential means of MC activation and of particular relevance to those MCs (MC_{TC}) within the ASM bundles.

As described above, HLMCs express the TLR-1-5, -7 and -9,⁴⁷ stimulation of which results in MC activation. Perhaps of most relevance to asthma is MC activation via TLR3 with double-stranded viral RNA ligand. Poly I:C, a synthetic activator of TLR3, induces the specific release of IFN α as does exposure to RSV and influenza virus.³⁵ Since viruses are a common cause for asthma exacerbations, the MC antiviral response may be an important contributor to the deteriorating airway physiology. Nakayama and co-authors investigated the role of TLR4 on MCs in allergic airway inflammation using MC-deficient Kit^{W/W^v} mice and TLR4^{-/-} mice in an asthma model.¹⁶⁸ Ovalbumin-induced eosinophilic inflammation in the lung was dramatically increased by the administration of LPS in wild-type mice, whereas the same increase was not observed in MC-deficient or TLR4-deficient mice. Adoptive transfer of bone marrow-derived MCs from wild-type, but not from TLR4-deficient mice restored the increased eosinophilic infiltration in the airway in MC-deficient mice.¹⁶⁸ LPS inhalation increased IL-5 production by lung MCs and exacerbated airway inflammation in mouse asthma model.¹⁶⁹ *In vitro* studies revealed that LPS treatment augmented levels of IL-5 and -13, as well as mRNA expression of IL-4, IL-5 and IL-13 and CCL24 (eotaxin-2) in wild-type bone marrow MCs, whereas no increase in IL-5 and eotaxin-2 was detected the case of TLR4^{-/-} MCs.¹⁶⁸ Interestingly, production of IL-6 and TNF- α was not significantly changed by LPS in wild-type MCs. Moreover, cotreatment of bone marrow MCs with LPS with IgE/antigen showed synergistic effects on the expression of IL-5 and -13 and CCL24. The TLR4 mediated signaling involved immediate NF-kappaB activation, followed

by increase in the levels of GATA1 transcription factor responsible for activation of the IL-4, IL-5 and IL-13 promoters. Furthermore, examination of the gene expression profile from human peripheral blood MCs using high density oligonucleotide probe arrays following activation with LPS via TLR4 compared to anti-IgE demonstrates that both induce a core response, plus an LPS or anti-IgE specific program of gene expression.¹⁷⁰ LPS-stimulated MCs specifically induced a subset of genes that included a Th2 cytokine and chemokines that recruit Th2 cells and eosinophils.¹⁷⁰ These data indicated that TLR4 in MCs may be involved in LPS-mediated enhancement of allergic airway inflammation.

MC TLRs also have the potential to improve asthma. Pretreating mice with the TLR9 ligand, immunostimulatory sequences (ISS) of DNA containing a CpG motif, significantly inhibited the accumulation of peribronchial MCs and the expression of IL-4 and IL-9 in lung, as well as reduced the increased airway hyperreactivity to methacholine; all caused by chronic antigen challenge.⁴⁸ In vitro experiments on MCs derived from the bone marrow of these mice showed that ISS did not affect histamine release, but induced IL-6 production.⁴⁸ These data suggest that stimulation of TLR-9 may have beneficial effect in asthma model.

Thymic stromal lymphopoietin is a cytokine released from airway epithelial cells in response to injury.^{171,172} Its overexpression in mice leads to asthmatic-type Th2-driven airway changes, while its inhibition attenuates the airway changes seen in mouse models of asthma.¹⁷³ One paradigm for the effects of TSLP is that it activates CD11c+ dendritic cells which in turn leads to the differentiation of CD4⁺ T cells into the Th2 phenotype through an interaction of OX40 with its ligand.^{174,175} However, it is well documented that T-cell-independent pathways also contribute to TSLP-dependent inflammation. In particular, the direct interaction of TSLP with MCs is highly relevant to the development of allergic-type airway inflammation. TSLP, derived from human epithelial cells activated with cytokines, peptidoglycan or trauma, induces the release of numerous cytokines and chemokines from human MCs, including IL-1.¹⁷² IL-13 production by MCs is inhibited when endogenous TSLP released by activated human epithelial cells is blocked.¹⁷² Furthermore, in a mouse model of rhinitis, MC activation was required for the increased TSLP production by airway epithelium.¹⁷⁶ MC-derived TSLP also has the potential to contribute to the development of airway inflammation.¹⁰⁴ TSLP is overexpressed in asthmatic airways,^{177,178} and so both MC-derived and epithelial-derived TSLP may be a key cytokine driving the MC in asthma.

Immunoglobulin (Ig) free light chains are present in serum in normal subjects and their production is augmented in inflammatory diseases such as rheumatoid disease. In mice Ig free light chains can confer MC-dependent hypersensitivity through an unknown mechanism,¹⁷⁹ and antigen-specific light chains can mediate MC-dependent bronchoconstriction following antigen challenge.¹⁸⁰ Concentrations of Ig free light chains are elevated in the sera of asthmatic compared to normal subjects suggesting they may be relevant to the pathophysiology of human asthma.¹⁸⁰

Genetic factors may also lower the threshold for MC activation in asthma and thus predispose to the development of the disease and/or its severity. For example, an important negative regulator of MC activation is the phosphatase SHIP.¹⁸¹ SHIP-deficient MCs exhibit markedly enhanced secretory responses and with respect to human basophils at least, cells that are "hyperreleasable" demonstrate a relative deficiency of this molecule.¹⁸²

Mast Cells as Initiators of Allergic Airway Inflammation

Conventionally, MC activation in asthma has been interpreted as an IgE-dependent event, but recent studies indicate that common allergens, many of which are serine proteases, can induce MC histamine and cytokine secretion directly through an IgE-independent mechanism.^{183,184} This suggests that MCs may not only contribute to the chronic airway inflammatory response and symptomatology, but may have a central role at the initiation of the allergic immune response, i.e., providing signals inducing B-cell IgE synthesis and Th2 lymphocyte differentiation.^{185,186}

Mast Cells in Animal Models of Asthma

Several models have been developed which aim to induce the airway features of asthma. The most widely reported is the mouse model using intraperitoneal antigen sensitization followed by antigen challenge of the airways. This most closely resembles the model of acute allergen challenge in the airways although the route of sensitization is obviously different. The dependency on MCs in these models with regard to the development of airway hyperresponsiveness and inflammatory changes in the airways is highly dependent on the model studied and the mode of antigen sensitization. Thus sensitization without adjuvant generates a MC-dependent model while sensitization with adjuvant creates a MC-independent model.¹⁸⁷

An alternative model uses airway sensitization without adjuvant from the outset and, to some extent, this is more physiological. In this setting, MCs are again an essential component required for the development of airway hyperresponsiveness, inflammation and remodeling (collagen deposition, goblet cell hyperplasia.¹⁸⁸⁻¹⁹⁰ Furthermore, mouse models allowed to estimate contribution of infections and TLR activation, as well as the nervous system^{42,168,169,191,192} to MC function in the development of asthma. However, there are inevitably a number of problems in relating these models to the human disease. For example, mouse airways contain very few MCs at baseline, so the changes seen following antigen challenge rely heavily on the recruitment of MC progenitors rather than the activity of resident cells. Therefore, it is perhaps not surprising that short term models using intraperitoneal sensitization do not reveal a role for MCs in the outcomes commonly measured. In addition, mice have relatively little smooth muscle in their airways and so there is no model described to date in mice which has recapitulated the infiltration of ASM by MCs; a feature that may be key to the development of asthma in humans. So while mouse models are useful for generating hypotheses regarding the pathogenesis of asthma, their findings may also be potentially misleading.

Mast Cells in Chronic Obstructive Pulmonary Disease (COPD)

COPD, one of the leading causes of death worldwide, is characterized by airflow limitation that is largely fixed and which is usually progressive. This is associated with an abnormal inflammatory response of the lungs to noxious particles or gases, e.g., tobacco smoke. Inflammation is followed by scarring and remodeling with airway thickened walls; the latter is followed by fibrosis and destruction of alveoli. In contrast to asthma where there is overwhelming evidence for MC involvement, their role in COPD is less obvious (Table 4). In COPD patients, the MC population in the lung undergoes changes in density and distribution related to a severity of the disease. In contrast to asthma, the total MC

density in lung, as well as MC_T in submucosa, progressively decreases compared with healthy lung, but MC activation and degranulation are increased as in asthma, coinciding with enhanced histamine release in advanced COPD.^{107,193} Similar to asthma, an increased MC_{TC} density has been reported in alveolar parenchyma and in ASM.^{107,109} In lungs from patients with very severe COPD, there is a several-fold increase in the MC_{TC}/MC ratio cells in small airways, small airway epithelium, pulmonary vessels and alveolar parenchyma, with MC_{TC} comprising ~95% of the total MCs in larger airways.¹⁰⁷ Of particular importance is the increased CD88 expression in both MC_{TC} and MC_T cells,¹⁰⁷ which coincides with increased expression of the CD88 ligand c5a in COPD patients¹⁶⁷ and which is likely to cause MC activation. The changes in MC_{TC} population also coincide with increased expression of TGF β and renin, but their functional importance in COPD has not been elucidated. Interestingly, increased MC_T and MC_{TC} numbers positively correlate with the lung function in COPD.¹⁰⁹ High levels of MC activation in COPD are also likely to be caused by elevated bronchial mucosal expression of TSLP and chemokines similar to that found in severe asthma.¹⁷⁷

Mast Cells in Interstitial Lung Disease

Interstitial lung diseases are characterized by the presence of pulmonary fibrosis occurring as a result of injury and aberrant repair. The most common form of pulmonary fibrosis encountered in the clinic is idiopathic pulmonary fibrosis (IPF) with a histological pattern of usual interstitial pneumonia (also known previously as cryptogenic fibrosing alveolitis) (reviewed in see ref. 194). While the initiating events and role of inflammation in the onset of IPF remain controversial, there is good evidence of ongoing damage to the alveolar epithelium, basement membrane and capillary endothelium, leading to the activation of repair mechanisms with infiltration of fibroblasts, MCs and other inflammatory cells. The chronicity of the insult leads to dysregulated repair with the generation of fibroblastic foci. These events are driven by several profibrotic mediators and cytokines including TGF β 1, PDGF and bFGF.¹⁹⁴ The key cell driving the development of fibrosis is the myofibroblast, which has an intermediate phenotype between fibroblasts and smooth muscle, in that it expresses α -smooth muscle actin (α SMA) and exhibits contractile activity, but is capable of the synthesis and deposition of extensive fibrotic matrix.¹⁹⁵ The myofibroblast is therefore a highly attractive target for the treatment of IPF.

In fibrotic lung disorders, there is an increased MC number in lung tissue compared with the control group, with MC counts significantly correlating with the degree of fibrosis (Table 4).¹¹⁰ In lungs of patients with idiopathic bronchiolitis obliterans organizing pneumonia, MC density and activation are also increased.¹¹² In addition increased MC density, with MC_{TC} correlated to accumulation of ASM cells and myofibroblasts, is found in idiopathic interstitial pneumonia.¹¹³ In both normal and fibrotic lung, the majority of MCs and fibroblasts in the alveolar septae are apposed.¹⁹⁶ A marked increase in MC density and MC hyperplasia has been described in the thickened fibrous alveolar septae, as well as in MCs also were found within the alveolar epithelial layer and alveolar lumina of patients with pulmonary fibrosis resulting from several diverse etiologies in contrast to control specimens, in which MCs were most frequently seen in subpleural and perivascular connective tissue.¹¹¹ These MCs often show reduced numbers of granules and disorganized granule content, suggesting that there is partial ongoing degranulation. In addition, about 10-fold-elevated histamine concentrations have been described in BAL from patients with IPF.¹⁹⁷ A specific feature of IPF is the abundant bFGF-expressing cells in lung

interstitium, which have been identified as MCs.¹¹⁴⁻¹¹⁶ These bFGF-containing MCs are surrounded by collagen, elastic fibers and smooth muscle cell/myofibroblast-like cells.¹¹⁶ Furthermore, the distribution of bFGF-containing MCs matches that of ECM deposition and correlates with the extent of fibrosis; morphometrically, providing circumstantial evidence that MCs contribute to the fibrotic process.

Alveolar hypoxia reportedly induces activation of MCs, leading to local generation of Ang II and consequently to systemic inflammation.¹⁹⁸ MC stabilization with cromolyn prevented the systemic response, as did ACE inhibitors and AngII receptor blockers, through interactions between leukocytes and endothelial cells. It is conceivable that similar mechanisms may activate MCs in pulmonary fibrosis and link MC activation with systemic inflammation in COPD. Fibroblasts/myofibroblasts interact intimately with MCs through cell-cell adhesion thereby inducing the release of various MC-derived mediators. For example, cultured rat embryonic skin fibroblasts phagocytose rat MC granules and this is followed by secretion of collagenase and β -hexosaminidase.¹⁹⁹ Histamine, TNF α , bFGF, TGF β and IL-4 promote fibroblast proliferation in humans.²⁰⁰⁻²⁰⁴ In coculture, human MCs adhere avidly to human fibroblasts through undefined receptors.²⁰⁵ MCs augment proliferation of human skin fibroblasts via MC-derived IL-4, independently of cell-cell contact.²⁰⁶ However, IL-4 could not be detected in coculture, suggesting that IL-4 was secreted by MCs in low amounts and strictly limited to cell-cell contacts with fibroblasts. We proposed that MCs may present IL-4 on their surface in order to confer local cytokine specificity.²⁰⁷ IL-4 is a chemoattractant for human fibroblasts and also induces human fibroblasts to secrete ECM proteins.^{208,209}

Among HLMC-derived mediators, bFGF is likely to have a major effect on lung fibroblasts. Heparin stabilizes bFGF structurally and preserves its bioactivity by protecting it from degradation.²¹⁰ Furthermore, heparin and/or heparan sulfate are required for binding of bFGF to its receptors²¹¹ and also release it from basement membranes where it is stored.²¹² Heparin may thus potentiate fibroblast activation and proliferation indirectly through the regulation of bFGF activity. In addition, MC tryptase induces proliferation of lung parenchymal and airway fibroblasts via activation of PAR-2.²¹³

In animal models, increased numbers of MCs and elevated lung histamine content have been documented in pulmonary fibrosis induced by bleomycin, radiation and asbestos.²¹⁴⁻²¹⁶ In the murine model of silica-induced pulmonary inflammation, MC hyperplasia is also seen, but MC-deficient mice develop significantly less severe lung lesions and have a reduced BAL neutrophilia and protein content.²¹⁷

MCs are also implicated in pulmonary capillary hemangiomatosis, causing pulmonary arterial hypertension with no effective medical therapy. Microarray analysis of lesions from patients with this rare disease has identified overexpression of MC-related genes for surfactant protein C and tryptase.²¹⁸ MCs, identified by CD117 immunostaining, confirmed an abundance of MCs with high PDGFR- β expression in this disease.

Lung Cancer

The role of MCs in cancer is discussed in more detail in Chapter 14. Lung cancer is the leading cause of death from malignancy worldwide with nonsmall cell lung cancer (NSCLC) accounting for the majority of these cases. There is extensive evidence indicating that the immune system plays a key role in the regulation of cancer development and progression,²¹⁹ and cells of the innate and adaptive immune responses have been implicated in both the progression and curtailment of tumor growth.²²⁰ The role of MCs in tumor

development and progression is controversial due to the nature of the mediators they generate.²²¹ Several lines of evidence have suggested that MCs promote tumor progression and metastasis,²²²⁻²²⁴ potentially through their ability to promote angiogenesis via the release of autacoid mediators and pro-angiogenic chemokines and growth factors.^{225,226} For example, the granular products of MCs, histamine and cannabinoids released during degranulation, have been demonstrated in coculture to enhance the migration of cervical cancer cells.²²⁷ Recently a peptide adrenomedullin implicated in cross-talk between MCs and cancer cells was localized in MCs within lung cancer.²²⁸

Increased MC numbers have been documented to correlate with poor outcome in patients with NSCLC, especially lung adenocarcinoma.²²²⁻²²⁴ and microvessel density,^{222-224,229-233} although exceptions have been noted.^{234,235} Nevertheless, other studies found positive correlations between MC intratumoral density and good prognosis,^{230,236,237} particular at early stages and specifically for the MC_{TC} type.^{233,234} MC counts also negatively correlate with the presence of metastatic lung cancer in lymphatic nodes.²³⁸ There are however reports of lack of correlation between lung cancer survival and MCs.^{232,235,239} Regardless of their findings, a problem with these studies is that none of them paid attention to the location of MC within NSCLC tumors. We therefore carefully examined the density of tryptase+ MC in the tumor stroma compared to the clusters of tumor islets in 175 patients with surgically resected NSCLC. These studies revealed that MC numbers were similar in the tumor stroma of patients with surgically resected NSCLC irrespective of survival status, but there was a marked survival advantage when MC were present in NSCLC tumor islets.²³⁷ Therefore, as in other locations and diseases, the microanatomical location of MC is likely to be key with respect to their biological activity within the tumor microenvironment.

CONCLUSION

There is overwhelming evidence, based largely on studies from humans, that MCs play a central role in asthma pathogenesis and are undoubtedly involved in many diverse pulmonary diseases. The goal of current research is to understand the mechanisms driving their chronic activation and cellular cross-talk, with a view to developing novel therapies which are effective when administered to patients long-term in vivo.

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