

STATE OF THE ART REVIEWS ON MECHANISMS OF ALLERGIC DISEASE

Mast cells in asthma – state of the art

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Summary

Mast cells (MCs) play a central role in tissue homeostasis, sensing the local environment through numerous innate cell surface receptors. This enables them to respond rapidly to perceived tissue insults with a view to initiating a co-ordinated programme of inflammation and repair. However, when the tissue insult is chronic, the ongoing release of multiple pro-inflammatory mediators, proteases, cytokines and chemokines leads to tissue damage and remodelling. In asthma, there is strong evidence of ongoing MC activation, and their mediators and cell–cell signals are capable of regulating many facets of asthma pathophysiology. This article reviews the evidence behind this.

Introduction

Mast cells (MCs) were first described in the thesis of Paul Ehrlich in 1878 [1]. They are present in all classes of vertebrates including, amphibians, reptiles, birds and mammals, and it is estimated that the storage of histamine in vertebrate MCs and its use as an inflammatory mediator was established in primitive reptiles (Lepidosauria) ~276 million years ago [2]. However, as MCs are also present in the sea squirt *Ciona intestinalis*, they are thought to have emerged > 500 million years ago, before the appearance of the chordates and long before the development of adaptive immunity. This is consistent with a role mediating functions essential for life rather than representing a vestigial remnant of the immune system, a view supported by the fact that humans with MC deficiency have never been described.

In health, MCs are present in all human tissues, both within connective tissue and at mucosal surfaces. They form a heterogeneous population of cells with differences apparent in their development, mediator content, ultrastructure and functionally in their ability to interact with their local environment. They store and secrete a vast arsenal of biological substances including the classical autacoid mediators (histamine, prostaglandin [PG]D₂, leukotriene [LT]C₄), proteases and cytokines.

Accumulating evidence suggests that their primary role is to act as sensors of the surrounding environment, with the ability to respond rapidly to a perceived tissue insult and initiate a co-ordinated programme of inflammation and repair. Examples of potentially beneficial roles of MCs include roles in the response to infection [3], and protection against kidney injury [4], the effects of envenomation [5] and in some circumstances, cancer progression [6]. However, when a tissue insult is repeated or continuous, the effects of MC mediators are potentially deleterious for the host, and it is therefore not surprising that MCs have been implicated in the pathophysiology of many diverse diseases including asthma and allergy, rheumatoid disease, atherosclerosis and fibrotic disorders such as scleroderma and idiopathic pulmonary fibrosis [7]. It is the ability of MCs to release a host of proinflammatory mediators in response to cross-linkage of surface bound IgE by allergens that first focused attention on their role in asthma and atopic disease. The aim of this article is to review several decades of research suggesting that MCs are likely to play a central role in asthma pathophysiology, through mechanisms that extend well beyond their interaction with IgE. We will begin with an overview of MC biology, highlighting areas of particular relevance to asthma; this will also demonstrate the numerous poten-

tial check points that might be effective for the development of anti-MC drugs. We will then discuss the evidence pointing directly at a role for MCs in asthma pathophysiology.

Mast cell biology

Mast cell progenitors and tissue homing

MCs are derived from CD34⁺ and CD117⁺ pluripotent haematopoietic stem cells which arise in the bone marrow and develop through a granulocyte/monocyte lineage (reviewed in [8]). Unlike many other haematopoietic cells which are released into the systemic circulation as recognizably mature cells, MC progenitors enter the systemic circulation as undifferentiated mononuclear cells through mechanisms which are, as yet, poorly defined. These progenitors subsequently migrate into tissue where they differentiate (Fig. 1).

There is a lack of information regarding the homing of MC progenitors to tissue in humans, and while there is some insight from animal studies, the inter-species heterogeneity in MC populations renders the significance of animal data to humans uncertain. For instance, while some studies using human cord blood-derived MCs report expression of the chemokine receptor CCR3 [9], other studies using murine cord blood-derived MCs found that CCL11 did not induce chemotaxis of MC progenitors [10]. Similarly, although human MCs expressing CCR3 have been found in asthmatic airways, the numbers are small [11], and CCR3^{-/-} mice display increased airway hyperresponsiveness (AHR) and increased MCs within the trachea [12]. Many MC chemoattractants have been identified, as summarized in Table 1, and suggest that MC migration occurs mainly via a regulated process of recruitment. It is likely that disease-related increases in MC numbers in tissue are at least in part due to the induced recruitment of progenitors although mature human skin, gut and lung MCs are capable of proliferating and migrating [13–15].

In mice, MC homing to the gut requires the expression of the $\alpha_4\beta_7$ integrin and the CXCR2 chemokine receptor on circulating MC progenitors, as well as expression of the adhesion molecules mucosal vascular addressin cell adhesion molecule (MAdCAM)-1 and vascular cell adhesion molecule (VCAM)-1 on the intestinal endothelium (reviewed in [16]). Unlike human lungs, mouse lungs do not contain many MCs at baseline, but numbers increase markedly with induction of experimental IgE-dependent inflammation. This increase in MC numbers is attributed to recruitment. Mice lacking VCAM-1 exhibit attenuated recruitment of MCs to the inflamed lung [17], and inflammatory recruitment of committed MC progenitors to the mouse lung requires the expression of MC progenitor $\alpha_4\beta_7$ and $\alpha_4\beta_1$ integrins. There is also involve-

ment of the CCR2/CCL2 pathway in these murine models [10]. It is possible that resident MCs contribute to progenitor recruitment as LTB₄, which is released from mouse MCs activated with anti-IgE, is a chemoattractant for mouse and human MC progenitors [18]. Similarly, PGE₂ is also chemotactic for mouse MC progenitors and is active in the conditioned medium from processed nasal mucosa that has been challenged with ovalbumin (OVA) in sensitized animals [19].

Whether this inflammation-dependent recruitment of MC progenitors in mouse airways reflects the constitutive recruitment present in healthy human airways is undetermined. For instance, the MC line HMC-1, human bone marrow-derived MCs and human lung MCs do not express the chemokine receptor CCR2 [20], although it is of course possible that early progenitors might. Furthermore, PGE₂ inhibits human lung MC migration through the E-prostanoid (EP)-2 receptor, but is chemotactic when this receptor is blocked [21]. As the EP2 receptor is relatively dominant in less mature human cord blood-derived MCs, it seems unlikely that PGE₂ is important for human MC progenitor recruitment to the airways [22].

Mast cell growth and survival

Growth factors. The growth and maturation of recruited MC progenitors within tissue occurs in response to the local cytokine milieu and interactions with resident cells such as fibroblasts and the tissue matrix. Experiments from the subcutaneous transplantation of human lung into congenitally athymic nude mice indicate that tissue MCs may survive for several months [23], and in keeping with this, primary human MCs isolated from skin [13] and lung [15] can be maintained in cell culture for several months in the presence of stem cell factor (SCF) and IL-6. Again, important differences exist between rodent and human MCs; for example, IL-3 is required for growth of murine MCs [24], whereas this is not obligate for human mast cells, although it enhances progenitor proliferation *in vitro* if included in the first week when culturing cord blood progenitors [25]. However, among mature human mast cells, there is significant heterogeneity with respect to IL-3. Human gut mast cells proliferate and exhibit enhanced Fc ϵ RI-dependent mediator release in the presence of IL-3 [26], but human lung MCs do not exhibit IL-3 receptor expression [27].

SCF is an obligate MC growth factor in humans and is produced predominantly by epithelial and mesenchymal cells in both soluble and membrane-bound forms [28–31]. CD117 (also known as Kit) is the receptor tyrosine kinase (RTK) for SCF and is encoded by the *c-kit* proto-oncogene [32]. Activation of Kit on MCs regulates expression levels of *c-kit* [33], induces immature cell

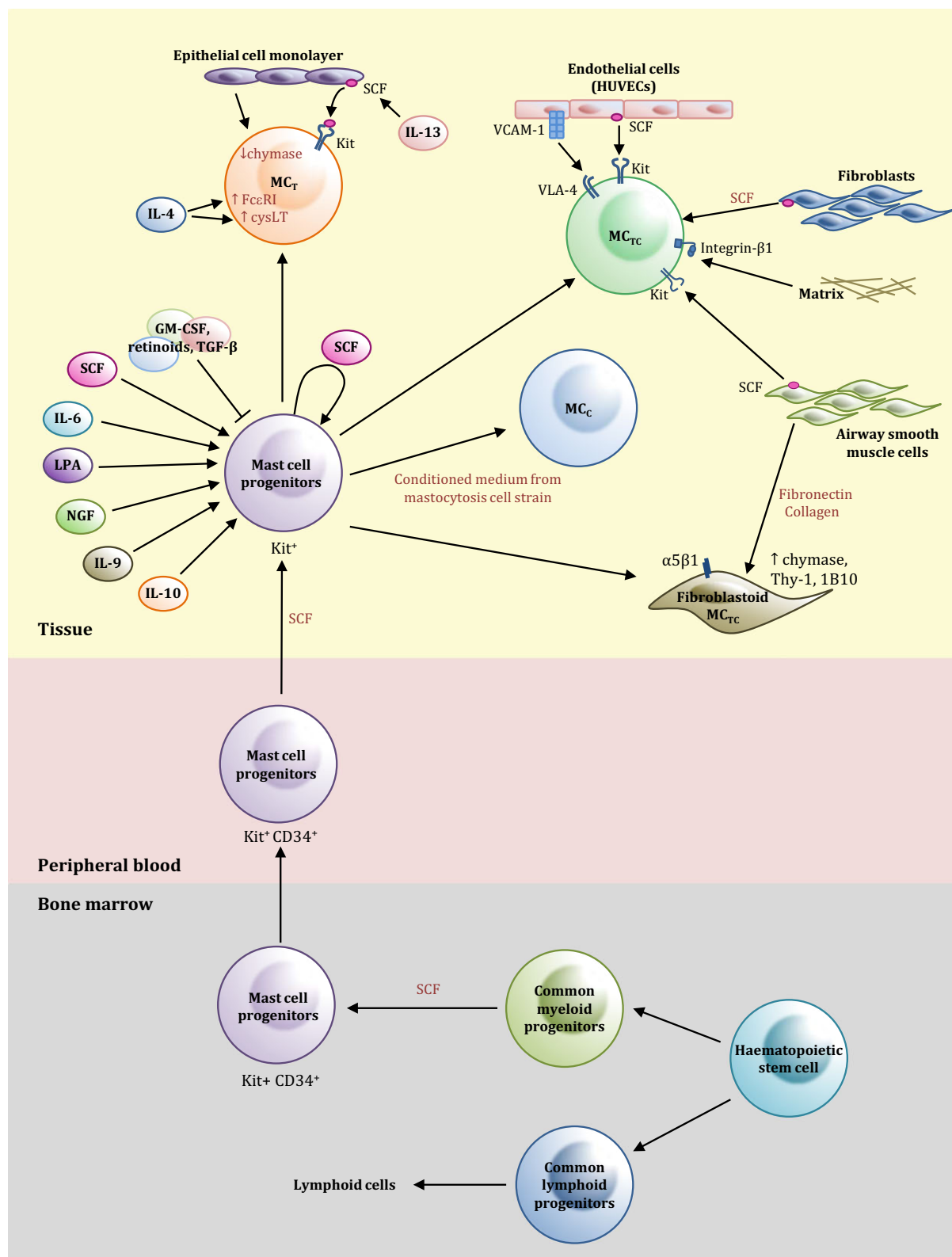


Fig. 1. Recruitment of mast cell progenitors and differentiation of mast cell phenotypes. Mast cell progenitors originate from haematopoietic stem cells in the bone marrow via the myeloid lineage. Progenitors then circulate in the blood stream and eventually migrate into tissues, whereupon interaction with structural cells and their cytokine environment determines their differentiation and maturation into distinct phenotypes. A change in the mast cell's environment in pathological conditions also contributes to mast cell heterogeneity. Key: ●: Membrane-bound SCF; ○: Soluble SCF.

Table 1. Human mast cell chemoattractants

Chemoattractant	Receptor	Study findings	References
Chemokines			
CCL5	CCR1, CCR3, CCR4	CCL5 is chemotactic for human cord blood-derived MCs and human lung MCs	[663, 664]
CCL11	CCR3	Induces chemotaxis of 4 week old human cord blood MC progenitors but not mature 9 week old MCs. Induces chemotaxis of human lung MCs	[9, 664]
CXCL12	CXCR4	Induces chemotaxis of 4 week old human cord blood MC progenitors, human cord blood-derived MCs and synergizes with histamine via the H4 receptor. Contributes to human lung MC chemotaxis towards human ASM supernatants	[9, 547, 665–667]
CXCL8 (IL-8)	CXCR1 and CXCR2	Blockade of CXCR1 attenuated human lung MC chemotaxis towards human ASM supernatants. HMC-1 cells migrate towards IL-8 via CXCR2. CXCR2 is not expressed on human lung MCs.	[547, 668]
CXCL9, CXCL10, CXCL11	CXCR3	See text, page 39. In addition, CXCR3 is expressed on MCs in patients with rheumatoid arthritis and is accompanied by elevated levels of CXCL9 and CXCL10 protein in the synovium.	[20, 547, 669]
CX3CL1 (fractalkine)	CX3CR1	CX3CL1 from human ASM cells induces HMC-1 migration via CX3CR1, but CX3CR1 is not expressed on human lung mast cells.	[20, 531]
Growth factors			
Stem cell factor (SCF)	c-Kit tyrosine kinase receptor (CD117)	SCF is chemotactic for MCs	[94, 670]
Transforming growth factor (TGF)- β	TGF- β serine/threonine type-I and type-II β receptors	TGF- β derived from human ASM cells, or present in asthmatic BAL, induces HMC-1 chemotaxis. TGF- β 1-3 all mediate chemotaxis of human cord blood-derived MCs.	[548, 671, 672]
Nonchemokine GPCR ligands			
PGE ₂	EP3	PGE ₂ is chemotactic for mouse MCs via the EP3 receptor. PGE ₂ inhibits human lung MC chemotaxis to ASM supernatants via EP2, but alone promotes migration of human lung MCs at high concentrations; this is enhanced when EP2 is blocked.	[19, 21]
LTB ₄	BLT1, BLT2	LTB ₄ promotes mouse MC and human cord blood progenitor migration, and chemotaxis of mature mouse MCs and human HMC-1 cells, but not mature human cord blood MCs. SCF down-regulates BLT1 and -2 expression and attenuates LTB ₄ -induced migration	[18, 673]
LTD ₄	cysLT1	LTD ₄ is chemotactic for human CD34 ⁺ progenitors	[674]
Platelet activating factor (PAF)	PAF receptor	PAF promotes intracellular Ca ²⁺ mobilization and HMC-1 chemotaxis	[675]
C3a, C5a	C3aR1, C5aR1 and 2	C3a and C5a induce HMC-1 chemotaxis at low nM concentrations. C5a induces migration of LAD2 cells via C5aR2, but this receptor is not expressed on human CD34 ⁺ cell-derived MCs	[234, 676]
5-hydroxytryptamine (5-HT; neurotransmitter serotonin)	5-HT receptors	5-HT receptors are expressed by human cord blood-derived MCs; and 5-HT induces migration via the 5-HT(1A) receptor.	[677]
Miscellaneous			
IgE	Fc ϵ RI	Mouse and human mast cells sensitized with antigen-specific IgE migrate towards the target antigen <i>in vitro</i> . Mouse MCs also migrate towards IgE	[678–680]
Catestatin	No specific receptor on MCs identified	This neuroendocrine antimicrobial peptide induces LAD2 and human peripheral blood-derived MC migration, degranulation and lipid mediator release	[681]
Adrenomedullin	No specific receptor on MCs identified	Adrenomedullin is chemotactic for HMC-1 cells. HMC-1 cells also produce adrenomedullin, and it is expressed by MCs within human tumours	[682]

proliferation, promotes chemotaxis, suppresses apoptosis and thus enhances MC survival, recruitment and growth [34]. Removal of SCF leads to rapid MC apoptosis, although several factors have the potential to delay this (see below). MCs can also undergo apoptosis in the presence of SCF as they have been found to express tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) receptors which initiate this [35].

Human MCs will grow *in vitro* from bone marrow, cord or peripheral blood-derived progenitors with SCF as the only exogenous factor, although several studies have reported that the cells remain relatively immature [14]. These cells are predominantly of the tryptase-only (MC_T) phenotype [14], but, as discussed later, the reported presence or absence of MC proteases in cultured MCs may depend to some extent on the sensitivity of the tools used to detect them and hence explain several discrepancies in the scientific literature. Cells grown in suspension culture in a medium supplemented with serum, SCF and IL-6 develop more mature granules and nuclear structure but are of the MC_T phenotype [36–38], whereas cells grown on a fibroblast or endothelial cell monolayer express both tryptase and chymase (MC_{TC} phenotype) and resemble skin MCs [14, 39, 40] (Fig. 1). In addition, human bone marrow-derived MCs and umbilical cord blood-derived MCs grown in conditioned medium from a cell strain derived from a patient with systemic mastocytosis together with SCF produced a culture of mature cells containing chymase only (MC_C phenotype) [41]. These observations indicate that factors other than SCF are required for complete MC maturation.

Several co-factors enhance or inhibit the effects of SCF depending on the origin and level of maturity of the MCs [42]. Lysophosphatidic acid (LPA) is a lipid mediator which exerts co-mitogenic effects with SCF in human cord blood-derived MCs [43], and nerve growth factor (NGF) [44], IL-3, IL-6, IL-9 [45] and IL-10 [36] potentially enhance SCF-dependent MC growth and/or promote survival. Conversely, granulocyte macrophage-colony stimulating factor (GM-CSF) [46], retinoids [47] and transforming growth factor (TGF)- β [47] prevent the growth and differentiation of MCs (Fig. 1). IL-4 inhibits the proliferation of immature human peripheral blood-derived MCs but potentiates the proliferation of more mature cells [48]. In addition, IL-4 promotes MC maturation, indicated by an increase in tryptase content and expression of *c-kit* and Fc ϵ RI [49, 50]. IL-5 and interferon (IFN) γ may prolong human cord blood-derived MC survival on SCF withdrawal [36, 51], but inhibit immature human peripheral blood-derived MC proliferation [48]. IL-6, through its ability to inhibit apoptosis, is important for the maintenance of human lung MC_T viability, but has no effect on the human lung MC_{TC} phenotype or skin MCs [52, 53]. Interestingly, monomeric

IgE promotes the survival of human lung MCs through the stimulation of IL-6 release which acts in an auto-crine fashion [53]. IL-33 may also prevent human skin MC apoptosis via up-regulation of the anti-apoptotic protein BCL-X_L [54] and prolongs human cord blood-derived MC survival following the removal of SCF [55].

Mast cell adhesion and CADM1. Cell adhesion is a fundamental mechanism through which cells communicate, permitting the delivery of specific cell–cell signals. Cell adhesion molecule-1 (CADM1; also known previously as TSLC1, IGSF4, SgIgSF, Necl2, Syncam1, RA175) is particularly important in this respect for MC function and signalling through Kit.

CADM1 is an adhesion receptor and member of the immunoglobulin (Ig) superfamily and its altered expression is implicated in several diseases including cancer (due to its roles in tumour suppression) [56, 57], autism spectrum disorder [58, 59], pulmonary fibrosis [60] and venous thromboembolism [61]. CADM1 consists of 3 extracellular Ig-like C2-type loops, a transmembrane domain and a 47 amino acid C-terminal intracellular domain [56, 62] (Fig. 2a). The extracellular Ig-like C2-type loops mediate cell–cell adhesion by forming either homophilic or heterophilic *trans*-interactions between adhesion molecules, which do not require the presence of Ca²⁺ [63] (Fig. 2b). CADM1 binds to itself (homophilic adhesion) and to heterophilic binding partners including CADM2, CADM3, Nectin-3, and cytotoxic and regulatory T cell molecule (CRTAM) [64–66], all of which are members of the same Ig superfamily. The intracellular domain of CADM1 contains two important sites required for protein binding. The first of these sites is a juxtamembrane protein 4.1/ezrin/radixin/moesin (FERM) binding motif, which interacts with DAL-1 (protein 4.1B) and anchors CADM1 to the f-actin cytoskeleton [67]. The second site is a PDZ-binding motif located within the C terminus of CADM1. This motif has been shown to mediate interactions with the PDZ domains of several other proteins including membrane palmitoylated protein (MPP)-3, MPP6 and calcium/calmodulin-dependent serine protein kinase (CASK), all members of the membrane associated guanylate kinases (MAGUK) family [64, 68, 69]. Members of this family of kinases play important roles in the cell surface expression and trafficking of many ion channels and receptors and also act as molecular scaffolds for the formation of signalling complexes [70]. The PDZ domain of CADM1 also binds Mint1, a protein which, together with CASK and another PDZ domain protein, mLin-7, forms heterotrimeric complexes that are important for the correct targeting of membrane proteins in many cell types [71]. FERM- and PDZ-binding domains are particularly important components of CADM1; if either the FERM-binding domain or PDZ-binding domain is deleted,

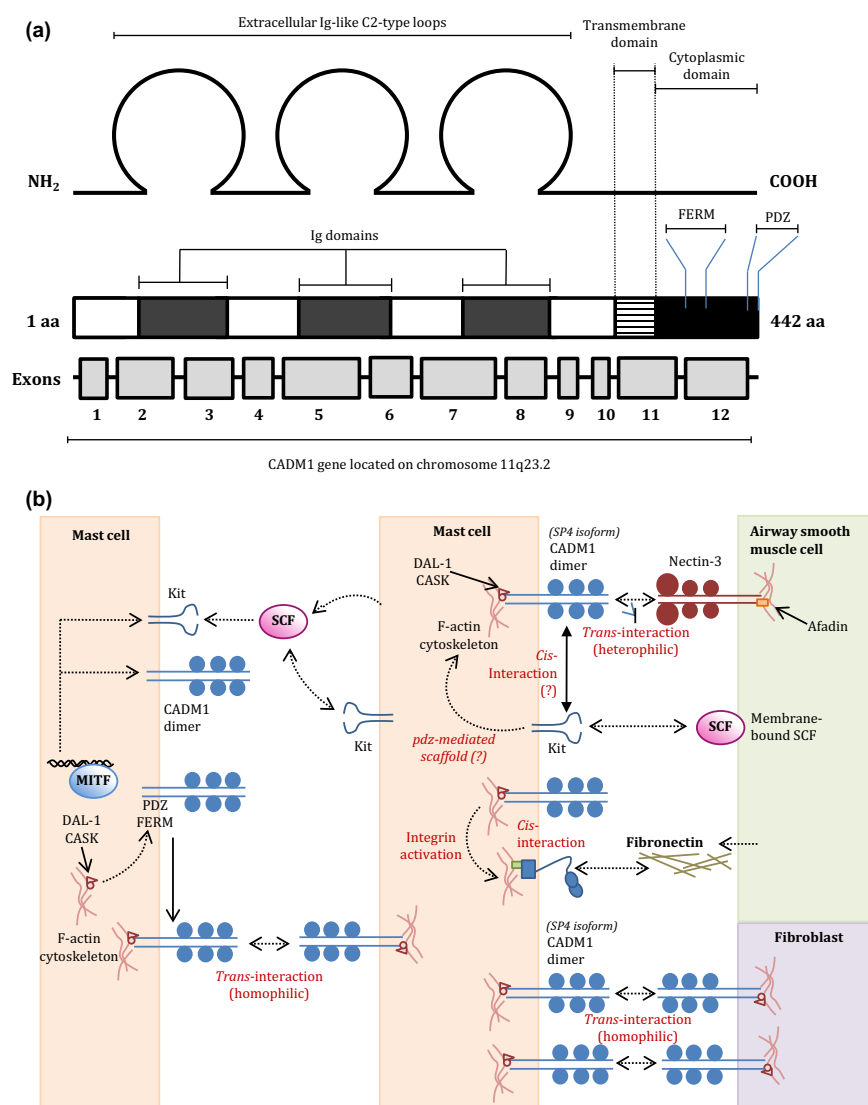


Fig. 2. The structure of CADM1 and its role in mast cell adhesion. (a) Predicted structure of CADM1 alongside its amino acid sequence and exon map of the CADM1 gene, located on chromosome 11q23.2. Exon number is indicated below each exon box. FERM- and PDZ-binding motifs are located within the C terminus of CADM1 and are considered vital for CADM1-mediated cell–cell adhesion (see text for references). (b) Mechanisms of CADM1-mediated mast cell adhesion. CADM1 facilitates homophilic (CADM1–CADM1) or heterophilic (e.g. CADM1–nectin-3) *trans*-interactions between MCs or between MCs and other structural cells such as airway smooth muscle cells and fibroblasts. In MCs, CADM1 proteins form a dimer, which complexes with DAL-1 and CASK proteins bound to the F-actin cytoskeleton. CADM1 dimers can bind to Nectin-3 expressed by airway smooth muscle cells, which are bound to afadin, connected to F-actin. CADM1 is also thought to be capable of forming *cis*-interactions with other receptors expressed by the same cell (see text for references). For instance, CADM1 is thought to stabilize Kit expression, which can subsequently interact with membrane-bound SCF on airway smooth muscle cells to promote MC adhesion and survival. CADM1 can also induce integrin activation, allowing integrins to bind to and interact with their extracellular matrix ligands secreted by structural cells. MITF: microphthalmia transcription factor.

CADM1 is no longer able to mediate cell–cell adhesion, and its tumour suppressor activity in epithelial cells is lost [57, 72]. In fact, mutants of these domains act in a dominant-negative fashion to increase tumour formation in nude mice [57, 72]. This suggests that there is an important co-operative interaction between the PDZ- and FERM-interacting proteins in tumour suppression, and inside-out signalling mediating cell adhesion.

CADM1 also plays an important role in cell motility [73].

CADM1 is expressed in epithelial cells, nerves, endothelial cells, fibroblasts and MCs, but not other leucocytes. The only exceptions are the malignant T cells in adult T cell leukaemia/lymphoma (ATLL) [74]. Several isoforms of CADM-1 exist due to variable splicing between exons 7–11 in the juxtamembranous

extracellular region [75, 76], although the factors regulating isoform expression are not known. Several functional CADM1 isoforms and several cryptic nonfunctional isoforms are detectable in the immature HMC-1 human MC line derived from a patient with MC leukaemia, the differentiated LAD2 human MC line derived from a patient with MC sarcoma and in primary human lung MCs [76, 77]. Based on the ability to clone CADM1 and detect it by Western blotting, the SP4 CADM-1 isoform, encoded by exons 1–8/11–12, is the only functional isoform present in the MC line HMC-1, whereas LAD2 cells and human lung MCs express approximately 80% of SP4, 16–18% of the longer SP1 isoform (exons 1–9/11–12) and approximately 2–5% of the longest SP6 (exons 1–12) isoforms. Real-time PCR has also detected the SP3 (exons 1–7/11–12) isoform in human lung MCs, but not SP2, soluble SP5 (exon 1–intron 7) or CADM-1b (exons 1–7/9/11–12) [77]. The failure to detect SP5 in human MCs is significant; SP5 has been cloned from mouse MCs, where it is proposed to function as a negative regulator of CADM-1 function [78].

The activities of each CADM-1 isoform in MCs differ. For instance, the SP4 isoform mediates homotypic (MC–MC) adhesion through a homophilic (CADM1–CADM1) *trans*-interaction and also promotes human lung MC and HMC-1 survival in mono-culture, although these two processes are not entirely interdependent [76]. In contrast, although the SP1 isoform also supports homotypic MC adhesion, it is less efficient than SP4, and SP1 overexpression acts in a dominant-negative manner to reduce HMC-1 and human lung MC survival. CADM1 down-regulation results in reduced viability and decreased expression of the pro-survival protein Mcl-1L, but not Bcl-2 or Bcl-XL, and increases caspase-3/7 activity in both HMC-1 cells and HLMCs [76]. This coincides with decreased basal Kit levels in HLMCs. Interestingly, SP4 expression, which promotes survival, is approximately fourfold higher in rapidly proliferating neoplastic HMC-1 cells than human lung MCs. Consequently, it is plausible that high SP4 expression and absent SP1 expression contributes to the neoplastic transformation of MCs in a manner similar to that evident in ATLL [74].

CADM1 expression not only promotes homotypic adhesion between MCs, but also facilitates the formation of heterotypic interactions between human MCs and other cell types. For example, CADM1 mediates adhesion of human lung MCs to primary human parenchymal lung fibroblasts [79] and, of relevance to asthma, primary human airway smooth muscle (ASM) cells [80] (Fig. 2b). This CADM1-dependent adhesive process promotes MC survival and proliferation in the absence of serum and exogenous SCF or IL-6 [79, 81]. Thus, CADM1 has a key role in MC biology. More

extensive experiments with human ASM cells have suggested that CADM1 and Kit have an intimate relationship in MCs. For example, CADM1 and Kit cooperate and contribute to human lung MC survival and proliferation [81]. In addition, both CADM1 and Kit exist under the transcriptional control of microphthalmia transcription factor (MITF) [33, 82], co-immunoprecipitate from HMC-1 cells [81] and co-localize in human lung MCs at points of adhesion to human ASM muscle cells, as determined by confocal imaging [81]. Down-regulation or overexpression of CADM1 also reduces or increases, respectively, surface Kit expression in both human lung MCs and HMC-1 cells [76, 83]). Thus, it is thought that CADM-1 plays an important role localizing Kit to the surface of MCs at points of cell–cell adhesion. This will facilitate the interaction between Kit and membrane-bound SCF expressed on cells such as fibroblasts and ASM cells, hence promoting MC survival.

CADM1 is also required for the efficient function of integrin-dependent adhesion to extracellular matrix (Fig. 2b). CADM1 regulates both E-cadherin and $\alpha_6\beta_4$ integrin function in other cell types [73, 84, 85], and down-regulation of CADM1 in HMC-1 cells results not only in reduced adhesion to human ASM cells, but also reduced adhesion to their extracellular matrix [83]. Time-course studies have demonstrated that CADM1 provides fast initial adhesion to human ASM cells and assists with slower adhesion to the extracellular matrix. However, as CADM1 down-regulation, but not antibody-dependent CADM1 inhibition, reduced MC adhesion to the extracellular matrix, it is thought that CADM1 indirectly regulates extracellular matrix adhesion.

Collectively these findings suggest that CADM1 is a key adhesion receptor which regulates net MC adhesion, both directly, through CADM1-dependent adhesion, and indirectly, through the regulation of other adhesion receptors. The latter is likely to occur via docking of Kit and polymerization of cortical F-actin. In light of these findings, a stepwise model of adhesion with CADM-1 has been suggested as a driving force for net MC adhesion [83].

In summary, CADM-1 is an important adhesion molecule that interacts with Kit in a co-operative manner to promote MC survival. Targeting MC-specific CADM1 signal transduction pathways or inhibiting the function of its counter-receptors on fibroblasts and ASM cells has the potential to become a novel strategy for the inhibition of MC function in asthma.

Ion channels. Like all cells, MCs maintain tight control of ion flux to achieve optimal growth and survival. TRPM7, a member of the transient receptor potential (TRP) melastatin (M) family of ion channels, is expressed by the human MC line HMC-1 and human

lung MCs [86]. The channel is involved in Mg^{2+} homeostasis and its activity is regulated by intracellular concentrations of Mg^{2+} and Mg^{2+} ATP, with increasing concentrations inhibiting the channel's gating. TRPM7 can also carry various trace divalent cations that are essential to normal human health [87]. In many systems, knockdown of TRPM7 induces cell death [88], inhibits cell proliferation [89] and inhibits or enhances cell adhesion depending on the model studied [90, 91]. Similarly, knockdown of TRPM7 in HMC-1 cells and human lung MCs leads to rapid cell death [86]. TRPM7 is an unusual channel in that it expresses an α -kinase in its N terminus. This enzyme is not essential for channel gating but phosphorylates annexin I and actomyosin, a cytoskeleton component, and thus couples TRPM7 to the cytoskeleton [90, 91]. As annexin I is itself implicated in the regulation of cell growth, differentiation and apoptosis [92, 93], this may provide a further link between TRPM7 and MC survival.

MCs express many other ion channels. The calcium activated K^+ channel $K_{Ca3.1}$ and the store-operated Ca^{2+} channel Orai1 do not appear to regulate MC survival or growth [94, 95], but blockade of a chloride channel in HMC-1 cells by tamoxifen markedly reduced HMC-1 proliferation and induced human lung MC death [15]. This raises the possibility that tamoxifen may be useful for the treatment of mastocytosis in humans and mastocytomas in dogs. A clinical trial of tamoxifen for the treatment of mastocytosis is currently in progress (<https://www.clinicaltrials.gov/ct2/show/NCT01334996>).

MS4A proteins. The membrane-spanning 4A (MS4A) gene family is located within chromosome 11q12-q13 and encodes a family of proteins with similar topology to tetraspanins [96]. The β chain of Fc ϵ RI (Fc ϵ RI β or MS4A2), encoded by the MS4A2 gene, serves to amplify Fc ϵ RI-dependent MC activation, in part through its ability to chaperone the α chain to the cell membrane and increase Fc ϵ RI surface expression [97]. However, two truncations of Fc ϵ RI β have been described recently with different functions (Fig. 3). Firstly, a truncation arising from inclusion of intron 5 results in an in-frame stop codon (MS4A2 variant 2) and has been detected in human cord blood-derived MCs and basophils [98]. This truncation decreases the expression of Fc ϵ RI by chaperoning the α chain to endosomes for degradation instead of to the cytoplasmic membrane, although the subsequent effects on cell function are not known. Secondly, we cloned a truncation of MS4A2 from HLMCs which lacks exon 3 (MS4A2_{trunc} [or t-Fc ϵ RI β]) [99]. t-Fc ϵ RI β gene expression was negatively regulated by SCF, and its expression was not detected in HMC-1 cells which have a gain-of-function mutation (s) in Kit. Unlike Fc ϵ RI β , t-Fc ϵ RI β does not traffic to the

cytoplasmic membrane, but instead associates with the nuclear membrane. Overexpression of t-Fc ϵ RI β induced human lung MC death and profoundly inhibited HMC-1 cell proliferation by inducing G₂-phase cell cycle arrest and apoptosis. These findings suggest that the MS4A2 gene and its product, Fc ϵ RI β , are not solely required for the regulation of acute allergic responses. Instead, MS4A2 silencing may contribute to pathological MC proliferation.

Another MS4A family member, MS4A4, is expressed by human lung and cord blood-derived MCs, and the HMC-1 and LAD2 human MCs lines [100]. In cord blood-derived MCs, expression increased as the cells matured over 8 weeks of culture. Silencing experiments in LAD2 cells demonstrated that MS4A4 regulates MC function by promoting the surface expression of Kit by facilitating Kit trafficking through endocytic recycling rather than degradation pathways, by a mechanism that involves recruitment of Kit to caveolin-1-enriched microdomains. MS4A4 also regulates signalling through the phospholipase (PL) C γ 1 pathway for Kit, possibly by recruitment of receptors to lipid rafts. Altered Kit trafficking and signalling with MS4A4 silencing resulted in an increase in AKT signalling, LAD2 cell proliferation and human cord blood-derived MC migration. These observations suggest that MS4A4 dysfunction in MCs may result in aberrant cellular responses and may help explain the observations that aberrant genetic expression of MS4A family members is associated with a range of disease processes. The regulation of human MC function by MS4A2 and MS4A4 is summarized in Fig. 3.

Mast cell heterogeneity and phenotype

MC heterogeneity exists across species, between different organs, and even within the same organ. This heterogeneity manifests as differences in cell structure, receptor expression, mediator content, immunological and nonimmunological activation and pharmacological responsiveness (summarized in Table 2; reviewed in [101, 102]). This marked variability in phenotype is driven by a number of different factors including interactions with the tissue matrix and resident cells such as epithelial cells, endothelial cells, fibroblasts and ASM cells [14, 31, 40, 103] (Fig. 1).

The protease content of MCs varies and this is commonly used to define MC subsets [104] (Table 2). MCs which contain only tryptase, based on immunohistochemical analysis in tissue, are typically designated MC_T. These cells are found predominantly at mucosal surfaces, such as within the bronchial and nasal airway epithelium in asthma and allergic rhinitis respectively [104–106], and in healthy and asthmatic bronchial lamina propria [105, 106]. In contrast, MCs which contain

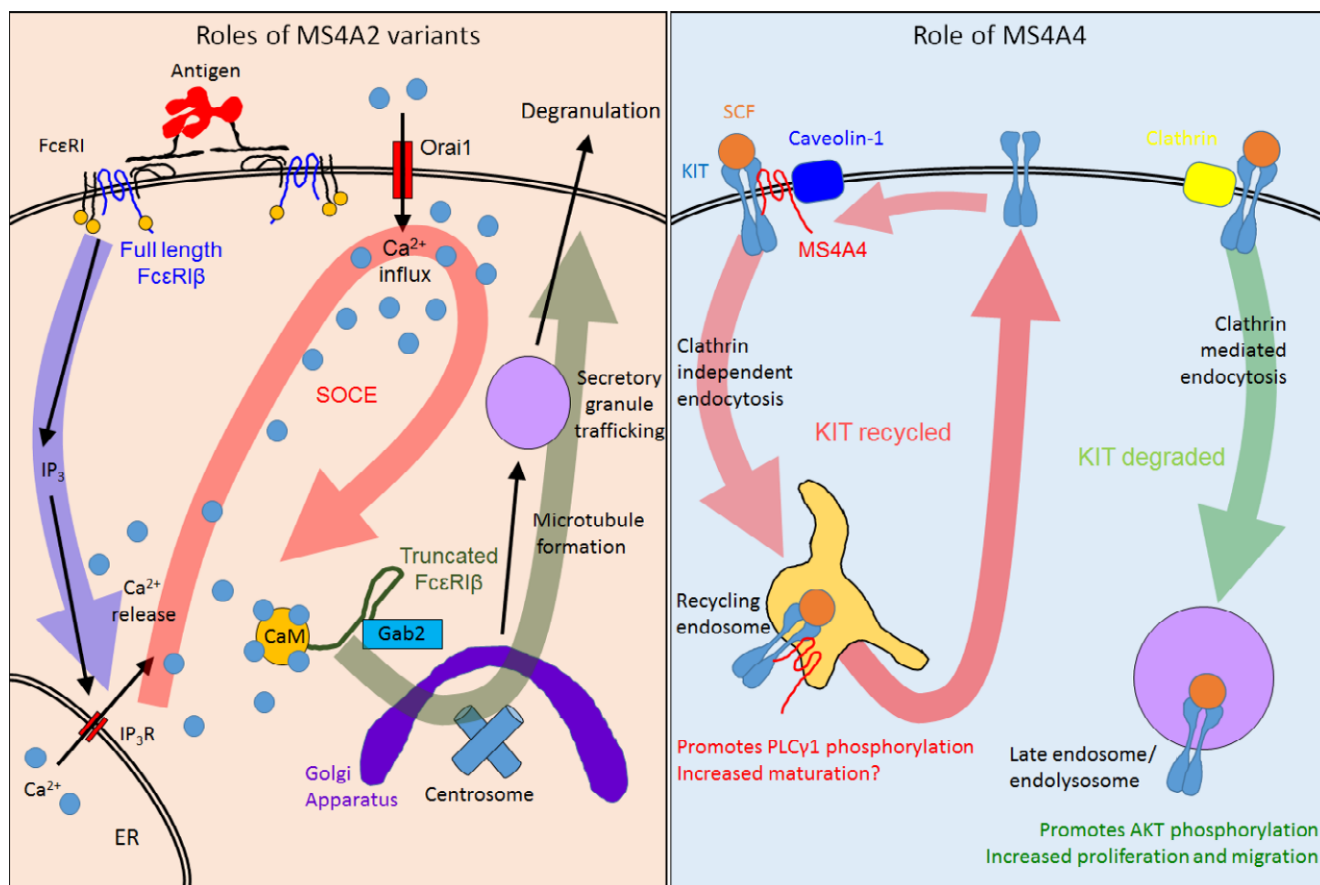


Fig. 3. The proposed roles of MS4A proteins in mast cell function. MS4A proteins appear to play important roles in cell signalling and receptor/protein trafficking pathways that critically affect mast cell functions. (a) MS4A2 splice variants encode FcεRIβ isoforms that traffic to different cellular compartments where they play critical roles in mast cell signal transduction and degranulation. The full length 4 pass transmembrane FcεRIβ protein (depicted in blue) amplifies FcεRI signalling via C-terminal immunoreceptor tyrosine-based activation motifs (ITAMs – orange circles) promoting generation of inositol triphosphate (IP₃), which induces calcium release from stores in the endoplasmic reticulum (ER). This release of calcium stores triggers store-operated calcium entry (SOCE) through Orai1 channels (orange arrow). Truncated FcεRIβ (depicted in green) localizes to intracellular compartments and the Golgi Apparatus, functioning downstream of calcium signals, regulating microtubule formation and granule translocation to the plasma membrane. The proposed mechanism is that truncated FcεRIβ translocates to the peri-centrosomal Golgi Apparatus in response to interactions with calcium loaded calmodulin (CaM) and Gab2. Therefore, full length FcεRIβ participates in signal transduction from FcεRI at the plasma membrane upstream of calcium signalling (blue arrow) and truncated FcεRIβ functions downstream of calcium to transmit the signal to the cytoskeleton (green arrow). (b) KIT receptors that regulate many mast cell functions are most likely endocytosed by at least two pathways and the proportion of KIT receptors that are endocytosed by each pathway may alter the strength or specificity of signals in response to ligand (SCF) binding to KIT. MS4A4 (depicted in red) promotes the recruitment of KIT receptors into caveolin-1-enriched membrane microdomains and thus alters the mechanism of KIT endocytosis directing a proportion of KIT receptors through caveolae-mediated endocytosis pathways. By altering the endocytosis of a proportion of KIT receptors, MS4A4 alters both trafficking and signalling of KIT, promoting receptor recycling and lipid raft-associated signalling (PLCγ1) (red arrows). Reduced MS4A4 expression results in increased KIT endocytosis, a loss of KIT recycling and reduced surface KIT expression (green arrow). In addition, reduced MS4A4 expression alters KIT signalling by reducing PLCγ1 phosphorylation, but increasing AKT phosphorylation that promotes mast cell proliferation and migration. Figure and concepts courtesy of Dr Glenn Cruse.

tryptase, chymase, carboxypeptidase A (CPA) and cathepsin G, identified by immunohistochemistry, have been labelled as MC_{TC} [104, 107] (Table 2). MC_{TC} cells are located within connective tissues including healthy skin [104], the ASM bundles in asthma [108] and atherosclerotic lesions [109]. They are also present in the nasal and bronchial lamina propria, particularly adjacent to blood vessels [106]. MCs containing chymase and CPA (MC_C) without tryptase have also been

observed [106, 110, 111], but vary in location and their function has yet to be elucidated.

While the above classification of human MCs based on protease content has proved convenient and contributed to our knowledge of MC function, this categorical division of MCs into MC_T and MC_{TC} has recently been challenged. With the development of PCR and more sensitive techniques for protein detection, MCs infiltrating the airway epithelium in asthma have been

Table 2. Characteristics of mast cell subtypes

Characteristics	Rodent MCs		Human MCs	
	MMC	CTMC	MC _T	MC _{TC}
Protease content	Chymase (rat MC protease 2)	Chymase (rat MC protease 1)	Tryptase	Tryptase, chymase, carboxypeptidase, cathepsin G
Proteoglycan content	Chondroitin sulphate	Heparin	Heparin	Heparin
Predominant granule patterning evident with electron microscopy			Scroll	Lattice
Common location	Mucosa	Submucosal tissues	Epithelium, lamina propria	Lamina propria, connective tissue, skin
Staining characteristics	Alcian Blue +	Safranin +	Safranin +	Safranin +
Relative LTC ₄ release	High	Absent	High	Skin low
Relative PGD ₂ release	Low	Low	High	Skin high
Tissue type-2 cytokine profile			IL-4 (low), IL-5 (high), IL-6 (high), IL-13 (low)	IL-4 (high), IL-13 (high)
Activated via FcεRI	Yes	Yes	Yes	Yes
Activated by substance P	No	Yes	No	Yes
Responds to C5a			No	Yes
Response to IL-4			Apoptosis	Resistant to apoptosis
Inhibited by sodium cromoglycate	No	Yes	Yes (weak effect)	No

shown to express both tryptase and CPA but not chymase [112]. In addition, it was found using PCR and flow cytometry rather than immunohistochemistry that all MCs derived from adult peripheral blood progenitors express the full repertoire of MC proteases [113]. Thus, it seems probable that all MCs arise from a common progenitor, and the level of protease expression is determined by the local cytokine milieu and cell–cell and cell-matrix interactions. This is supported by several lines of evidence. For instance, IL-13-stimulated bronchial epithelial cells producing increased quantities of SCF supported the survival of MCs expressing tryptase and CPA, but suppressed chymase expression [112]. Similarly, human cord blood-derived MCs developed in the presence of SCF, IL-6 and IL-10 expressed both chymase and tryptase, but chymase expression was lost after co-culturing these cells for 2 days with human bronchial epithelial cells [31]. IL-4 significantly up-regulates chymase expression in human cord blood-derived MCs [49], and human umbilical vein endothelial cells support survival of human gut MCs that express both tryptase and chymase [14]. Hence, MC protease expression appears to be highly labile and regulated by local factors.

While all MCs may express all MC proteases to varying degrees, the MC_{T/TC} classification is still useful for several reasons, but should now be used in the context of MCs that are protease-high or -low rather than with respect to positive or negative. High chymase expression correlates with other functional differences in human MCs. For example, > 99% of skin MCs are MC_{TC}

and respond with a secretory response to the complement component C5a. A homogenous population of human lung MCs will not respond to C5a, but if the CD88 (C5a receptor)⁺ fraction are isolated, these cells, which are MC_{TC}s, will behave in the same way as human skin MCs when challenged with C5a. Heterogeneity also occurs in terms of cytokine expression, which associates with protease expression defined by immunohistochemistry in tissue (Fig. 4); for instance, IL-4 and IL-13 expression occur predominantly in MC_{TC} cells in the bronchi, nasal mucosa and conjunctiva, whereas IL-5 and IL-6 are almost exclusively localized to MC_T cells [106, 114].

Further marked intra-tissue heterogeneity within the lung has been demonstrated by Andersson and colleagues who examined the expression of several receptors, enzymes and growth factors by MCs in situ [115]. They identified clear differences in MC size and shape, and in the expression of tryptase, chymase, FcεRIα, IL-9R, histidine decarboxylase, 5-lipoxygenase, LTC₄ synthase, renin, VEGF and basicFGF in MCs located in the large airways, small airways, alveoli, pulmonary vessels and pleura.

It is not surprising that there is such extensive heterogeneity among MCs. A MC interacting with an ASM cell is likely to have different goals to one interacting with an epithelial cell, for example. However, the precise roles of MC_T and MC_{TC} cells are uncertain, particularly when considering the potential combinations of receptor, cytokine and enzyme expression. The absence of MCs in patients with defective T cell func-

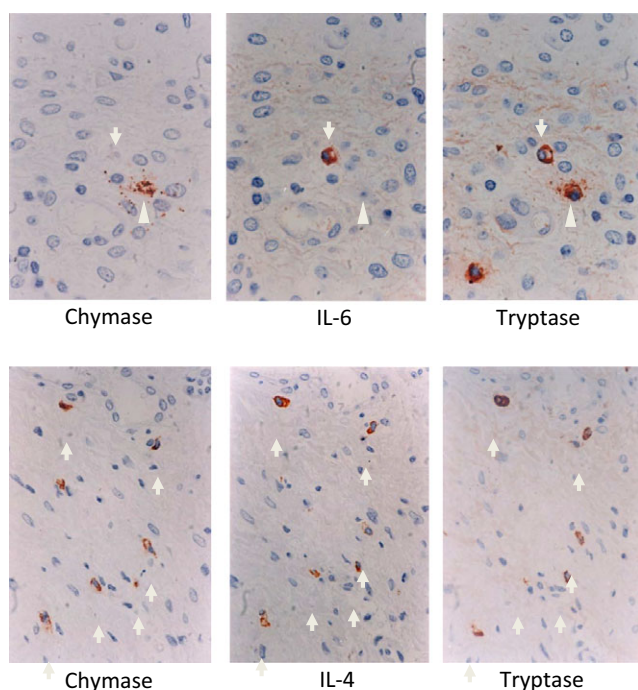


Fig. 4. The heterogeneity of mast cells: cytokine content. Three sequential 2 mm sections of nasal mucosa from a patient with allergic rhinitis stained for (a) chymase, IL-4 and tryptase (bottom row), demonstrating that nearly all chymase-positive MCs contain IL-4, and (b) chymase, IL-6 and tryptase (top row), demonstrating co-localization of IL-6 to tryptase-positive, chymase-negative MCs (arrows). A chymase-positive MC does not contain IL-6 (arrowheads). Images from Professor Peter Bradding, with permission (reproduced from Bradding et al. [106]).

tion has led to the suggestion that MC_T cells at mucosal surfaces interact predominantly with the immune system and regulate host defence [116]. In contrast, MC_{TC} cells are proposed to be involved in tissue repair, fibrotic reactions and angiogenesis [117, 118]. Of note, gene expression in a relatively homogenous cord blood-derived MC population is highly specific to the stimulus [119], and MC protein expression changes in disease states, with up-regulation of IL-4 in asthma [120], and increased expression of basicFGF in idiopathic pulmonary fibrosis [121]. So ultimately, the biological functions of these cells will depend largely on the stimuli they respond to and the profile of mediators they release in response to this.

Mast cell structure

MCs are readily identified by their relatively large, membrane-bound intracellular granules. These contain a proteoglycan matrix which in human MCs consists predominantly of heparin sulphate [122], and to a lesser extent, chondroitin E. Neutral proteases, acid hydrolases and histamine molecules are attached to the to the proteoglycans by ionic linkage to the

sulphate groups on the glycosaminoglycans (GAGs) [123]. The GAG sulphate groups generate an acidic environment within the granules, and this maintains the mediators in an inactive state [124]. Heparin is essential for the stabilization of the β -tryptase tetramer [125], both heparin and heparan sulphate, a glycosaminoglycan present on the extracellular side of cell membranes, bind several cytokines and chemokines. This has several potential consequences such as facilitating the local paracrine presentation of cytokines to adjacent cells, protecting them from proteolytic degradation, and potentially interfering with cytokine detection by ELISA and immunohistochemistry [126–129].

There are ultrastructural differences in the membrane-bound intracellular granules in MC_{TC} cells compared to MC_T cells, with scroll patterns dominating in MC_T granules compared to lattice patterns in MC_{TC} granules [130]. IgE-dependent activation of MCs induces granule swelling, crystal dissolution and fusion of the granules to both surrounding granules and also the cell membrane. This is followed by exocytosis and subsequent release of mediators into the extracellular space [131], a process termed 'compound exocytosis' or 'anaphylactic degranulation'. MC activation through this pathway is not a cytotoxic event, and even after almost complete degranulation human lung MCs are able to re-granulate over a period of 48 h [132, 133]. Once released from the acidic environment of the granules, the neutral pH of the extracellular space generates active mediators [131, 134]. However, in many diseased tissues including asthmatic bronchial mucosa, MCs typically demonstrate piecemeal degranulation [135–137], where there is variable loss of granule contents with the granules and their membranes remaining intact (Fig. 5). The mechanisms leading to piecemeal degranulation in MCs are poorly understood and require further research.

Once released, the effects of preformed mediators often remain localized. Histamine activity is short-lived *in vivo* due to degradation by histamine-N-methyltransferase (HMT), while the active tetramer of tryptase rapidly dissociates into inactive monomers in the absence of heparin [138]. It is also appears that the activity of many cytokines occurs in close proximity to the releasing MC due to retention on cell surface proteoglycans and/or protease degradation of 'free' cytokine [139].

Following IgE-dependent activation, stored granule-derived mediators are released from MCs alongside newly formed metabolites of the phospholipid arachidonic acid. Arachidonic acid is liberated from the cell membrane, nuclear envelope or lipid bodies during immunologic activation and is rapidly oxidized by either the cyclooxygenase or the lipoxygenase path-

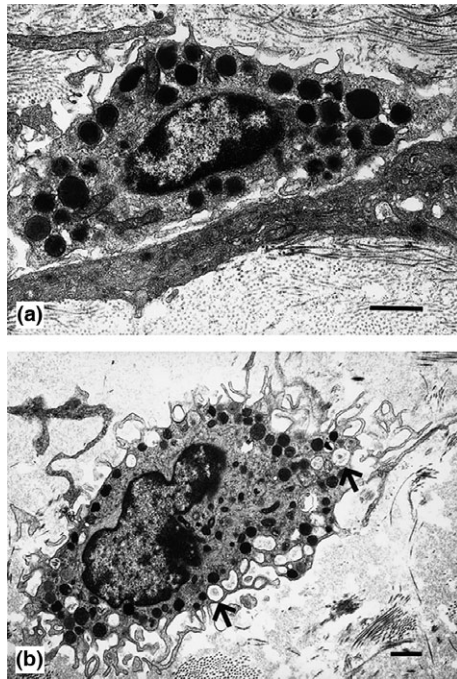


Fig. 5. Piecemeal degranulation of mast cells. Scanning electron micrographs of MCs. (a) Normal airway MC at rest showing electron-dense granules and a mono-lobed nucleus with little condensed chromatin. (b) MC in an asthmatic airway showing evidence of piecemeal degranulation with variable, selective loss of granule contents (arrows). Images reproduced from Bradding P, Cruse G. Mast cells: biological properties and role in health and allergic diseases. In: *Allergy and Allergic Diseases* (2nd edition). Kay AB, Kaplan AP, Bousquet J, Holt P (Eds). Blackwell Scientific Publications, Inc. 2008; 217–57.

ways to form the eicosanoids PGD_2 and LTC_4 , respectively [140, 141]. In addition, MCs are also capable of synthesizing and secreting numerous cytokines and chemokines depending on the stimulus (see below). The principal biological properties of MC autacoids (histamine, PGD_2 , LTC_4), proteases and cytokines/chemokines are summarized in Tables 3–5.

Mechanisms of mast cell activation

FcεRI-dependent activation

MC activation via the high affinity IgE receptor $\text{Fc}\epsilon\text{RI}$ is the most extensively studied mechanism of MC activation and long considered the most relevant to allergic disease (reviewed by [142, 143]). The majority of downstream signalling pathways identified following $\text{Fc}\epsilon\text{RI}$ engagement have been obtained in rodent models, with relatively little known about events in human MCs. Where human cells have been investigated, some important differences in the signalling molecules present and the response to cell activation are evident when compared to mice [144–149]. However, this also

applies to human MCs from different sources, again highlighting the importance of MC heterogeneity [50, 150–153].

$\text{Fc}\epsilon\text{RI}$ is a tetrameric structure from the multichain immune recognition receptor (MIRR) family. It is comprised of a 45 kDa α chain ($\text{Fc}\epsilon\text{RI}\alpha$) which binds IgE, a 30 kDa β chain signalling subunit ($\text{Fc}\epsilon\text{RI}\beta$ or MS4A2) and two 10 kDa γ subunits which exist as a disulphide-linked homodimer signalling subunit ($\text{Fc}\epsilon\text{RI}\gamma$). Trafficking of the α chain to the cell surface is enhanced by expression of the β chain [97], and the tetramer is stabilized on the cell surface by the binding of IgE [154]. In keeping with this, therapeutic anti-IgE, which binds to the receptor binding domain of IgE and thus prevents IgE binding to the α chain, reduces cell surface $\text{Fc}\epsilon\text{RI}$ expression *in vivo* [155]. $\text{Fc}\epsilon\text{RI}$ expression is also increased further by IL-4 and IL-13, which enhance $\text{Fc}\epsilon\text{RI}$ -dependent secretion [154, 156].

A detailed description of the multiple signalling cascades activated following receptor activation is beyond the scope of this article but is summarized in Fig. 6 (for reviews see [142, 143]). In terms of the proximal signalling pathways, the γ signalling subunits contain an immunoreceptor tyrosine-based activation motif (ITAM) [157] within their cytoplasmic C-terminal domains, which bind to Syk tyrosine kinases initiating phosphorylation. The β chain of $\text{Fc}\epsilon\text{RI}$ also contains an ITAM. However, the $\text{Fc}\epsilon\text{RI}\beta$ ITAM contains a noncanonical tyrosine residue which prevents binding of Syk kinase. Instead, $\text{Fc}\epsilon\text{RI}\beta$ signals through the activation of the lipid raft-associated Lyn tyrosine kinase which in turn activates Syk kinase (Fig. 6) [143]. However, full length $\text{Fc}\epsilon\text{RI}\beta$ held in the cell cytoplasm may also act as a negative regulator of $\text{Fc}\epsilon\text{RI}$ -dependent signalling through its ability to capture important signalling molecules such as Lyn [158].

Signalling *in vivo* is initiated when multivalent allergen binds to allergen-specific IgE bound to the $\text{Fc}\epsilon\text{RI}\alpha$ chain. This promotes $\text{Fc}\epsilon\text{RI}$ aggregation and can be mimicked *in vitro* by the use of anti-IgE antibodies. Receptor aggregation initiates the association of the receptor with lipid rafts containing Lyn, a membrane anchored member of the Src family of protein tyrosine kinases (reviewed by [159]). Lyn kinase transphosphorylates tyrosine residues in the ITAMs before binding strongly to the phosphorylated $\text{Fc}\epsilon\text{RI}\beta$ ITAM through the Src homology 2 (SH2) domain (reviewed by [160]). Syk protein tyrosine kinases are recruited to the rafts and bind the doubly phosphorylated ITAMs. They are themselves phosphorylated by recruited Lyn and Syk kinases promoting the Syk activation loop which results in fully activated Syk with adjacent tyrosine phosphorylation that begins a cascade of events leading to the generation of inositol triphosphate (IP_3) via $\text{PLC}\gamma$. IP_3

Table 3. Classical human mast cell mediators and proteases and their biological effects (see text for references)

Mediator	Stored or newly synthesized	Biological effects
Histamine	Stored	Induces bronchoconstriction and tissue oedema; increases vascular permeability, mucus secretion, fibroblast proliferation, collagen synthesis and endothelial cell proliferation; activates dendritic cells
Heparin	Stored	Anticoagulant effects; provides proteoglycan-based storage matrix for mediators in MC granules; sequesters growth factors to prevent their degradation; activates fibroblasts; induces endothelial cell migration
Tryptase	Stored	Degrades respiratory allergens, cross-linked IgE and neuropeptides; generates C3a and bradykinin; enhances bronchial hyperresponsiveness and ASM contractility; indirectly activates collagenase; increases fibroblast proliferation and collagen synthesis; activates epithelial cells and TGF- β molecules; enhances MC histamine release; recruits neutrophils, cleaves and inactivates CCL5 and -11
Chymase	Stored	Increases mucus secretion; degrades ECM and several cytokines including IL-4; facilitates processing of type-1 pro-collagen molecules; induces synthesis of angiotensin II; reduces adhesion of T cells to ASM; activates IL-1 β ; releases membrane-bound SCF; enhances IL-33 activity through proteolytic cleavage
PGD ₂	Newly synthesized	Induces bronchoconstriction and tissue oedema; increases mucus secretion; activates dendritic cells; acts as chemoattractant for eosinophils, Th2 cells and basophils via the CRTh2 receptor, activates ILC2 cells
LTC ₄ (and extracellularly derived D ₄ and E ₄)	Newly synthesized	Induces bronchoconstriction and tissue oedema; enhances mucus secretion, IL-13-dependent ASMC proliferation and tissue fibrosis; promotes maturation and recruitment of dendritic cells; induces IL-4 secretion from eosinophils, and IL-5, IL-8 and TNF- α release from MCs; chemoattractant for MCs; induces IL-4, IL-5 and IL-13 production by mouse ILC2 cells

induces Ca²⁺ mobilization from intracellular rough endoplasmic reticulum (RER) stores, which initiates the influx of extracellular Ca²⁺ *via* Orai1 store-operated Ca²⁺ channels (Fig. 6). In terms of IgE-dependent mediator release in both rodents and humans, influx of extracellular Ca²⁺ is a critical requirement for the release of both pre-formed and newly generated mediators [161, 162]. PLC γ also activates the Grb2/Sos/Ras pathway which leads to increased extracellular regulated kinase (ERK) activity which in turn initiates arachidonic acid metabolism via activation of phospholipase A₂ (PLA₂) and subsequent eicosanoid production and release. Studies in knockout mice or pharmacological inhibitors demonstrate that a PI-3 kinase (PI-3K) δ signalling pathway also contributes to MC degranulation, eicosanoid production and cytokine release [163]. Sphingosine-1-phosphate (S1P) is a further autocrine amplifier of Fc ϵ RI-dependent mediator release (reviewed in [164]).

The truncation of Fc ϵ RI β (t-Fc ϵ RI β or MS4A2_{trunc}) described earlier is not only important for regulating human MC survival and proliferation, but is also important for microtubule formation and degranulation in the LAD2 human MC line. In this respect, t-Fc ϵ RI β appears to function by trafficking adaptor molecules

and kinases to the pericentrosomal and Golgi region in response to Ca²⁺ signals [145]. t-Fc ϵ RI β contains a putative calmodulin-binding domain, and mutagenesis studies suggest that calmodulin binding to t-Fc ϵ RI β in the presence of Ca²⁺ is critical for t-Fc ϵ RI β function. Thus gene targeting of t-Fc ϵ RI β in LAD2 cells attenuated microtubule formation, degranulation and IL-8 production downstream of Ca²⁺ signals [145]. Thus the function of the MS4A2 gene extends beyond the production of Fc ϵ RI β and Fc ϵ RI expression and potentially plays a crucial role in the regulation of both human MC survival and activation.

Ion channel regulation of Fc ϵ RI-dependent mediator release

Ion channels have vital roles in the physiological activity of all living cells and form a group of proteins that are embedded within the plasma membranes of cells and allow the passage of charged particles, such as Na⁺, K⁺ and Ca²⁺ ions, across the membrane (Fig. 7a). The process of Ca²⁺ entry through the plasma membrane as a result of the depletion of Ca²⁺ from internal stores is termed capacitive Ca²⁺ entry. The Ca²⁺ current passing through the plasma membrane is known as the Ca²⁺

Table 4. Human mast cell-derived chemokines and their biological effects *in vitro*

Chemokine and known stimuli	Target cells	Biological effects
-CC-		
CCL1 [209, 683–685] FcεRI IgE	T cells	T cell recruitment
CCL2 [681, 683–688] Constitutive SCF FcεRI Dengue virus IL-1β Catestatin	Mast cells Epithelial cells Fibrocytes T cells Eosinophils Monocytes Basophils	Chemotaxis Chemotaxis, proliferation Chemotaxis Induces Th2 phenotype Chemotaxis Chemotaxis Activation, mediator release
CCL3 [209, 361, 681, 683–686] FcεRI Nod1 ligand Dengue virus Catestatin IgE	Mast cells T cells Macrophages Neutrophils Eosinophils Monocytes Basophils	Activation, mediator release Chemotaxis (selective for Th1), induces Th1 phenotype Differentiation Chemotaxis (in vivo), cytotoxicity Chemotaxis Chemotaxis Activation, mediator release
CCL4 [361, 681, 683–686] FcεRI Nod1 ligand Dengue virus Catestatin	T cells Eosinophils Neutrophils	Chemotaxis (selective for Th1), induces Th1 phenotype Chemotaxis Chemotaxis (in vivo)
CCL5 [683, 684, 689] FcεRI Dengue virus	Mast cells T cells Eosinophils Monocytes	Chemotaxis Chemotaxis (selective for Th1), induces Th1 phenotype Chemotaxis Chemotaxis
CCL7 [683–685] FcεRI	Eosinophils Monocytes Basophils	Chemotaxis Chemotaxis Activation, mediator release
CCL8 [684, 686] Constitutive Dengue virus	T cells Basophils B cells Eosinophils	Chemotaxis Chemotaxis, histamine release Chemotaxis Chemotaxis
CCL13 [684] Constitutive	Eosinophils Basophils Monocytes Immature dendritic cells T cells Endothelial cells Fibroblasts	Chemotaxis Histamine release, chemotaxis Chemotaxis Chemotaxis Chemotaxis Chemotaxis Inhibits apoptosis
CCL17 [684] Constitutive	T cells	Chemotaxis (selective for Th2)
CCL18 [684, 685] FcεRI	Epithelial cells T cells B cells Immature dendritic cells Basophils Macrophages	Epithelial mesenchymal transition Chemotaxis Modulates activation and chemokine-induced responses Chemotaxis Chemotaxis, histamine release Chemotaxis, activation
CCL19 [129, 684] Constitutive	Airway smooth muscle cells	Chemotaxis
	Dendritic cells T cells	Chemotaxis Chemotaxis

1)

Table 4 (continued)

Chemokine and known stimuli	Target cells	Biological effects
CCL20 [684, 690] FcεRI Pseudomonas	T cells	Chemotaxis (selective for Th2)
CCL22 [683, 684] Constitutive		
CCL23 [684] FcεRI	Monocytes	Chemotaxis, adhesion, mediator release
	T cells	Chemotaxis
	Neutrophils	Chemotaxis
	Myeloid progenitor cells	Suppressed development
	Dendritic cells	Chemotaxis
CCL25 [683, 684] Constitutive	Periosteal progenitor cells	Chemotaxis
	Lymphocytes	Adhesion
	Mesenchymal stem cells	Chemotaxis
CCL28 [684] Constitutive	Lymphocytes	Adhesion
	T cells	Migration
	Haematopoietic progenitor cells	Growth and survival
-CXC-		
CXCL2 [684] FcεRI	Neutrophils	Chemotaxis, adhesion
	Airway smooth muscle cells	Migration
CXCL3 [684, 685] FcεRI	Airway smooth muscle cells	Migration
CXCL4 [684] Constitutive	T cells	Chemotaxis
	Endothelial cells	Angiogenesis
	Monocytes	Impairs chemotaxis
	Neutrophils	Adhesion
	Neutrophils	Chemotaxis
CXCL5 [684] Constitutive		
CXCL7 [686] Constitutive Dengue virus	Neutrophils	Chemotaxis, degranulation
CXCL8 [55, 208, 273, 361, 666, 684–686, 691–694] Constitutive	Mast cells	Reduces chemotaxis and mediator release
	Endothelial cells	Chemotaxis, proliferation, survival, angiogenesis
	Neutrophils	Chemotaxis
	Eosinophils	Chemotaxis following priming with IL-3, IL-5 or GM-CSF
IgE FcεRI CXCL12 Substance P TSLP + IL-1β IL-33 Nod1 ligand Dengue virus		
CXCL10 [684, 686] Constitutive Dengue virus	Mast cells	Chemotaxis
CXCL14 [684] Constitutive	Bacteria	Antimicrobial action
	Monocytes	Chemotaxis
	Dendritic cells	Chemotaxis
CXCL16 [684] Constitutive	T cells	Chemotaxis
	Monocytes	Chemotaxis
CXCL17 [684] Constitutive	Bacteria	Antimicrobial action
	Dendritic cells	Chemotaxis
	Monocytes	Chemotaxis
-C-		
XCL1 [684]	T cells	Chemotaxis, activation apoptosis
-CX3C-		
CX3CL1 [684]	Mast cells	Chemotaxis
	Leucocytes	Chemotaxis, adhesion
	Fibroblasts	Increased MMP2 production

Table 5. Human mast cell-derived cytokines and some important biological effects

Cytokine and known inducing stimuli	Target cells	Important biological effects
GM-CSF [209, 239, 273, 283, 403, 408]	Eosinophils	Eosinophil growth, adhesion, transendothelial migration, chemotaxis, activation
Constitutive	Mast cells	↓ tryptase expression, ↑ FcεRI-dependent histamine release
IgE		
FcεRI		
FcγRI		
TLR2		
TSLP + IL-1β		
Eosinophil contact		
IL-1β [119, 239, 692, 695]	Mast cells	Induces histamine release from adenoidal MCs, ↑ uterine MC adhesion
Constitutive	T cells	Activation
FcγRI	B cells	Augments proliferation and Ig synthesis
TLR2, -4, -5, -9	Hepatocytes	Acute phase protein release
IFN-γ	Endothelial cells	Adhesion molecule expression
	Airway smooth muscle	Uncouples β ₂ -adrenoceptor
	Airway epithelium	GM-CSF secretion, ↑ ICAM-1 expression, ↑ neutrophil and eosinophil adhesion
IL-3[692, 696]	Mast cells	Growth, differentiation and survival of bone marrow progenitor cells
FcεRI	Eosinophils	Eosinophil growth, adhesion, transendothelial migration, chemotaxis, activation
	Basophils	Survival, activation
IL-4 [106, 117, 120, 316, 369, 387, 388, 451, 457, 516, 540, 620, 697–707]	Mast cells	↑ FcεRI and releasability, ↑ ICAM-1 expression, ↑ sensitivity to adenosine, apoptosis of MC _T but not MC _{TC}
FcεRI	B cells	Proliferation, increases MHC class II, CD40 and CD25 expression, IgE class switching and secretion, ↑ IL-6 release
Lectins	T cells	Proliferation, promotes a Th2 cell phenotype
Proteases	Monocytes/macrophages	Reduces H ₂ O ₂ and O ₂ production, attenuates parasite killing and tumoricidal activity, induces monocyte to macrophage differentiation, ↓ IL-1, IL-6, IL-8, TNF-α and 15-lipoxygenase production, ↑ MHC class II and CD23 expression
Fibroblast contact		
constitutive mRNA		
expression in asthmatic airways	Eosinophils	Transendothelial migration, activation, primes for CCL5-dependent migration
	Fibroblasts	Proliferation and chemotaxis, transformation to myofibroblasts, ↑ ICAM-1 expression and extracellular matrix deposition
	Endothelial cells	Proliferation, ↑ VCAM-1 expression, ↓ ICAM-1 expression
	Airway smooth muscle	Inhibits human ASM cell proliferation, ↑ CCL11 release
	Epithelial cells	↑ NO and periostin production, CCL11, IL-8, GM-CSF, TSLP and TGF-β ₂ release, ↑ 15-lipoxygenase expression, ↑ mucus production
IL-5 [120, 238, 239, 273, 451, 457, 697, 698, 704, 708, 709]	Eosinophils	Growth, adhesion, transendothelial migration, chemotaxis, activation
FcεRI		
FcγRI		
TLR2, -4		
TSLP + IL-1β		
IL-6 [52, 53, 119, 120, 240, 273, 692, 693, 697, 710]	B cells	Promotes Ig secretion and enhances IgE production
Constitutive	T cells	Growth and differentiation
FcεRI	Mast cells	Supports growth, inhibits IL-4 dependent apoptosis of human lung MC _T s
FcγRI	Airway smooth muscle	Enhances contractility of human ASM cells
IgE	Epithelial cells/submucosal glands	Mucus hypersecretion
TLR4		
IFN-γ		
SCF		
TSLP + IL-1β		

1)

Table 5 (continued)

Cytokine and known inducing stimuli	Target cells	Important biological effects
IL-8 (CXCL8) [55, 208, 273, 361, 666, 685, 686, 691–694] Constitutive IgE FcεRI CXCL12 Substance P TSLP + IL-1β IL-33 Nod1 ligand Dengue virus	Neutrophils Mast cells Endothelial cells	Chemotaxis and activation Chemotaxis Angiogenesis
IL-9 [692, 711] FcεRI	Mast cells Eosinophils Neutrophils Airway smooth muscle cells Airway epithelial cells Monocytes T cells	Induces VEGF expression and release from human LAD2 cell line, promotes MC progenitor growth Enhances differentiation, survival and IL-5 receptor expression Induces IL-8 production and release Potentiates ERK-dependent release of IL-8 and CCL11, and thus induces eosinophil chemotaxis Induces goblet cell hyperplasia during repair after injury or during differentiation, and increases mucin expression and release Reduces the oxidative burst and LPS-induced TNF-α and IL-10 release Promotes growth, prevents apoptosis of a subset of Th2 cells induced by growth factor withdrawal
IL-10 [119, 238, 240, 712, 713] Constitutive FcεRI TLR2, -4 IFN-γ	Mast cells T cells	↓ FcεRI-dependent lipid mediator and cytokine release, supports progenitor growth Inhibits Th1 differentiation
IL-11[707] FcεRI	T cells Macrophages Epithelial cells	Promotes Th2 differentiation Inhibition of LPS-dependent pro-inflammatory activity Supports tumour growth
IL-13 [55, 273, 387, 390, 714] FcεRI FcγRI TSLP + IL-1β IL-33	Mast cells B cells T cells Monocytes/macrophages Eosinophils Endothelial cells Epithelial cells Airway smooth muscle T cells	↑ FcεRI and releasability, ↑ human lung MC proliferation IgE class switching and secretion Promotes Th2 responses Same as IL-4 (see above) Activation, survival Increases VCAM-1 expression ↑ NO and periostin production, CCL11, GM-CSF, TSLP and TGF-β2 release, ↑ mucus production Enhances sensitivity to contractile agonists Th1 chemotaxis, inhibits Th2 activation
IL-16 [692, 715] FcεRI	Epithelial cells Airway smooth muscle Neutrophils	Enhances mucus secretion Enhances sensitivity to contractile agonists Indirectly promotes recruitment through chemokine up-regulation
IL-17A [716–718] TNF-α IgG complexes C5a TLR4 (LPS)	Lymphocytes	↑ IFN-γ production from Th1, CD8 and NK cells
IL-18 [692] Constitutive	Keratinocytes	Epidermal hyperplasia, antimicrobial peptide secretion, ↑ VEGF secretion promoting angiogenesis
IL-22 [718] Stimuli not known, mRNA expressed constitutively in psoriatic skin	Epithelial cells Airway smooth muscle	Proliferation, antimicrobial peptide secretion, inhibits IL-25 release by airway epithelial cells Enhances sensitivity to contractile agonists, prevents apoptosis, ↑ migration

Table 5 (continued)

Cytokine and known inducing stimuli	Target cells	Important biological effects
IL-24 [719]	Keratinocytes	↑ secretion of pro-inflammatory mediators
T cell-derived microvesicles		
TNF- α [119, 120, 361, 720–722]	Monocytes/macrophages	Increases cytotoxicity, chemotaxis and prolonged survival
Fc ϵ RI	T cells	Promotes class II antigen and IL-2 receptor expression, and increases proliferation
Fc γ RI		
TLR4, -5, -9	Neutrophils	Increases cytotoxicity and chemotaxis
IFN- γ	Eosinophils	Increases cytotoxicity and oxidant production
Nod1 ligand	Endothelial cells	Increases E-selectin, ICAM-1 and VCAM-1 expression, and enhances extravasation of most leucocytes
	Fibroblasts	Growth and chemotaxis, reduces collagen production, increases collagenase, IL-6 and IL-8 production
	Epithelial cells	↑ eosinophil adhesion, ↑ mucus production
	Airway smooth muscle	↑ sensitivity to contractile agonists, ↑ cytokine and chemokine release, induces corticosteroid resistance in combination with IFN- γ
	Mast cells	Promotes histamine and tryptase release
SCF [723]	Mast cells	Growth, differentiation, survival, chemotaxis. Uncouples β_2 -adrenoceptor
Constitutive		
NGF [724, 725]	B cells	Differentiation, proliferation, increased Ig synthesis
Constitutive	T cells	Differentiation, proliferation
	Eosinophils	Proliferation
	Basophils	Activation, mediator release
	Neutrophils	Chemotaxis, survival, mediator release
	Monocytes/macrophages	Proliferation, mediator release
	Fibroblasts	Contraction, migration
	Airway smooth muscle	Migration, contraction, proliferation
	Mast cells	Differentiation, survival, activation, mediator release
TGF- β [726, 727]	Airway smooth muscle	Differentiation, ↑ α -smooth muscle actin, ↑ contraction, activation
Constitutive	Fibroblasts	Myofibroblast differentiation, collagen secretion, contraction
	Epithelial cells	Inhibition of proliferation, EMT
	Endothelial cells	Increases angiogenesis
FGF-2 [115, 121, 128, 727, 728]	Fibroblasts	Proliferation
Fc ϵ RI	Airway smooth muscle	Proliferation
	Endothelial cells	Increases angiogenesis
VEGF [729–732]	Endothelial cells	Angiogenesis
Substance P		
Adenosine		
PGE ₂		
IFN- α [239]	Natural killer cells	Increases cytotoxicity
TLR3	Macrophages	Development, maturation
	Dendritic cells	Activation, maturation, increased IFN- γ production
	T cells	Increases survival of activated T cells, promotes Th1 phenotype, inhibits Th2 phenotype
TSLP [392, 452, 593]	Dendritic cells	Induces Th2 immunity
Fc ϵ RI	ILC2	Activation
	Mast cells	Increases IL-5 and -13 expression
Amphiregulin [453, 614]	Fibroblasts	Induces proliferation
Fc ϵ RI	Epithelial cells	Increased mucin gene expression

NGF, nerve growth factor; FGF, fibroblast growth factor; TSLP, thymic stromal lymphopoietin; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein.

release activated Ca²⁺ current (I_{CRAC}), and this plays a central role in many physiological processes such as gene transcription, proliferation and cytokine release [165]. I_{CRAC} has been well characterized electrophysio-

logically in several cells including rodent MCs [166], and more recently, human MCs [95]. The six transmembrane protein Orai1 (also known as CRACM1) forms hexamers which create the channels carrying I_{CRAC}

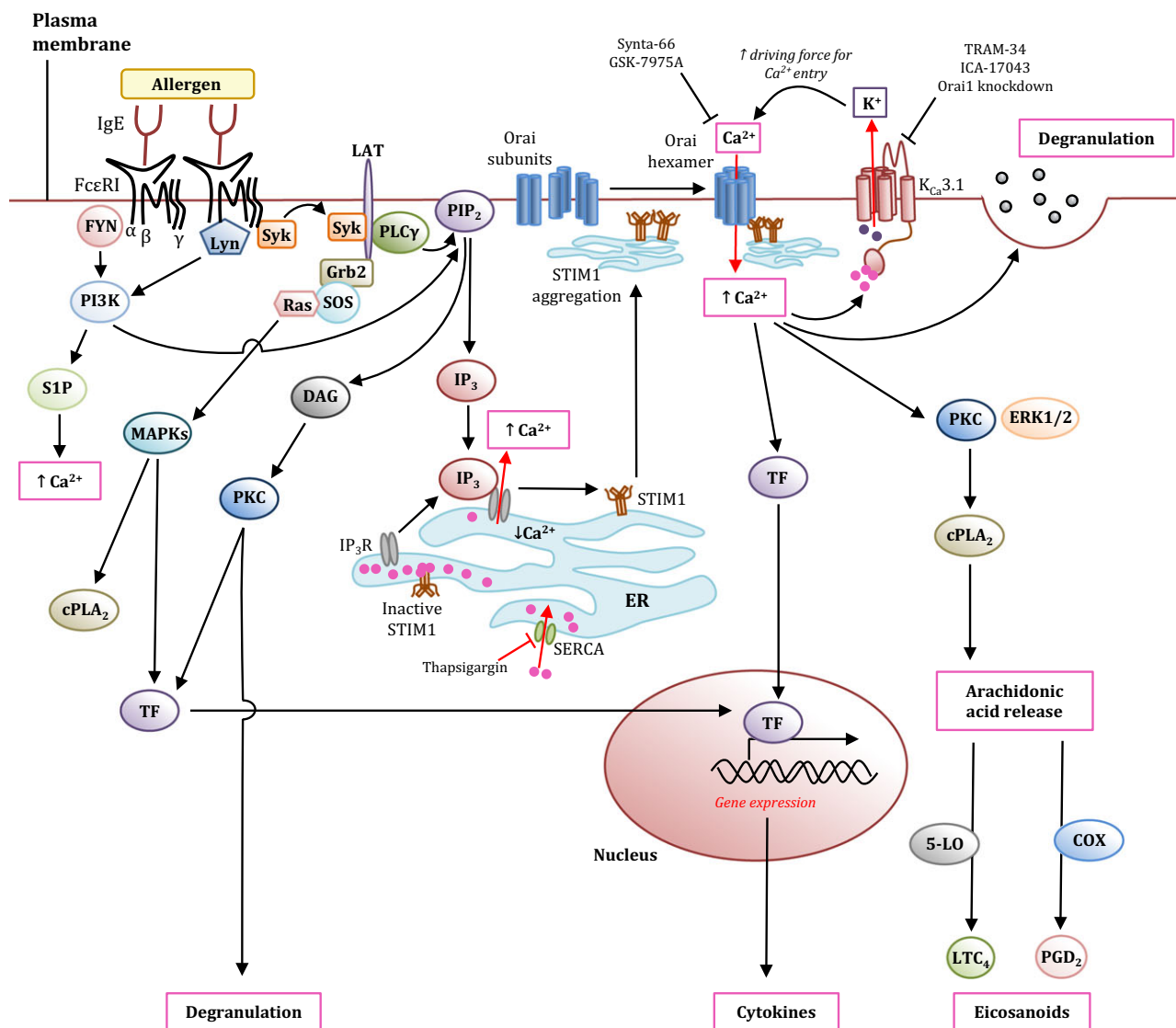


Fig. 6. FcεRI-dependent activation of mast cells. Allergen binding to FcεRI-bound IgE causes FcεRI aggregation and cross-linking. This triggers the phosphorylation of the adaptor molecule LAT by Lyn and Syk kinases. Phosphorylated LAT binds to the signalling enzyme PLCγ, which subsequently hydrolyses PIP₂, a phospholipid component of cell membranes, into the secondary messengers IP₃ and DAG. Phosphorylated LAT is also able to bind to the cytosolic adaptor molecule GRB2, which binds to SOS. Activated SOS promotes the activation of Ras, and this initiates a Ras-MAPK cascade. The MAPK pathway induces TF activation and subsequent cytokine production and also promotes arachidonic acid metabolism and the generation of eicosanoids (such as LTC₄ and PGD₂) via cPLA₂. TF activation, gene expression, cytokine production and MC degranulation also occur via the PKC pathway, which can be induced by the secondary messenger DAG, and via increased mobilization of intracellular Ca²⁺. A biphasic increase in Ca²⁺ mobilization is triggered by binding of IP₃ to its receptors on the Ca²⁺ stores of the ER. This leads to translocation and aggregation of STIM1 near the plasma membrane, which promotes the aggregation of Orai subunits and the formation of hexameric Orai channels. Activation of Orai channels by STIM1 binding leads to Ca²⁺ influx, and this initiates MC degranulation, TF activation and gene expression. Ca²⁺ influx through Orai channels also induces the activation of cPLA₂ and 5-LO via Ca²⁺-dependent PKC and ERK1/2, leading to arachidonic acid metabolism and eicosanoid generation by 5-LO and COX enzymes. Ca²⁺ influx via Orai can be augmented by KCa3.1 channel activation; binding of Ca²⁺ to KCa3.1 via its Ca²⁺-sensing domain, calmodulin, leads to KCa3.1 activation and rapid K⁺ efflux. This causes membrane hyperpolarization and enhances the driving force for Ca²⁺ entry via Orai, thus modulating downstream effects of Ca²⁺ influx such as MC degranulation. Ca²⁺ mobilization is also thought to be induced by a SK-S1P pathway: FcεRI aggregation is thought to activate SK via Lyn and Fyn, resulting in S1P formation. SK activity is regulated by PLD, which is activated by PI3K following FcεRI aggregation. Hence, PI3K and S1P may be involved in Ca²⁺ mobilization and thus modulate Ca²⁺-dependent processes such as degranulation in MCs. Red arrows indicate the movement of free ions; black arrows represent a pathway or sequence of events. LAT, linker for activation of T cells; PLCγ, phospholipase Cγ; PIP₂, phosphatidylinositol 4,5-bisphosphate; IP₃, inositol-1,4,5-triphosphate; DAG, diacylglycerol; GRB2, growth factor receptor-bound protein 2; SOS, son of sevenless homologue; MAPK, mitogen-activated protein kinase; TF, transcription factor; LTC₄, leukotriene C₄; PGD₂, prostaglandin D₂; cPLA₂, Ca²⁺-dependent phospholipase A₂; ERK, extracellular signal-regulated kinase; 5-LO, 5-lipoxygenase; COX, cyclooxygenase; ER, endoplasmic reticulum; STIM1, stromal interaction molecule-1; SK, sphingosine kinase; S1P, sphingosine-1-phosphate; PLD, phospholipase D; PI3K, phosphoinositide 3-kinase.

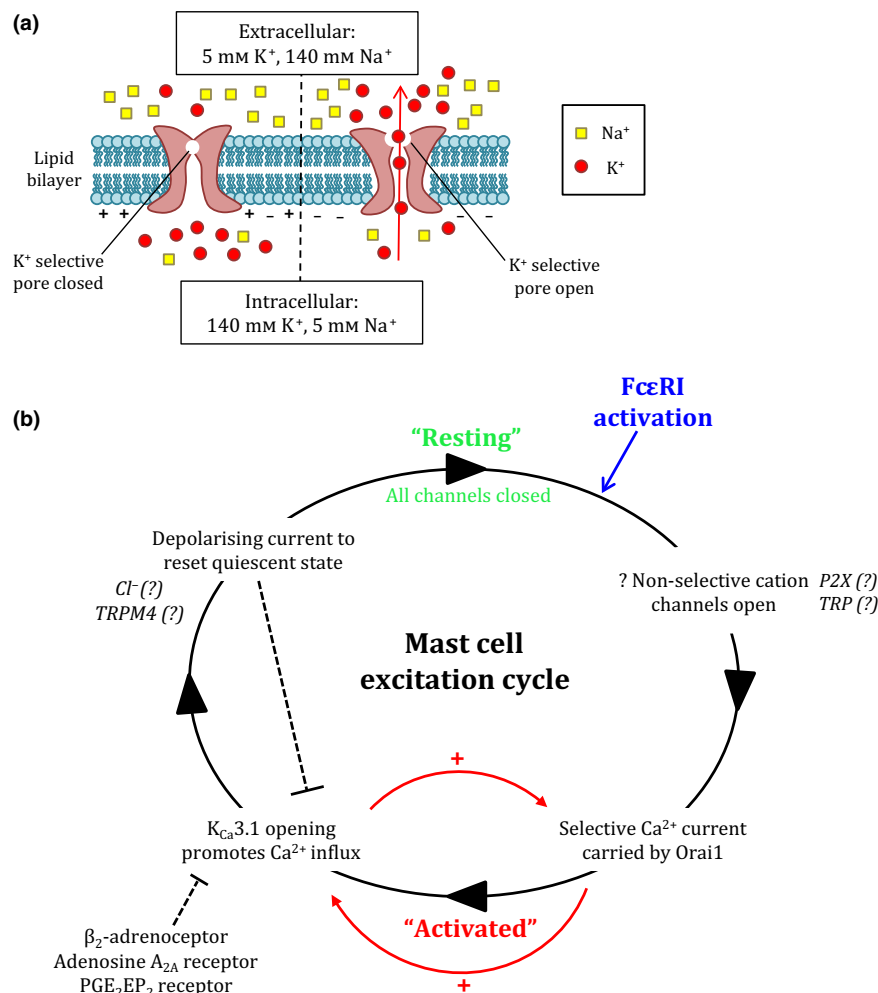


Fig. 7. Ion channels in mast cells. (a) The driving force of ion channel activity across plasma membranes consisting of a lipid bilayer. Ion channels bypass the hydrophobicity of the plasma membrane by providing a selective hydrophilic aqueous pore. Different channels selectively allow specific ionic species to pass through by discriminating between the size and charge of the molecule, thus allowing ions to be maintained at highly explicit concentrations within the cell. The movement of ions through an open ion channel is rapid and occurs passively down the ionic concentration and electrical gradient. (b) Putative human mast cell electrical excitation cycle.

[167–170]. Two closely related channels, Orai2 and Orai3 have also been identified [171]. These are also able to carry CRAC currents, but exhibit distinct functional properties, and may form heterodimeric channels with Orai1 [171].

Work using Orai1 knockout mice demonstrated that Orai1 is essential for mouse MC degranulation, LTC₄ release and TNF-α production following IgE-dependent activation, while in parallel experiments Orai2 regulated T cell responses [172]. In human lung MCs, mRNA for all three Orai channel subunit homologues and protein for Orai1 and Orai2 are present. Human lung MC viability *in vitro* is unaffected by the expression of dominant-negative Orai subunit mutants which ablate Orai currents, and so Orai channels do not appear to play a

role in lung MC survival. Pharmacological inhibition of Orai channels in human lung MCs attenuated degranulation, LTC₄ release and cytokine production [95], while knockdown studies showed that Orai1 is the dominant Orai channel regulating the release of these mediators [173]. Block of Orai channels with the compound Synta66 also inhibited allergen-dependent ASM contraction in isolated bronchial rings [95] (Fig. 6). This suggests that pharmacological block of Orai channels in asthmatic subjects will inhibit the MC-dependent early reaction following bronchial allergen challenge.

Similar consequences on activated MCs have been seen following knockdown of stromal interaction molecule (STIM)-1, a 77 kDa protein that couples store depletion with Ca²⁺ influx through Orai channels [174,

175]. Upon store depletion, STIM1 translocates towards the plasma membrane, where it facilitates clustering of Orai subunits and the subsequent activation of Orai channels (Fig. 6). STIM1-deficiency in fetal liver-derived MCs attenuates FcεRI-induced Ca^{2+} influx, degranulation and cytokine release following FcεRI activation [176]. Thus, both Orai channels and STIM1 are key proteins facilitating the Ca^{2+} influx required for FcεRI-mediated MC activation and subsequent mediator release.

K^+ channels regulate Ca^{2+} influx in many cells due to their ability to hyperpolarize cell membranes when open. This increases the electrical driving force for Ca^{2+} (Fig. 6), but more importantly, Orai channels are inwardly rectifying, and conduct larger currents at more negative (hyperpolarized) membrane potentials [166]. In both RBL cells and rat IL-3-dependent bone marrow-derived MCs, an inwardly rectifying K^+ channel (Kir2.1) is open when the cells are at rest [177, 178]. However, the K^+ channels present in human MCs differ to those in rodents highlighting a good example of species-dependent heterogeneity. Of note we have never seen a Kir current in a human MC from any source [144, 179–181]. Thus the majority of human lung MCs and human peripheral blood-derived MCs are electrically 'silent' at rest with a resting membrane potential of around 0 mV.

Following FcεRI-dependent activation, human MCs open the intermediate conductance Ca^{2+} -activated K^+ channel $\text{K}_{\text{Ca}3.1}$ (Fig. 7b) [144, 180]. This was also observed in rodent MCs relatively recently [182]. $\text{K}_{\text{Ca}3.1}$ channels indirectly enhance Ca^{2+} influx and histamine release in both human lung MCs and mouse bone marrow MCs, but are not critical for secretion and can thus be considered as increasing the gain of an immunological stimulus [144, 180]. Conversely, FcεRI-dependent $\text{K}_{\text{Ca}3.1}$ channel opening in human lung MCs has a critical requirement for influx of extracellular Ca^{2+} via Orai1 channels [144, 183]. In fact, $\text{K}_{\text{Ca}3.1}$ interacts physically with Orai1 but not Orai2, suggesting the channels operate in a tight spatiotemporal signalplex [183]. $\text{K}_{\text{Ca}3.1}$ is closed in human lung MCs by Gs-coupled ligands which inhibit MC secretion such as β_2 -adrenoceptor agonists [184], adenosine [185] and PGE_2 [21], providing a mechanism for the coupling of receptor activation and impaired secretion. Interestingly, this effect appears to act through a cAMP-independent mechanism. In mice, further studies of signalling pathways influencing $\text{K}_{\text{Ca}3.1}$ activity have shown that PI-3KC2β is also essential for FcεRI-dependent activation of $\text{K}_{\text{Ca}3.1}$ and Ca^{2+} influx in MCs [186]. The tripartite motif containing protein TRIM27 functions as an E3 ligase to ubiquitinate and inhibit PI3KC2β's kinase activity and, via this effect, functions

to negatively regulate $\text{K}_{\text{Ca}3.1}$ channel activity and FcεRI-dependent Ca^{2+} influx both *in vitro* and *in vivo* [186]. A diagrammatic representation of the putative human MC electrical excitation cycle can be seen in Fig. 7b.

$\text{K}_{\text{Ca}3.1}$ is widely expressed in the lungs, in MCs, T cells [187–189], airway epithelial cells (unpublished), fibroblasts [190–192] and ASM cells [193, 194] and contributes to potentially pro-inflammatory and remodelling activity. This makes $\text{K}_{\text{Ca}3.1}$ an attractive target for asthma as its inhibition may target several dysfunctional airway elements. Two animal models of asthma support this view. In an OVA mouse model, the $\text{K}_{\text{Ca}3.1}$ blocker TRAM-34 delivered subcutaneously, significantly attenuated OVA-induced airway eosinophilia, AHR and several parameters of remodelling including a reduction in ASM mass, subepithelial fibrosis and peribronchial fibrosis [195]. As we do not have any treatments that currently target airway remodelling, this might be a very important observation. In a house dust mite-driven sheep model, the $\text{K}_{\text{Ca}3.1}$ blocker Senicapoc (ICA-17043) delivered orally, significantly reduced resting lung resistance, nonspecific AHR to carbachol and the early-phase bronchoconstrictor response to allergen challenge, and reduced BAL eosinophilia and vascular remodelling [196]. A phase IIa allergen challenge study of a $\text{K}_{\text{Ca}3.1}$ blocker (ICA-17043, Senicapoc) has not been published in full, but was reported by Icagen, Inc. to reduce the late asthmatic response by approximately 30% [197]. These studies suggest that targeting $\text{K}_{\text{Ca}3.1}$ channels expressed by MCs and other airway cells, may improve both the inflammatory and remodelling components of asthma. An *in vitro* study of corticosteroid resistance also suggested that $\text{K}_{\text{Ca}3.1}$ blockers may reverse this [194].

Activation of nonselective cation channels including some members of the transient receptor potential (TRP) family results in depolarization of cell membranes due to the cellular exit of K^+ and entry of Na^+ down their respective concentration gradients. In MCs this would be expected to reduce the driving force for Ca^{2+} influx through CRAC channels and therefore the amount of Ca^{2+} entry. This appears to be the function of TRPM4 channels in mouse MCs [198]. TRPM4 carries Na^+ and K^+ and is activated by Ca^{2+} although it does not conduct it. Bone marrow-derived MCs from a TRPM4 knockout mouse showed both increased Ca^{2+} entry and increased release of mediators (histamine, leukotrienes and $\text{TNF-}\alpha$) compared to their wild-type counterparts [41]. In addition, MC migration was impaired in bone marrow-derived MCs from the TRPM4 knockout due to inhibition of the Ca^{2+} dependent cytoskeletal re-arrangement required for cell migration [199]. TRPM4 channels therefore provide a

potential regulatory negative feedback loop between TRPM4 and Orai channels.

Mast cell activation by monomeric IgE

In addition to the cross-linking of FcεRI-bound IgE molecules by allergen, the binding of IgE alone to FcεRI can initiate intracellular signalling events with protein phosphorylation, Ca²⁺ influx and subsequent mediator release [200–208]. In rodent MCs IgE stimulates the release of granule-derived mediators and the secretion of cytokines including IL-6 [200, 203, 205]. In human cord blood-derived MCs IgE induces the release of the cytokines CCL1, CCL3 and GM-CSF without histamine release [209]. However, in human lung MCs IgE induces the secretion of histamine, LTC₄ and IL-8, which is markedly enhanced in the presence of SCF [208]. With both rodent MCs and HLMCs, the release of IL-6 in the presence of IgE prolongs cell survival after SCF withdrawal [53, 200]. Interestingly, in both rodent MCs and HLMCs, ongoing signalling requires the presence of 'free' IgE, and this ceases immediately when free IgE is removed, suggesting that these findings are physiologically relevant [208, 210]. The mechanisms behind this are uncertain but in part are thought to involve FcεRI aggregation and are described in detail in a recent review [211].

The ability of IgE alone to activate MCs and promote their survival is potentially highly relevant to asthma because both SCF and free IgE concentrations are elevated in asthmatic airways, and there is a robust correlation between total serum IgE, and the presence of asthma and AHR [212–216]. This provides a potential mechanism for the ongoing activation of MCs through FcεRI in the absence of allergen and could explain in part the efficacy of anti-IgE therapy in chronic allergic disease [155, 217].

Superantigens

Human lung and/or heart MCs can be activated through FcεRI by bacterial, viral and endogenous superantigens including several Staphylococcal enterotoxins, Peptostreptococcus protein L [218], protein Fv (released from the liver during infection with hepatitis viruses A, B, C and E) [219] and the HIV-1 protein gp120 [220] (and reviewed in [221]). Each of these superantigens is capable of binding and cross-linking IgE bound to FcεRI, leading to classical FcεRI-dependent mediator release (reviewed in [221], Fig. 8). Increased exposure to Staphylococci has been documented in patients with atopic eczema, asthma and nasal polyposis and has been linked to asthma severity [222–227]. It is therefore postulated that Staphylococ-

cal superantigens may contribute to both the pathogenesis and severity of these diseases and engage MCs in the process.

FcεRI-independent activation

MCs express numerous classes of receptors which mediate their activation by diverse stimuli independently of IgE (Fig. 8). Stimuli include proteases (including tryptase) [228, 229], cytokines (e.g. SCF, TNF-α, IFN-γ, IL-33, TSLP) [55, 230–233], complement [234, 235], platelet activating factor [236], adenosine [237], toll-like receptor ligands [238, 239], bacterial products [240], neuropeptides (particularly skin MCs) [230, 241] and hyperosmolality [242–244]. Many of these are likely to contribute to MC activation in the complex environment of the asthmatic airway, and some examples are discussed in the following section.

Proteases. Histamine release from human MCs in several tissues, including the lung, can be induced by human tryptase [228, 229, 245]. In addition, tryptase inhibitors reduce FcεRI-dependent human lung MC histamine release, suggesting that tryptase amplifies this response in an autocrine fashion [228]. *In vivo* studies in dogs and sheep have demonstrated that exogenously administered tryptase induces bronchoconstriction and AHR [246, 247] and *in vitro*, tryptase potentiates the contractile response of sensitized bronchi to histamine [248]. *In vivo*, pre-treatment with antihistamines block these effects of tryptase, suggesting that MC activation is likely to play a role.

Complement. The C5a receptor CD88 is expressed on the MC_{TC} subset of human lung MCs [249]. Elevated C5a concentrations have been identified in the induced sputum of asthmatic subjects [250], thus providing an alternative means of MC activation in chronic disease, and of particular relevance to those MCs (MC_{TC}) within the ASM bundles (see below).

SCF and Kit. The engagement of Kit by SCF augments human lung MC activation. At 10 ng/mL SCF potentiates IgE-dependent MC mediator and cytokine release, while at 100 ng/mL it directly activates the cells for histamine, LTC₄ and PGD₂ release [208, 251–255]. SCF also enhances LTD₄-induced Ca²⁺ mobilization and expression of inflammatory genes such as TNF-α in the LAD2 human MC line [256], and LTD₄ and SCF together induce the synergistic activation of MC Kit [256].

Another interesting feature of SCF is its ability to rapidly inhibit β₂-adrenoceptor (β₂-AR) signalling in HLMCs and HMC-1 cells through the phosphorylation of the β₂-AR on Tyr350 within 2 min of exposure

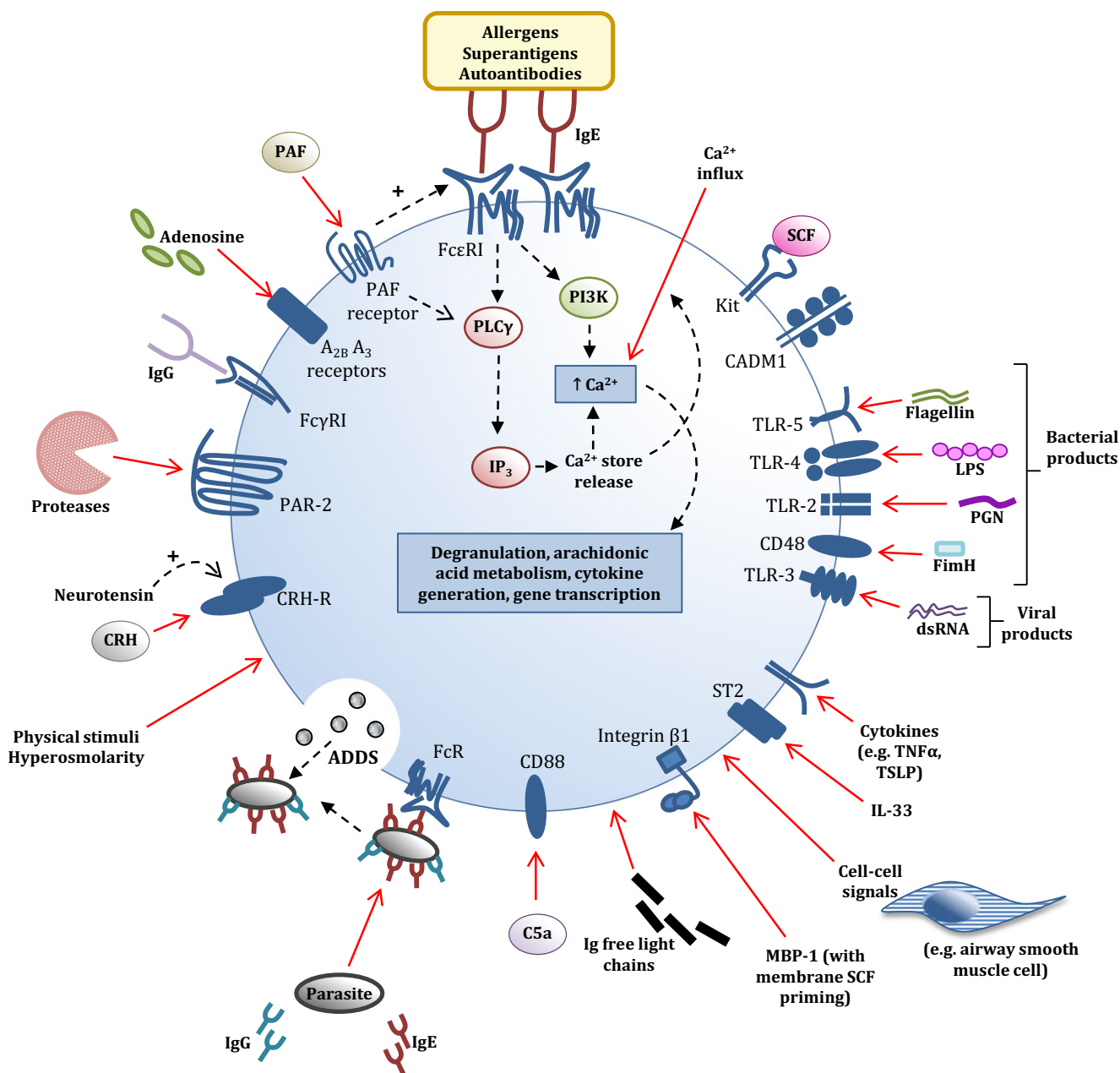


Fig. 8. Receptors capable of modulating human mast cell activation. Human MCs express a broad range of receptors, allowing them to respond to a diverse range of stimuli, including via IgE-independent mechanisms. Downstream pathways of each individual receptor are not fully understood, but ultimately lead to MC degranulation, gene transcription, arachidonic acid metabolism and cytokine production. Among the well-known signalling pathways are the PLC γ and PI3K pathways which induce an increase in intracellular Ca²⁺ mobilization and Ca²⁺ influx. PAF, platelet activating factor; PI3K, phosphoinositide 3-kinase; PLC γ , phospholipase C γ ; IP₃, inositol-1,4,5-triphosphate; TLR, toll-like receptor; LPS, lipopolysaccharide; PGN, peptidoglycan; MBP-1, major basic protein-1; FcR, Fc receptor; CRH, corticotropin-releasing hormone; CRH-R, CRH receptor; PAR, protease-activated receptor.

[257]. This reduces β_2 -AR-dependent inhibition of both histamine and LTC₄ release and prevents β_2 -AR agonists from closing the K_{Ca}3.1 ion channel [257]. Interestingly, in the presence of both IgE and SCF, β_2 -AR agonists actually increase histamine release in a dose-dependent manner [257]. If this occurs in vivo, it might explain

why β_2 -AR agonists used regularly sometimes reduce asthma control [258, 259] and enhance both MC mediator release and the MC-dependent early asthmatic response following allergen challenge [260, 261]. As SCF and Kit expression are increased in asthmatic airways [262, 263], and MCs within the ASM bundles are

exposed to membrane-bound SCF present on the surface of ASM cells, such a mechanism is highly plausible. Thus targeting SCF-Kit might not only exert anti-inflammatory effects through the inhibition of MC function, but also remove the potentially detrimental effects of β_2 -AR agonists.

TNF- α . *In vitro*, TNF- α either directly stimulates or potentiates human MC degranulation [264–266]. Furthermore, following IgE-dependent activation the release of preformed MC-associated TNF- α has been shown to serve as a positive autocrine feedback signal to augment NF-kappaB activation and further production of TNF- α and other cytokines, including GM-CSF and IL-8 [232]. A small double-blind placebo-controlled crossover study of Etanercept for 10 weeks in patients with severe refractory asthma resulted in a marked reduction in sputum histamine concentration in parallel with improvement of several clinical parameters [267]. This suggests that the *in vitro* effects of TNF- α on MC mediator release may be relevant *in vitro*, although it should be noted that larger studies of anti-TNF- α treatment in unselected patients with asthma have failed to show clinical benefit.

IL-33. IL-33 is expressed by several structural cells within asthmatic airways, with increased expression evident in both the ASM [268] and airway epithelium [269]. IL-33 is released from ASM cells by TNF- α and IFN- γ [268]. Human cord blood-derived MCs express the receptor for IL-33 known as ST2 [55] and respond to IL-33 stimulation with the release of several cytokines and chemokines including IL-5, IL-8, IL-13 and TNF- α [55, 270]. In the presence of IgE, IL-33 may induce human cord blood MC degranulation and PGD₂ generation [270], but this is not a consistent finding [55]. In HMC-1 cells and mouse bone marrow-derived MCs, IL-33R-dependent signalling was shown to induce and require cross-activation with Kit, with evidence of a physical association between the two receptors [271].

It has been suggested that IL-33 derived from ASM may play a key role in the activation of ASM-infiltrating MCs and the propagation of ASM dysfunction in asthma. However, chronic exposure (> 72 h) of human cord blood MCs to IL-33 resulted in substantially reduced Ca²⁺ mobilization and degranulation in response to Fc ϵ RI-dependent activation [272]. This reprogramming to a hyporesponsive phenotype occurred due to the MyD88-dependent attenuation of signalling processes necessary for MC activation, including Fc ϵ RI-mediated Ca²⁺ mobilization and cytoskeletal reorganization, potentially as a consequence of PLC γ 1 and Hck down-regulation. These find-

ings suggest that IL-33 may play a protective rather than a causative role in MC activation under chronic conditions. The potential outcomes of targeting IL-33 in asthma are therefore uncertain.

TSLP and IL-1 β . TSLP and IL-1 β derived from activated human epithelial cells and ASM cells work co-operatively to induce the release of numerous cytokines and chemokines from human MCs, including IL-5 and IL-13 [233, 273, 274], but do not induce degranulation. This is discussed in more detail later. Several further chemokines and cytokines have consistently failed to induce degranulation of HLMCs or enhance the IgE-dependent activation of these cells after brief incubation [254, 275].

Fc γ RI-dependent activation. IFN- γ up-regulates the expression of Fc γ RI on human peripheral blood-derived MCs and human lung MCs [276–278]. Aggregation of Fc γ RI on human MCs after IFN- γ exposure leads to degranulation, generation and release of both PGD₂ and LTC₄, and the up-regulation of mRNA expression for several cytokines. The relevance of this to asthma however is unclear.

Immunoglobulin free light chains. Immunoglobulin (Ig) free light chains are present in serum in healthy 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, and antigen-specific light chains can mediate MC-dependent bronchoconstriction following antigen challenge [279]. Concentrations of Ig free light chains are elevated in the sera of asthmatic compared to healthy subjects and may be relevant to the pathophysiology of human asthma [279].

Toll-like receptors and microbial products. Toll-like receptors (TLRs) 1–7 and –9 play an important role in the innate host response to pathogens, activating diverse programmes of gene expression depending on the stimulus. Many of these are expressed by both rodent and human MCs [119, 238, 239, 280–283]. These are described further in the section detailing the role of MCs in host defence.

Platelet activating factor. Platelet activating factor (PAF) activates human MCs, either indirectly in the skin, or through a direct receptor-mediated pathway in the lungs [236]. Activation of human skin MCs by PAF requires a neural reflex [284] and isolated human skin MCs do not degranulate in response to PAF directly [236, 285]. In contrast, exposure of human lung and peripheral blood-derived MCs to PAF at con-

centrations measured in human blood during anaphylactic reactions induces rapid histamine release and enhances IgE-dependent mediator release [236]. PAF also induces release of PGD₂ and to a lesser extent CXCL8 and transiently up-regulates mRNA expression for several other chemokines in human peripheral blood-derived MCs [236]. These processes are partially dependent on Ca²⁺ influx and involve signalling via the PAF receptor coupled to G_{αi} with activation PLCγ1 and PLCβ2.

PAF is implicated in the pathogenesis of anaphylaxis [286], a condition characterized by the systemic activation of MCs. The reason for the systemic spread of MC activation has been assumed to relate to the systemic spread of allergen, but this is questionable, and amplification mechanisms should be considered. In humans PAF concentrations are markedly elevated in the plasma following anaphylactic reactions, and these correlate strongly with the severity of the response [286]. Furthermore, the chief metabolizing enzyme for PAF, PAF acetylhydrolase, demonstrates reduced expression in patients with anaphylaxis [286]. The generation of PAF in anaphylaxis therefore provides a potential mechanism whereby PAF directly amplifies the effects of allergen exposure on MC mediator release in the airways and lung parenchyma. The source of PAF in human anaphylaxis is uncertain but in part might come from MCs [287]. Thus it has been proposed that PAF generated locally by MCs and perhaps other cells in response to MC activation at the point of allergen contact may lead to an exaggerated MC response at more distal sites, such as the airway [236]. In turn, skin MCs may be activated indirectly by PAF through neural reflexes [284]. Such a PAF amplification loop may be a key factor in the generation of systemic anaphylaxis.

Adenosine. Adenosine is a purine nucleoside generated by numerous cell types in response to cell stress and hypoxia. It modulates human MC secretion through the adenosine A_{2A} and A₃ GPCRs and is of particular relevance to asthma [288–290], but the effects of adenosine on human lung MC secretion both *in vitro* and *in vivo* are complex. At relatively low concentrations *in vitro* (10⁻⁶ M), adenosine may enhance IgE-dependent human lung MC secretion, but the reported effects vary widely [291–296]. Because adenosine induces cytokine secretion from a human MC line through the A_{2B} receptor [297], it was also thought that potentiation of secretion from human lung MCs is mediated via the A_{2B} receptor. However, a more recent study using human lung MCs has provided strong evidence that this involves the A₃ receptor [296].

At higher concentrations of adenosine (10⁻⁵ to 10⁻³ M), there is more consistent dose-dependent inhi-

bition of MC secretion [185, 291, 292, 295, 298]. This is mediated predominantly via the A_{2A} receptor [299], although in human skin MCs adenosine influx via equilibrative nucleoside transporter 1 (ENT1) also plays an inhibitory role [300]. *In vivo*, AMP (which rapidly breaks down to adenosine) induces histamine and tryptase release from resident MCs when delivered locally the airways [301], and adenosine induces bronchoconstriction in subjects with asthma but not healthy subjects when delivered by aerosol [302]. It is thought that these *in vivo* effects are mediated directly on MCs although it remains possible that intermediaries and neural reflexes are also involved [303].

A proposed mechanism of action for the bronchodilator theophylline is thought to be the antagonism of adenosine receptors. This is suggested by the finding that theophylline provides better inhibition against adenosine-induced bronchoconstriction than histamine, potentially via A_{2B} receptor blockade [304]. Additionally, AHR in adenosine deaminase-deficient mice, which exhibited elevated levels of adenosine in the lungs, can be reversed by theophylline [305].

Stress. Psychological stress is thought to induce MC activation in different tissues via the stimulation of peripheral nerves and the release of neuropeptides and hormones such as substance P and corticotropin-releasing hormone (CRH), respectively [306–308]. This has been studied predominantly in animals, with all the usual caveats that this brings, but there is also indirect evidence from human studies. Human cord blood-derived MCs and HMC-1 cells express CRH receptors and their activation by CRH induces the selective release of VEGF without degranulation [309, 310]. Neutensin, a neuropeptide secreted under stress, can also trigger human cord blood-derived MC degranulation and induces CRH-R1 expression, thus augmenting MC activation [311]. These findings implicate a role for MCs in pathophysiological situations that are thought to derive from stress and provide a hypothetical link between stress and asthma exacerbations [312].

Altered threshold for mast cell activation. There may be genetic factors which lower the MC threshold for activation in asthma. For example, an important negative regulator of MC activation is src homology 2-containing inositol phosphatase (SHIP) [201]. SHIP-deficient rodent MCs exhibit markedly enhanced secretory responses, and human basophils that are 'hyper-releasable' demonstrate a relative deficiency of this molecule [313]. However, human peripheral blood-derived MCs from healthy controls and patients with atopic asthma are activated to a similar extent via FcεRI, suggesting that genetic factors directly regulating FcεRI-dependent releasability in asthma are less likely to be relevant

[314]. Whether this is true for other mechanisms of activation is not known.

Directional mast cell mediator release. A novel directional mechanism of MC degranulation, termed the antibody-dependent degranulatory synapse (ADDS), has recently been described by Joulia et al. [315]. In this study, human peripheral blood-derived MCs were examined with live cell imaging, using fluorochrome-labelled avidin as a probe which binds to heparin during degranulation. The first observation of note was that granule contents including tryptase and chymase were retained on the mast cell surface for several hours after FcεRI-dependent activation. This is in keeping with earlier work suggesting that mast cells express IL-4, a heparin/heparan-binding cytokine, on their cell surface [120, 316], and that they are capable of presenting functionally active proteoglycan-bound molecules such as IFN-γ on their cell surface when added exogenously [317].

Joulia et al. then showed that IgE already bound to CD20 on B cells was able to activate FcεRI on human MCs in co-culture leading to MC degranulation, and this degranulation localized predominantly at points of MC-B cell contact. Similar results were seen with IgG-bound B cells through activation of FcγRIIA on human MCs and also in the presence of IgG opsonized parasites. This highlights the ability of mast cells to establish discrete directional cell–cell crosstalk.

Inhibitory receptors on mast cells. MCs express several receptors which contain immunoreceptor tyrosine-based inhibition motifs (ITIMs). These regions are phosphorylated upon receptor activation and recruit phosphatases which subsequently dephosphorylate important signalling molecules, thus suppressing cell activation (for a detailed review see reference [318]). They are especially active when co-ligated with FcεRI and/or Kit, leading to inhibition of FcεRI and/or Kit-dependent signalling. Inhibitory molecules expressed by human MCs with functional activity include siglec-7 [319], siglec-8 [320], CD300a [321] (does not contain classical ITIM) and CD72 [322]. There is therefore the potential to inhibit MC activation through the administration of bi-specific antibodies targeting both the ITIM receptor and FcεRI or Kit [323]. With respect to FcεRI, whether such an approach would be more effective than the currently available anti-IgE molecule Omalizumab is uncertain. The tetraspanin CD151 is expressed on human MCs, and its deficiency in mouse MCs enhances FcεRI-dependent cytokine production but not degranulation [324].

Resolving mediators of inflammation include lipoxins, resolvins, protectins and maresins. They promote the resolution of inflammation and potentially prevent the development of fibrosis, acting via G_i-coupled

receptors. Pre-incubation of human lung MCs with resolvin D1, D2 or lipoxin A₄ for 10 min, produced a marked dose-dependent inhibition of subsequent FcεRI-dependent histamine release in the dose range 10⁻¹² to 10⁻⁶ M [325]. Even at 10⁻¹² M, human lung MC histamine release was reduced by about 50% with each of these mediators compared to vehicle control. In further support of these findings, lipoxin B4 reduced MC degranulation in vivo in a mouse model of allergic inflammation [326].

Mast cell interactions with the immune system

Host defence

MCs are activated by diverse pathogens via a variety of different mechanisms the results of which can have both protective and detrimental effects on the host. Consequently, the presence of MCs can be seen as a double-edged sword; while they have the potential to contribute to host defence against infection, the release of their potent mediators and cytokines can aggravate lung pathology and lead to a decline in airway function.

Defence against parasite infection. MCs were long considered to provide the primary defence against parasite infections and be important for the expulsion of parasites (reviewed in [327, 328]). However, although neutralization of IL-3 and IL-4 in mice infected with *Nippostrongylus brasiliensis* abrogates the parasite-induced gut MC hyperplasia and associated IgE synthesis, it does not alter the clinical course [329]. Hence, the role of MCs in immunological responses to parasites is far from clear.

The benefits of MC activation during parasite infection are implied by the presence of elevated levels of parasite antigen-specific IgE, increases in the number of MCs at infection sites and detection of activated MCs in proximity to the infectious agents [330–332]. Helminth infections often induce an increase in MC numbers in infected tissue, and antibodies targeting SCF or Kit prevent effective removal of *Trichinella spiralis* in mice [333, 334]. Similarly, IgE-deficient mice were slower to eliminate *Trichinella spiralis* and exhibited larger numbers of larvae 28 days postinfection than wild-type animals [335]. Mouse MC protease-6 (mMCP-6, a form of tryptase) appears to provide a critical role in the mouse immune response to *Trichinella spiralis* through its ability to recruit eosinophils to the site of larval infection [336].

Joulia et al. showed that human cord blood-derived MCs are activated by IgG opsonized *Toxoplasma Gondii*, with polarized degranulation occurring at the point of pathogen contact [315]. This in turn led to *T. gondii*

killing through a tryptase-dependent mechanism. Thus, MCs may well play a role in the immune response against parasite infection in humans *in vivo*.

Defence against bacterial infection. The strategic positioning of MCs in the skin and at mucosal surfaces and their ability to recognize pathogens via several mechanisms suggests that they contribute to immune defence against bacteria. A critical role for MCs in the host response to acute bacterial infection was first indicated by studies in MC-deficient mice. Experiments from several laboratories using a diverse range of bacteria and experimental models demonstrated that TNF- α release from resident MCs at various tissue sites was an essential prerequisite for neutrophil recruitment and activation, which was required to control the infection [282, 337, 338]. Further MC mediators such as leukotrienes, proteases, IL-6 and neurolysin have since been identified as protective factors in similar infective models [339–343]. MCs can also limit the toxicity of bacterial infection in a model of peritoneal sepsis by producing endothelin-1, a peptide with potent vasoconstrictor activity, and they carefully regulate the levels of this peptide by degrading it with MC-derived proteases [344]. MCs may also protect against the adverse effects of infection by modulating the activity of other immune cells. In a mouse model of life-threatening *Francisella tularensis* infection, TLR2-dependent IL-4 production by recruited MCs significantly inhibited bacterial uptake and growth within macrophages [345, 346].

MCs may also have detrimental effects during infection. Some recent studies demonstrated that MC activation and TNF- α production can also potentially increase mortality in septic mice [341, 347]. The critical factor appears to be the severity of sepsis induced, which reiterates the point that while it is likely that the principle role of MCs is to initiate a programme of inflammation and repair, their activation may have potentially adverse effects. So the demonstration of either protective or adverse effects in models of sepsis should not come as a surprise.

In vitro systems indicate that rodent and human MCs can directly reduce bacterial viability through several mechanisms [240, 348–350]. For example, human cord blood-derived MCs can internalize and kill several bacteria potentially opsonized by circulating antibodies or complement components in serum, and in parallel, release TNF- α within 30 min of exposure [350]. However, *Staphylococcus aureus* are also able to enter human cord blood-derived MCs and evade destruction [351], in part through a CD48-dependent mechanism. FimH, a mannose-binding subunit on type-1 fimbriae expressed by *Escherichia coli* and other enterobacteria, also binds to CD48 on rodent MCs [352]. Under

opsonizing conditions it was reported that this triggers both degranulation and MC phagocytosis and destruction of the bacteria by both oxidative (sequestering oxygen radicals to produce an oxidative burst) and nonoxidative (acidifying the endocytosed capsule by fusing with lysosomes) bactericidal systems [353, 354], in much the same way as professional phagocytes. However, other studies have shown that CD48 triggers the formation of bacteria-encapsulating caveolar chambers in mouse bone marrow MCs represents, where bacteria remain viable and escape intracellular killing mechanisms [355]. More consistently, MCs kill bacteria through the release of antimicrobial peptides known as cathelicidins [240, 349] and the generation of extracellular traps [348].

MCs are activated by bacteria, bacterial products or innate host components through several mechanisms including TLR-dependent pathways (for detailed review see [356], Fig. 8). Several formalin-fixed bacteria induced histamine and PGD₂ production from human lung MCs and human tonsillar MCs, with differences compared to Fc ϵ RI-dependent release with respect to kinetics, temperature, pH profiles and sensitivity to Ca²⁺ deprivation and metabolic inhibitors [357]. Subsequent studies have demonstrated that human cord blood-derived MCs respond to ligands for TLR2 (the receptor for bacterial peptidoglycan), TLR4 (the receptor for LPS), TLR5 (the receptor for bacterial flagellin) and TLR9 (the receptor for CpG oligodeoxynucleotides). For example, bacterial peptidoglycan and other TLR2 ligands consistently stimulate human cord blood-derived MC cysLT release although the reported effects on degranulation vary [238, 239, 283]. Cytokines released following TLR2 stimulation include IL-1 β , IL-5, IL-10, IL-13, GM-CSF and TNF- α , although there are again some inconsistencies across studies. TLR4 is also expressed by human MCs although the effects of TLR4 stimulation have not been consistent, with some studies demonstrating degranulation, some indicating that there is release of cysLTs, and variable generation of cytokines including IL-5, IL-10, IL-13 and TNF- α [238, 239, 283]. In one study flagellin stimulated TNF- α and IL-1 β but not cysLT production, whereas CpG induced TNF- α , IL-1 β and cysLT release [239].

Examination of the gene expression profile from human cord blood-derived MCs using high density oligonucleotide probe arrays following activation with LPS compared to anti-IgE demonstrated that both induced a core response, plus an LPS or anti-IgE-specific programme of gene expression [119].

MCs also express several NOD-like receptors (NLRs). These receptors recognize intracellular pathogen-associated molecular patterns (PAMPs) and 'danger' signals [358]. Two of the main families of NLRs are the NALPs and the NODs. NALP1, -2 and -3 form the central

scaffold of caspase-1-activating complexes known as inflammasomes. Inflammasomes may be activated by a number of diverse stimuli including the pore-forming toxins of several bacteria [358]. The consequence of caspase-1 activation is the conversion of IL-1 β to its active form. The NOD family members recognize cytoplasmic microbial PAMPs including peptidoglycan in bacteria. Mouse MCs express NALP-3, ASC and caspase-1, an inflammasome activated by the NALP-3 ligand R837 [359]. Human MCs in the bowel of patients with Crohn's disease demonstrate increased expression of NOD2 compared to controls [360]. Human MCs, including human lung MCs, up-regulate NOD2 expression in response to IFN- γ , and specifically up-regulate CXCL10 in response to the NOD2 agonist muramyl dipeptide (a component of peptidoglycan) [360]. In addition, human cord blood-derived MCs are activated by the NOD1 agonist M-TriDAP and release TNF- α , CXCL8, CCL3 and CCL4, but do not degranulate [361].

Pneumolysin is the principle toxin and virulence factor expressed by pneumococci. Human lung MCs kill wild-type pneumolysin-expressing pneumococci in co-culture, but not an isogenic pneumolysin-deficient pneumococcus [240]. This is driven by the pneumolysin-dependent activation of human lung MCs which results in the release of LTC₄ but not histamine, and the release of preformed cathelicidin LL-37 which is responsible for pneumococcal cell death. However, at high bacterial concentrations, all pneumococcal strains tested reduced human lung MC viability through a combination of pneumolysin and hydrogen peroxide-dependent mechanisms. This suggests that MCs provide an early warning system and potentially limit pneumococcal dissemination early in the course of invasive pulmonary pneumococcal disease, but there is the potential for pneumococci to overwhelm this protective mechanism.

Defence against viral infection. There is less *in vivo* evidence that MCs contribute to host defence against viruses, although this is emerging, and there is no doubt that MCs respond to the presence of viruses *in vitro*. Respiratory viruses with double-stranded RNA (a TLR3 ligand) such as influenza virus (PR8), type-1 retrovirus and respiratory syncytial virus (RSV) induce the synthesis and release of IFN- α from HCBMCs, a response mimicked by the TLR3 ligand, polyI:C [239]. Activation of TLR3 with polyI:C does not induce TNF- α , IL-1 β , IL-5 or GM-CSF production, or degranulation, but does stimulate low level release of cysLTs [239].

In vivo studies suggest MCs may either provide protection against viral infection or aggravate the effects of infection, likely due to the same reasons as discussed earlier for bacteria. For example, in a mouse model of viral myocarditis, MC deficiency reduced myocardial necrosis and inflammation [362]. In a mouse model of

influenza infection, *in vitro* co-culture of MCs with influenza A resulted in histamine release, LTB₄ and cytokine/chemokine production [363]. LTB₄ and cytokine/chemokine production involved signalling in MCs via the pattern recognition receptor RIG-1 but histamine release occurred independently of this. Interestingly, histamine release occurred in two waves, one peaking at 30 min and over by 60 min, consistent with acute degranulation, the second more modest starting after 6 h but sustained, perhaps indicative of enhanced synthesis and 'constitutive' release. *In vivo*, the influenza-dependent disease and immunopathology was markedly attenuated in the MC-deficient mice. In contrast, in a mouse model of pulmonary cytomegalovirus infection, MCs played an important role in controlling the infection through their ability to recruit protective CD8⁺ T cells [364]. MCs also appear to protect against the consequences of infection with dengue virus through the recruitment of NK and NKT cells [365].

Defence against fungi. Fungal sensitization (IgE) and colonization are common in asthma, and *Aspergillus fumigatus* in particular is associated with severe asthma and poor lung function and causes the associated syndrome of allergic bronchopulmonary aspergillosis [366, 367]. The precise contribution of MCs to the host response to fungal exposure is not currently known, but there are interactions between MCs and fungi beyond activation via IgE. For example, yeasts and hyphae of the diploid fungus *Candida albicans* induce rat peritoneal and mouse bone marrow MC degranulation and the production of a range of cytokines including TNF- α , IL-6 and CCL4 via activation of the receptor Dectin-1 independently of IgE [368]. In addition, fungal elements from *Aspergillus fumigatus* activate rodent MCs in the absence of IgE. For example, *Aspergillus* protease induced degranulation and IL-4 release from RBL cells [369], while mature *Aspergillus fumigatus* hyphae induced RBL cell degranulation through an undefined contact-dependent mechanism [370]. Fungal zymosan was also shown to stimulate the release of LTC₄ but not histamine from human cord blood-derived MCs [371].

IgE-dependent activation of MCs is also thought to play a role during fungal infections. For instance, *in vivo* studies in mice found that inhalation of *Aspergillus fumigatus* extract increased levels of IgE and numbers of MCs within the airways accompanied by elevated levels of systemic MC protease-1 in mice [372]. Thus, both IgE-dependent and -independent mechanisms may contribute to MC-mediated defence against fungi. Conversely, fungi may suppress MC activity during infection; gliotoxin, released by pathogenic fungi, suppressed both Fc ϵ RI-dependent and -independent rodent MC degranulation and LTC₄, IL-13 and TNF- α release [373].

Mast cell-immune cell crosstalk

Mast cells and T cell-dependent allergen sensitization. Both rodent and human MCs express class II MHC antigens, and rodent MCs can present soluble antigens to T cells resulting in T cell proliferation [374–377] (Fig. 9). This is enhanced by both IL-4 and GM-CSF and inhibited by IFN- γ [376]. It has been postulated that the release of IL-4 from MCs could provide the correct local environment for the differentiation of Th2 T cells [316], which is evident in experiments employing rat cells [378]. It is therefore plausible that antigen presentation and accompanying IL-4 release by MCs could contribute to Th2 differentiation at the onset of an immune response. This could occur in the absence of specific IgE because many allergens including bee venom PLA₂, *Der p* I and III and Schistosomal protease induce the release of histamine and IL-4 from human lung MCs in the absence of cell-bound IgE [369, 379]. Phospholipases, proteases or both are associated with many allergens including those from house dust mites, cockroaches, fungal spores, grain dust, plant pollens, cats and insect venoms. Proteases release mediators from MCs independently of IgE via the protease-activated receptor PAR2 [228, 380]. The fact that allergen sensitization does not occur in everyone can be explained by both environmental factors (e.g. level of

allergen exposure) and genetic factors (e.g. MC releasability, epithelial integrity/permeability, local anti-protease activity and regulation of cytokine production).

MCs also influence dendritic cell development and their ability to activate T cells (Fig. 9). For example, histamine increases IL-10 and decreases IL-12 production by mature dendritic cells, with the result that naïve T cells become polarized towards a Th2 phenotype [381]. Similar effects have been observed with PGD₂ [382], and MC dependence for the generation of Th2-promoting dendritic cells is evident in mice *in vivo* [383]. MC exosomes induce immature dendritic cells (DCs) to up-regulate MHC class II, CD80, CD86 and CD40 molecules and to acquire potent Ag-presenting capacity to T cells [384]. MC-derived TNF- α is important for dendritic cell migration during immune responses [385], and of particular relevance to allergy, a population of Fc ϵ RI⁺ dendritic-like cells also emerge in parallel with developing MCs from human hip bone marrow cultures [181].

Mast cell regulation of IgE synthesis. MC-derived IL-4 and IL-13 have the potential to regulate IgE production by B cells (Fig. 9). Gauchat et al. demonstrated that HLMCs express CD40 ligand (CD40L) and are able to provide the cell-cell signal required for IgE production

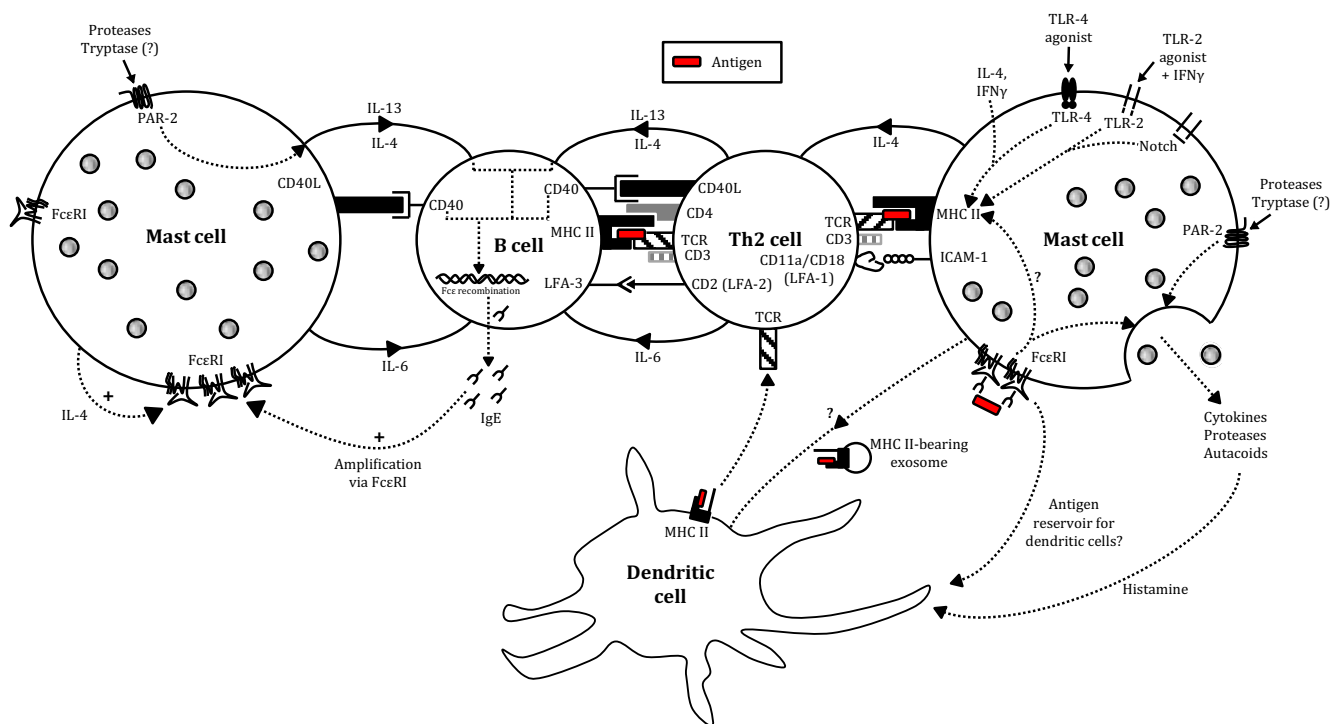


Fig. 9. Interactions between mast cells, T cells and B cells. Potential MC interactions with B cells and T cells at the initiation of a specific immune response to antigen. Adapted from Bradding & Holgate [7].

through the interaction with CD40 expressed on B cells [386]. The MCs were not activated in these experiments, and the addition of exogenous IL-4 was required to induce IgE secretion.

MCs purified from nasal biopsies of dust mite-sensitive patients with allergic rhinitis also induced the secretion of IgE from tonsillar B cells when incubated with allergen [387]. This MC-dependent IgE secretion was partially inhibited by neutralizing monoclonal antibody to IL-4 and completely inhibited by neutralizing antibody to IL-13 and CD40 ligand. This appears relevant clinically, because allergen challenge of atopic nasal tissue *ex vivo* induces IgE mRNA expression in resident B cells, in parallel with increased IL-4 and IL-13 expression in T cells and MCs [388]. Local IgE production is also evident in both atopic and nonatopic asthmatic bronchial mucosa [389]. As both IL-4/IL-13 and IgE induce the expression of FcεRI on MCs [154, 156, 390], it is likely that secretion of IL-4 from MCs produces positive feedback for FcεRI expression both in an autocrine IL-4-dependent manner and via B cell IgE secretion. This in turn should amplify the MC secretory response because in both mice and humans, up-regulation of FcεRI permits mediator release at lower concentrations of specific antigen [33, 63], mediator release through IgE binding in the absence of antigen, and also primes MCs to produce markedly elevated levels of IL-4 and other cytokines [33, 63].

Based on these considerations, Fig. 9 provides a model for the potential interaction of MCs, T cells, B cells and dendritic cells at the initiation of the immune response to aeroallergens.

Type-2 innate lymphoid cells

There has been much interest recently in the role of type-2 innate lymphoid (ILC2) cells in asthma and allergic disease [391]. These cells produce relatively large quantities of IL-5 and IL-13, and in animal models appear important for the development of Th2-driven disease. However, they are difficult to identify through their lack of lineage markers. They express Kit but not FcεRI and are assumed therefore not to be MCs, although FcεRI-low MCs undoubtedly exist [115]. A population of CD45⁺ IL-13⁺ cells that were not common airway leucocytes were present in asthmatic airways [392], and lineage negative cells expressing limited putative ILC2 markers have been identified in lung [393–395] and nasal mucosa, where they formed 1% of the CD45⁺ population in patients with chronic rhinosinusitis [393]. ILC2 cells in nondiseased human lung must either be relatively rare or express very low levels of Kit, because isolation of Kit⁺ cells from human lung tissue using immunomagnetic affinity selection

provides a > 99% pure MC population as assessed by metachromatic staining and staining for MC-specific proteases [396].

MCs produce PGD₂ which has important effects on *in vitro* culture-derived ILC2 cells [395, 397] (Fig. 10). Acting via its CRTh2 receptor, PGD₂ induces the release of type-2 cytokines from ILC2 cells and acts a chemoattractant for them [397]. PGD₂ present in the supernatants of activated human cord blood-derived MCs exerts the same effects. In addition, cleavage of IL-33 by either tryptase or chymase generates IL-33-derived peptides that are 30-fold more potent than full length IL-33 with respect to ILC2 activation [398]. MCs therefore have the ability to promote ILC2 cell recruitment and activation in disease.

Mast cell–eosinophil interactions

MCs and eosinophils are principle effector cells in allergic disease and their close co-existence at sites of allergic inflammation suggests that they are likely to influence each other's survival and activity (Fig. 10). This view is supported by several observations (reviewed in [399]). IL-3, IL-5, GM-CSF, TNF-α and tryptase are produced by MCs and modulate eosinophil activation, growth, survival and differentiation [400–403]. Histamine, PGD₂ and many chemokines mediate eosinophil chemotaxis [404–406]. The production of SCF and basic proteins by eosinophils promotes MC activation [407].

In diseased tissues, including asthmatic airways, MCs and eosinophils are found in close approximation [408]. In co-culture, human cord blood-derived MCs and peripheral blood eosinophils form contacts with 5 min, with contacts lasting from 3–4 min. These MCs embrace eosinophils with pseudopodia-like membrane structures, with tight interactions evident with transmission electron microscopy. Under these conditions, the presence of MCs enhances eosinophil survival when compared to eosinophils in monoculture with GM-CSF and prevents corticosteroid-dependent eosinophil apoptosis. In contrast, eosinophils did not significantly enhance mast cell survival in this study. The survival-enhancing effect of MCs on eosinophils was partially due to soluble GM-CSF, and partially due to adhesion mediated via MC CD48 and its ligand 2B4 expressed on eosinophils. Kit was not involved. There is therefore evidence of important crosstalk between human MCs and eosinophils, and this has led to the concept of the 'allergic effector unit' [408].

Mast cells in asthma

Asthma is a complex and heterogeneous chronic respiratory disease characterized by the presence of variable

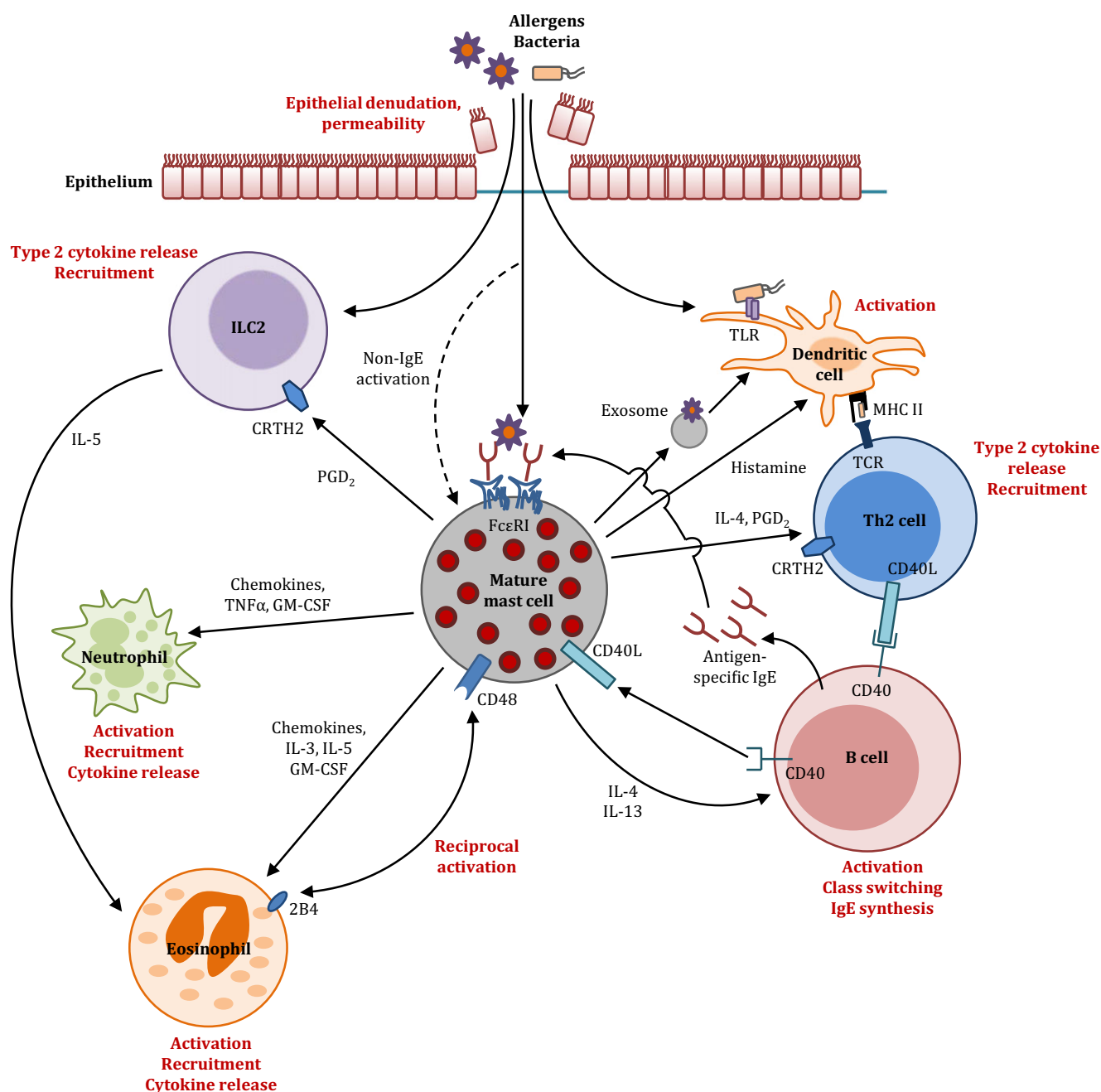


Fig. 10. Potential interactions between MCs and airway immune cells.

airflow obstruction resulting in the symptoms of wheeze, dyspnoea, cough and chest tightness. This obstruction is reversible, at least in part, either spontaneously or with pharmacological intervention. A number of different stimuli can predominate in any individual and may contribute to both ongoing background symptomatology as well as triggering severe exacerbations. The major processes producing airflow obstruction in asthma are contraction of the ASM on a background of AHR [409, 410], excessive mucus secre-

tion [411, 412], mucosal oedema due to increased vascular permeability, airway inflammation and various structural changes referred to as airway wall remodelling (reviewed in [413]). Features of airway wall remodelling include denudation of the epithelial layer, goblet cell and mucous gland hyperplasia and hypertrophy, subepithelial fibrosis, abnormal extracellular matrix deposition, vascular proliferation and increased ASM mass [413]. The precise interactions of airway inflammation, AHR and airway wall remodelling and

their influence over the clinical phenotype remain unclear. MCs, traditionally regarded as simple effectors of the acute airway changes that take place following allergen exposure, express an impressive toolkit endowing them with the potential to play a prominent role in the initiation and propagation of the complex pathophysiological processes that underlie asthma.

So far in this review we have demonstrated important aspects of MC biology and outlined how many of these might be relevant to asthma pathophysiology. In this section we will concentrate on the evidence which places MCs as central effector cells in asthma pathophysiology as determined by (i) the profile and biology of the mediators they release, (ii) evidence of their ongoing activation and release of these mediators and (iii) their infiltration of, and interaction with, key structures within the airways.

Evidence of mast cell activation in asthma

Acute allergen-induced asthma. Allergen exposure plays an important role in asthma pathophysiology but is only one of many factors. In patients with asthma, approximately 90% of patients with asthma under the age of 30 are atopic [414], with most reactions occurring in response to the house dust mite *Dermatophagoides pteronyssinus* or *farinae*. In contrast, the prevalence of atopy in subjects developing asthma over the age of 40 is similar to that found in the general population. The level of dust mite exposure in early life is a strong risk factor for the development of asthma, and some pollen allergic individuals only develop symptoms during the pollen season. Clusters of acute asthma exacerbations also occur following the acute release of pollen particles and fungal spores into the atmosphere following thunderstorms [415]. In addition, the clinical efficacy of the anti-human IgE monoclonal antibody omalizumab in severe atopic asthma and its ability to markedly reduce airway inflammation [155] points to the importance of IgE in ongoing disease. However, omalizumab does not cure the disease, and symptoms often persist albeit at a lower level. Furthermore, allergen avoidance usually has only a minor effect on the state of established disease which therefore appears to become 'self-perpetuating'. This is typified in cases of occupational asthma, where asthma will persist on removal of the sensitizing agent if exposure is not prevented early in the course of the disease.

Because of the evidence that asthma is at least in part an IgE-dependent disease, the method 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 there is a rapid fall in pulmonary function (e.g. FEV₁) at 10–20 min in nearly all atopic

asthmatics and many atopic nonasthmatics, which gradually recovers over the following 2 h, defined as the early asthmatic reaction (EAR). In about 50% of subjects, between 4–6 h there is a further fall in FEV₁, the late asthmatic reaction (LAR). This may last up to 12 h and in some individuals may be followed by recurring airway obstruction for several days or even weeks [416, 417]. For reasons not understood, the LAR is more severe following house dust mite challenge than grass challenge [418].

The early-phase asthmatic reaction. The EAR in response to allergen challenge is driven by the rapid release of a variety of vasoactive and spasmogenic mediators such as histamine, PGD₂ and LTC₄. These mediators induce bronchoconstriction, mucosal oedema and mucus secretion. Their relative rate of release from human lung MCs *in vitro* is histamine > PGD₂ > LTC₄ with half maximal release occurring at 2, 5 and 10 min, respectively [419]. This is paralleled *in vivo* where recovery of these mediators in bronchoalveolar lavage fluid (BAL) following local bronchial allergen challenge occurs within 5–10 min [420–426]. Calculations indicate that the concentrations of mediators generated *in vivo* are similar to those required to produce bronchoconstriction *in vitro* [425] or when delivered by nebulizer [423]. However, more direct evidence for their role is provided by studies using potent and selective receptor antagonists, which demonstrate that the EAR is significantly attenuated by antagonists of histamine [427, 428], LTC₄ [429, 430] and to a lesser extent PGD₂ [431].

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* is similar to 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 [422, 426]. Thirdly, β_2 -adrenoceptor agonists such as salbutamol which inhibit MC degranulation when applied acutely *in vitro* [432], completely abolish the early reaction and the associated increase in plasma histamine levels [433]. Lastly, the EAR is markedly attenuated after treatment with anti-IgE (omalizumab) [434, 435], indicating that IgE-dependent signalling is critical.

The late-phase asthmatic response. In contrast to the acute mediator-induced bronchoconstriction and mucosal oedema characteristic of the EAR, the LAR is associated with inflammatory cell accumulation and activation. This late inflammatory response is thought to account for the ensuing airway obstruction and associated increase in AHR which accompanies the LAR.

These features of the LAR are considered by many to mimic the pathological situation present in chronic asthma, but some caution is needed in extrapolating the results of applying a single large dose of allergen to the airways, on the background natural disease, to the natural disease itself. The contribution of MCs to the pool of bronchospastic mediators present during the LAR is more difficult to define than during the EAR due to the recruitment and activation of many cell types including eosinophils, CD4⁺ T cells, macrophages and small numbers of basophils.

While the LAR also displays increased levels of histamine, PGD₂ and LTC₄, the ratios of these mediators are different to that seen in the EAR. This could indicate that cells other than MCs, such as macrophages and eosinophils [425, 426], act as sources of these mediators. Furthermore, a role for MCs in the LAR has been questioned due to a decrease in tryptase levels at this time [426]. However, MC degranulation is not an 'all-or-nothing' process [240, 436–439]. In fact, various conditions can induce differential mediator release. For example, GM-CSF which is released following allergen provocation [440], inhibits expression of tryptase in the immature MC line, HMC-1, but does not attenuate histamine release [441], and in HLMCs may in fact potentiate IgE-dependent histamine release [442]. Recovery from degranulation may also explain disparity between MC mediators in the LAR; studies with rodent MCs demonstrate that following activation and secretion, mRNA transcripts encoding factors such as proteases remain low, suggesting that regranulation of MCs is a slow process [443].

The LAR is attenuated markedly by anti-IgE therapy (omalizumab) [434], indicating that MC activation during the EAR almost certainly initiates events leading to the LAR. It is therefore likely that the secretion of MC mediators and cytokines following allergen challenge contributes to both the pathophysiology and symptomatology of the LAR.

Mast cells in chronic atopic asthma. Asthma is a chronic and heterogeneous disease whose symptoms vary over time. In 'stable' atopic asthma, with 'atopic' defined as sensitization to a common aeroallergen, assessment of MC morphology using electron microscopy indicates that there is ongoing MC degranulation within asthmatic airway epithelium, lamina propria and ASM bundles [135–137, 444]. Many studies have found increased numbers of MCs [420, 445–447] and increased concentrations of histamine and/or tryptase [422, 446–449] in bronchoalveolar lavage fluid (BAL) from stable asthmatics compared to healthy controls. BAL MCs from symptomatic asthmatic subjects may differ from healthy BAL MCs by exhibiting greater constitutive histamine release [446, 447, 449]. Broide and

co-workers [449] found that IgE-dependent activation of MCs from symptomatic asthmatics did not produce any significant increase in histamine release on a background of very high constitutive release, in contrast to MCs from asymptomatic asthmatic subjects. This might suggest that high constitutive release in the presence of symptoms is related to IgE-dependent activation *in vivo*. Both Flint et al. [446] and Casolaro et al. [450] reported increased IgE-dependent histamine release from atopic asthmatic BAL MCs compared to nonatopic healthy controls. In addition, strong correlations have been observed between the severity of AHR and MC numbers, histamine concentrations and spontaneous histamine release in BAL [446–448, 450].

MCs within the bronchial mucosa in atopic asthma express mRNA and/or protein for several cytokines including IL-4, IL-5, IL-6, IL-13, TNF- α , TSLP and amphiregulin, with disease-related increases in expression for many of these [120, 316, 392, 451–453]. Increased mRNA and protein expression suggests these MCs are in a heightened state of activation. Strong correlations have been described between numbers of eosinophils and IL-4⁺, IL-5⁺ and TNF- α ⁺ MC densities in these studies. This supports the view that MC-derived cytokines make a major contribution to the pathophysiology of asthma.

MCs are therefore in a state of continuous activation in chronic atopic asthma, with some evidence to suggest that they may exhibit increased mediator releasability, both 'spontaneously' and in response to IgE-dependent activation. When this information is coupled with the epidemiological data implicating allergen exposure in the development of asthma and the efficacy of omalizumab, it is reasonable to hypothesize that the atopic asthmatic phenotype results in part from the everyday interaction between allergens, IgE and hyper-reactive MCs.

Mast cells in nonatopic asthma. The majority of people with asthma that appears for the first time in adulthood are not sensitized to common aeroallergens. These patients used to be described as having 'intrinsic' asthma, but are now referred to as nonatopic. This form of the asthma is often more severe and persistent, and more often associated with nasal polyposis and aspirin sensitivity. However, in spite of the different phenotypic clinical picture, the pattern of airway inflammation in nonatopic asthma is virtually identical to that found in atopic asthma [454]. This suggests that common mechanisms may be at play in the development and propagation of these asthma phenotypes. This hypothesis is supported by several pieces of evidence which also implicate a role for MCs.

Firstly, Fc ϵ RI⁺ cells are present in increased numbers in the bronchial mucosa of both atopic and nonatopic

asthmatic subjects compared to healthy controls [455]. Although total MC counts are the same in these three groups, the majority of the FcεRI⁺ cells are MCs, indicating that FcεRI density is greatly increased on MCs in both atopic and nonatopic asthma. Secondly, there is increased expression of both mRNA and protein for the Th2 cytokines IL-4 and IL-5 in nonatopic asthma [456]. As in atopic asthma, MCs in nonatopic asthma account for a significant proportion of the cells expressing mRNA and protein for both of these cytokines [457], again suggesting ongoing MC activation and cytokine secretion. Both IL-4 and IgE are powerful inducers of FcεRI expression on MCs, and IL-4 is of course a key cytokine involved in the promotion of IgE synthesis. There are increases in epsilon germline gene (Iepsilon) and mature epsilon heavy chain (Cepsilon) RNA⁺ B cells in the bronchial mucosa in both atopic and nonatopic asthma, suggesting that there is local IgE synthesis [389]. This is consistent with the finding that the concentration of IgE in BAL is increased 24 h after bronchial allergen challenge [458]. Thus it is likely that IgE production occurs locally within the bronchial mucosa in nonatopic asthma, promoting the increased expression of FcεRI, and perhaps explaining the absence of high serum concentrations. As nasal MCs can induce IgE synthesis by B cells in an IL-4 and IL-13 dependent manner [387], lower airway MCs might also contribute to local bronchial IgE production. If so, omalizumab therapy may also be effective in nonatopic asthma.

Occupational asthma. Occupational asthma can be defined as asthma which develops, or is exacerbated, following specific exposure in the workplace and excludes nonspecific stimuli which induce bronchoconstriction in any asthmatic subject. The prevalence of occupational asthma varies, but in some regions may account for 15–20% of adult asthma [459]. Over 300 agents are recognized as inducers of occupational asthma, and common to all of these compounds is that they are inhaled (which probably informs us about the induction of atopic and nonatopic asthma). They fall into three main groups. (i) Those associated with the generation of specific IgE antibodies. Compounds in this IgE-dependent group are either directly immunogenic proteins such as high-molecular-weight enzymes, or low-molecular-weight (LMW) compounds which bind to body proteins and act as haptens, for example the acid anhydrides and complex halogenated platinum salts. (ii) Those that are thought to produce sensitization through as-yet undefined immunological mechanisms, with specific IgE antibodies usually absent. Examples from this group include the LMW chemicals plicatic acid (present in the dust of western red cedar) and the isocyanates. (iii) Irritant gases, fumes or chemicals

which are capable of producing asthma after a single large exposure (reactive airways dysfunction syndrome or irritant-induced asthma).

The pathology of occupational asthma (with the exception of irritant-induced asthma) is remarkably similar to that present in atopic and nonatopic asthma, including occupational causes where IgE-independent mechanisms are involved such as western red cedar asthma (WRCA) and toluene diisocyanate (TDI) asthma [460, 461]. For example, in TDI asthma, MC numbers are increased in the bronchial epithelium when compared to healthy controls, and electron microscopy demonstrates that the majority of MCs are degranulated [461]. Subjects who develop occupational asthma after a short period of exposure to TDI (2 years) have more MCs in their airway mucosa than subjects who develop asthma after a long period of exposure (22 years) [462]. Whether this is a predisposing factor for the early onset of TDI asthma or a secondary effect due to different levels of exposure is uncertain.

Bronchial challenge with plicatic acid results in the rapid release of histamine into the airways of patients with WRCA but not healthy subjects [463]. *In vitro*, plicatic acid releases histamine from MCs present in BAL and bronchial mucosal biopsies collected from patients with WRCA but not those with atopic asthma [464]. This occurs via an undefined IgE-independent mechanism, which is in keeping with the observations that patients with WRCA do not usually have specific IgE to plicatic acid [464].

In summary, MCs appear to contribute to the pathophysiology occupational asthma in a similar manner to atopic and nonatopic asthma.

Exercise-induced asthma. Exercise-induced asthma is defined by transient bronchoconstriction after exercise and should be considered a manifestation of suboptimal disease control rather than a separate disease entity. It can be mimicked by hyperventilation of cold dry air. Before the widespread use of inhaled corticosteroids, it occurred in up to 80% of asthmatic subjects [465]. Typically bronchoconstriction occurs 5–10 min after exercise and usually recovers within 30 min. Approximately half of these patients exhibit a refractory period for about 1 h following exercise during which additional exercise does not induce further bronchoconstriction. Exercise-induced asthma is probably caused by the effects of airway cooling and water loss during exercise and may be mimicked by hyperventilation of cold dry air (reviewed by [466]).

Both MCs and basophils release histamine in a hyperosmolar environment [243, 244], and this release is attenuated by disodium cromoglycate, providing evidence that could link changes in airway osmolarity to bronchoconstriction. Some studies found increased con-

centrations of circulating histamine in the serum of asthmatic subjects following exercise [467–469], while analysis of induced sputum following exercise has demonstrated increased concentrations of histamine, cysteinyl leukotrienes and tryptase, indicative of MC activation [470]. In further support of this, terfenadine and clemastine, both H1 receptor antagonists, flurbiprofen, a potent cyclooxygenase inhibitor and the LTD₄ receptor antagonists MK 571 and ICI 204219, all significantly attenuate exercise-induced bronchoconstriction [471–474].

The refractory period following exercise remains unexplained. Interestingly, challenge of the airways with adenosine-5-monophosphate (AMP), which induces bronchoconstriction indirectly via activation of MCs, exhibits cross-refractoriness with exercise, implicating a similar mechanism [475]. Depletion of MC mediators has been put forward as an explanation, but this seems unlikely. In summary, there is strong evidence implicating MC activation as an important component of the bronchoconstrictor response to exercise.

Nonsteroidal anti-inflammatory (NSAID)-induced asthma. Asthma is exacerbated by aspirin and other NSAIDs in 5–10% of all asthmatics [476, 477], although this reaches 25% in subjects with a history of nasal polyposis [477]. This is more common in women with late onset disease, and the asthma is often severe, requiring oral corticosteroids for control [478]. Asthmatic symptoms usually start 1–2 h after drug ingestion and may be life-threatening. This occurs with all NSAIDs suggesting that alterations of arachidonic acid metabolism are important in the aetiology. A conformational change in cyclooxygenase-2 (COX-2) induced by nonselective NSAIDs may promote the formation of HETE's and leukotrienes via the lipoxygenase pathways resulting in bronchospasm, increased mucus production and airway oedema [479]. This is supported by the finding of increased LTC₄ in nasal secretions [480], increased LTE₄ in the urine [481] and the protection provided by LTC₄ receptor antagonists [482] following ingestion of aspirin in aspirin-sensitive asthmatics. Studies examining mediator release either following aspirin challenge or in the stable state identified histamine, tryptase and PGD₂ generation suggesting MC involvement [483–487]. *In vitro*, aspirin-induced mediator release from rat basophilic leukaemia (RBL) cells [369], and lysine aspirin-induced Ca²⁺ influx and PGD₂ release from human cord blood-derived MCs [488]. Further evidence that MCs contribute to NSAID-sensitive asthma comes from the observations that there are increased numbers of MCs in the airways of these patients and that an increased proportion of these cells express COX-2 [489]. In addition, MCs are

the predominant cells expressing LTC₄ synthase in the airways of NSAID-sensitive asthmatics [490].

The role of mast cells in asthma exacerbations. Acute exacerbations of asthma on a background of chronic disease represent a major cause of asthma-related morbidity and death. Acute deteriorations in lung function can be triggered by allergens via MC-dependent mechanisms, as described in previous sections of this review, but the majority of asthma exacerbations develop over several days [491]. In these cases, exacerbations are often precipitated by infections with viruses such as the rhinovirus [492, 493].

Naturally acquired viral infection in the human nasal mucosa promotes the accumulation of MCs in the nasal lamina propria during the first days of a symptomatic infection [494]. In contrast, following experimental rhinoviral infection, bronchial MC numbers in the lower airway lamina propria were reduced 4 days and 6 weeks following infection, although there was a non-significant upward trend in epithelial MC numbers over the same period [495]. However, the greater the airway lamina propria MC number at baseline, the greater the fall in FEV₁ following infection in those patients with asthma [495].

As described above, human MCs respond to TLR3 engagement with the production of interferons and cysLTs, and dual stimulation through TLR3 and FcεRI further enhances the release of cysLTs and induces the release of IL-1β, TNF-α and IL-5 [239]. Several lines of evidence suggest this is relevant clinically. Firstly, asthmatic subjects who are both sensitized and exposed to common aeroallergens suffer from more severe viral-induced exacerbations than nonexposed sensitized subjects, demonstrating a potentially important interaction between virus and allergen [496]. Secondly, experimental nasal infection in humans with live rhinovirus (RV16) increases the frequency of late asthmatic reactions after inhaled allergen challenge [497, 498], and after local bronchial allergen challenge, enhances both the immediate release of histamine into blood [498] and BAL [499], and the later recruitment of eosinophils into the airway [499]. Lastly, anti-IgE therapy (omalizumab) significantly reduces the rate of severe exacerbations in atopic asthmatic subjects [217, 500]. Taken together this provides circumstantial evidence that MCs contribute directly to viral-induced asthma exacerbations in atopic subjects.

Irrespective of atopy, viral infection can also result in the production of virus-specific IgE, and the intensity of this antibody response has been correlated with changes in airway function during the acute infection [501, 502]. An *in vivo* animal model of asthma and RSV infection resulted in MC degranulation through the cross-linking of virus-specific IgE on MCs, with

associated increases in AHR [503]. RSV-specific IgE also promoted the development of AHR and Th2-type inflammation in a MC-dependent manner on re-infection with RSV in new-born mice [504]. Viruses may also promote human MC mediator release indirectly although IgE-independent mechanisms as RSV infection of lung cancer-derived A549 epithelial cells increased TNF- α release from with the human MC line HMC-1 [505]. Overall, current data suggest that MC activation may occur during respiratory viral infection *in vivo*, through a combination of IgE-dependent, TLR-dependent and further undefined mechanisms.

Bacteria are also implicated in asthma exacerbations and may interact with viruses (reviewed in [506]). There is also the potential for bacteria to interact with MCs via TLRs, secreted toxins [240, 507], and through Fc ϵ RI via bacteria-specific IgE or superantigens as described earlier. Specific IgE to *Staphylococcus aureus*, *Chlamydia pneumoniae*, *Haemophilus influenzae*, *Streptococcal pneumoniae* and *Mycoplasma pneumoniae* has been detected in several studies, and in some cases linked either to the presence of asthma or asthma severity [222–225, 508–513]. It is therefore plausible that on reinfection, a simultaneous engagement of TLRs, other bacterial-specific receptors and Fc ϵ RI causes a synergistic activation of MCs, further amplified by pro-inflammatory signalling from surrounding cells in lung tissue.

Further evidence linking direct involvement of MCs to asthma exacerbations arises from pathological studies of asthma death which are discussed further in a later section.

Air pollution and mast cells in asthma. Exposure to outdoor air pollution is associated with an increased risk of developing asthma and asthma exacerbations, with particulate matter (PM), ozone, nitrogen dioxide and sulphur dioxide important factors [514]. HMC-1 MCs released several proinflammatory cytokines and chemokines including G-CSF, IL-1 β , IL-6, CCL3, CCL4 and CXCL8 when exposed to PM10 particulate matter [515]. There was a synergistic enhancement of PM10-dependent cytokine release when HMC-1 cells were co-cultured with A549 epithelial cells and/or THP-1 macrophages. In addition, nasal exposure to allergen and diesel exhaust particles induces a rapid up-regulation of IL-4 within human nasal mast cells [516] and diesel exhaust particles potentiate allergen-induced histamine release [517].

Exposure of sheep to ozone and healthy humans to NO₂ or SO₂ increased MC numbers in BAL fluid [518–521]. In mouse studies, MCs played a central role in the airway inflammation and damage induced by ozone [522, 523], but did not contribute to ozone-induced AHR. In a group of mild steroid-naïve asthmatic subjects, ozone did not induce acute MC mediator release

in the nose, although it did enhance eosinophil influx after subsequent allergen challenge [524]. In explanted human nasal tissue obtained at surgery for septoplasty and reduction of the inferior turbinates, both ozone and NO₂ increased histamine release into the culture supernatant over 24 h [525]. This correlated with the number of degranulated MCs in the tissue. Ozone exposure in patients with asthma increased the magnitude of the MC-dependent early response to allergen challenge, accompanied by an increase in histamine and tryptase concentrations in induced sputum [526]. In patients with asthma using inhaled corticosteroids, ozone induced a rapid increase in the numbers of MCs in the bronchial mucosa [527], but this was not evident in bronchial biopsy studies of ozone-exposed healthy subjects [528].

Taken together, these observations provide evidence that environmental pollutants activate airway MCs, which may lead to a reduction in asthma control and contribute to severe asthma exacerbations.

Mast cell distribution within the airways in asthma – implications for structural cell dysfunction and airway remodelling

MCs are present in all healthy human tissues, and in healthy airways, they reside in close proximity to blood vessels and throughout the lamina propria [104, 120]. Although there is some inconsistency in studies, total MC numbers in the lamina propria of steroid-naïve asthmatic airways are similar to those in healthy controls [120, 529, 530]. However, assessing total MC numbers is misleading; it is their compartmental sublocalization and state of activation in the airways that is key to understanding their role in asthma pathogenesis. MCs infiltrate three distinct sites in the airways of asthmatic subjects: the epithelium [112, 120, 530] (Fig. 11a), the mucosal glands [411] (Fig. 11b) and the ASM bundles [108, 136, 392, 531–536] (Fig. 11c). This micro-anatomical redistribution places MCs within these key dysfunctional airway elements, suggesting that the local delivery of their mediators is likely to be central to the disordered airway physiology. A summary of key interactions is shown in Fig. 12.

Mast cell infiltration of airway smooth muscle in asthma: a key feature of asthma

The proposal that MC infiltration of the ASM bundles is a key contributor to the disordered airway physiology of asthma arose from a comparison of the immunopathology of asthma and eosinophilic bronchitis (EB) [108]. Eosinophilic bronchitis is responsible for up to 15% of cases of cough referred to respiratory specialists [537], is characterized by the presence of a

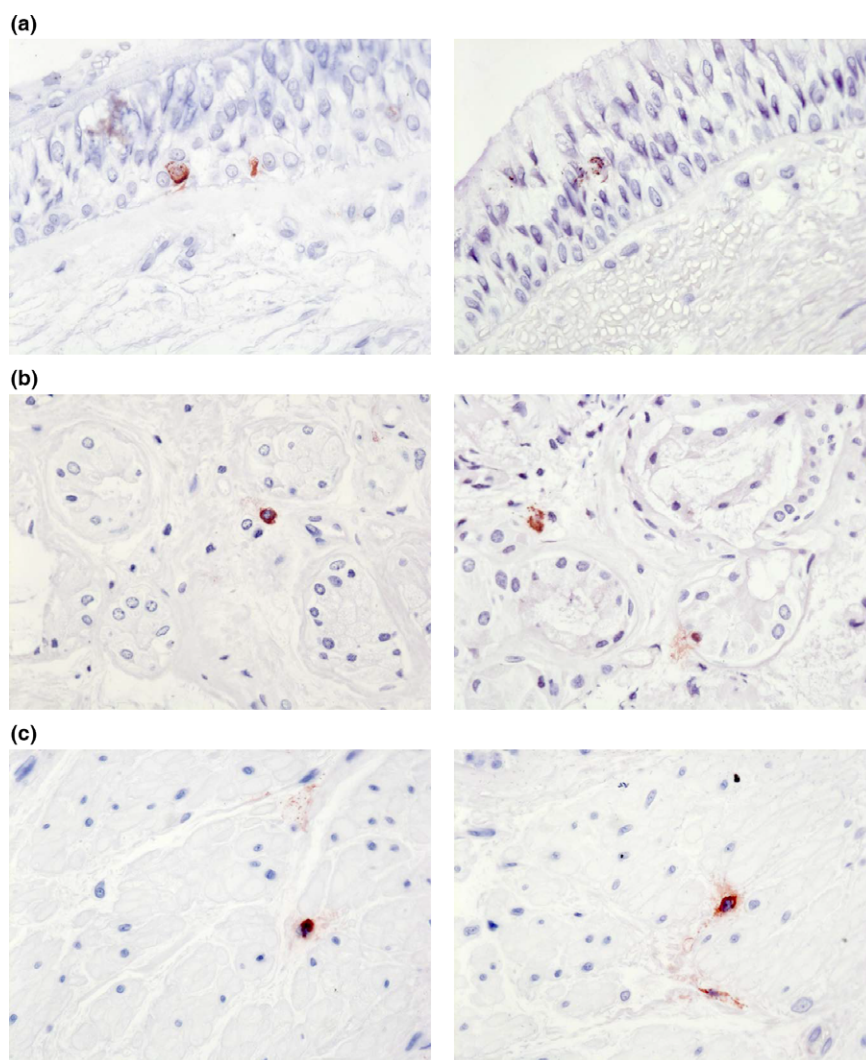


Fig. 11. Mast cells infiltrate the airway submucosal glands, smooth muscle and epithelium. MCs, identified with an antibody against MC tryptase, infiltrate (a) the airway epithelium, (b) the submucosal glands and (c) airway smooth muscle in bronchial biopsy specimens from patients with asthma. Magnification $\times 400$.

sputum eosinophilia occurring in the absence of variable airflow obstruction or AHR [537] and usually responds to treatment with inhaled corticosteroids. Asthma and EB appear identical in terms of the mucosal inflammatory cell infiltrate, mucosal IL-4 and IL-5 expression and the degree of subbasement membrane collagen deposition [108, 538–540]. Furthermore, in BAL and/or induced sputum, mediator concentrations including histamine and PGD_2 , and numbers of IL-4-expressing T cells are similar [538, 541]. This suggests that many of the Th2-related changes in asthmatic airways which have long been considered fundamental in disease pathogenesis, may not be so important for the development of airflow obstruction, AHR and airway wall remodelling as previously thought.

The striking difference between the pathology of asthma and EB lies within the ASM bundles. Although

ASM dysfunction is a disease-defining feature of asthma, it had been largely ignored in many previous immunopathological studies of the bronchial mucosa. In asthmatic bronchial biopsies, it is evident that there are numerous MCs present within the ASM bundles, but these are usually absent in the ASM of healthy subjects and patients with EB [108]. In contrast, we found no T cells or eosinophils within the ASM in any of these subject groups although numerous cells were evident in the surrounding mucosa. The increase in ASM MC numbers in asthmatic compared to healthy control subjects has been confirmed in several further studies from both Leicester and elsewhere [136, 392, 531–536]. This difference between asthma and EB suggests that MC infiltration of the ASM may be very important for the development of AHR and variable airflow obstruction. This hypothesis is supported by the

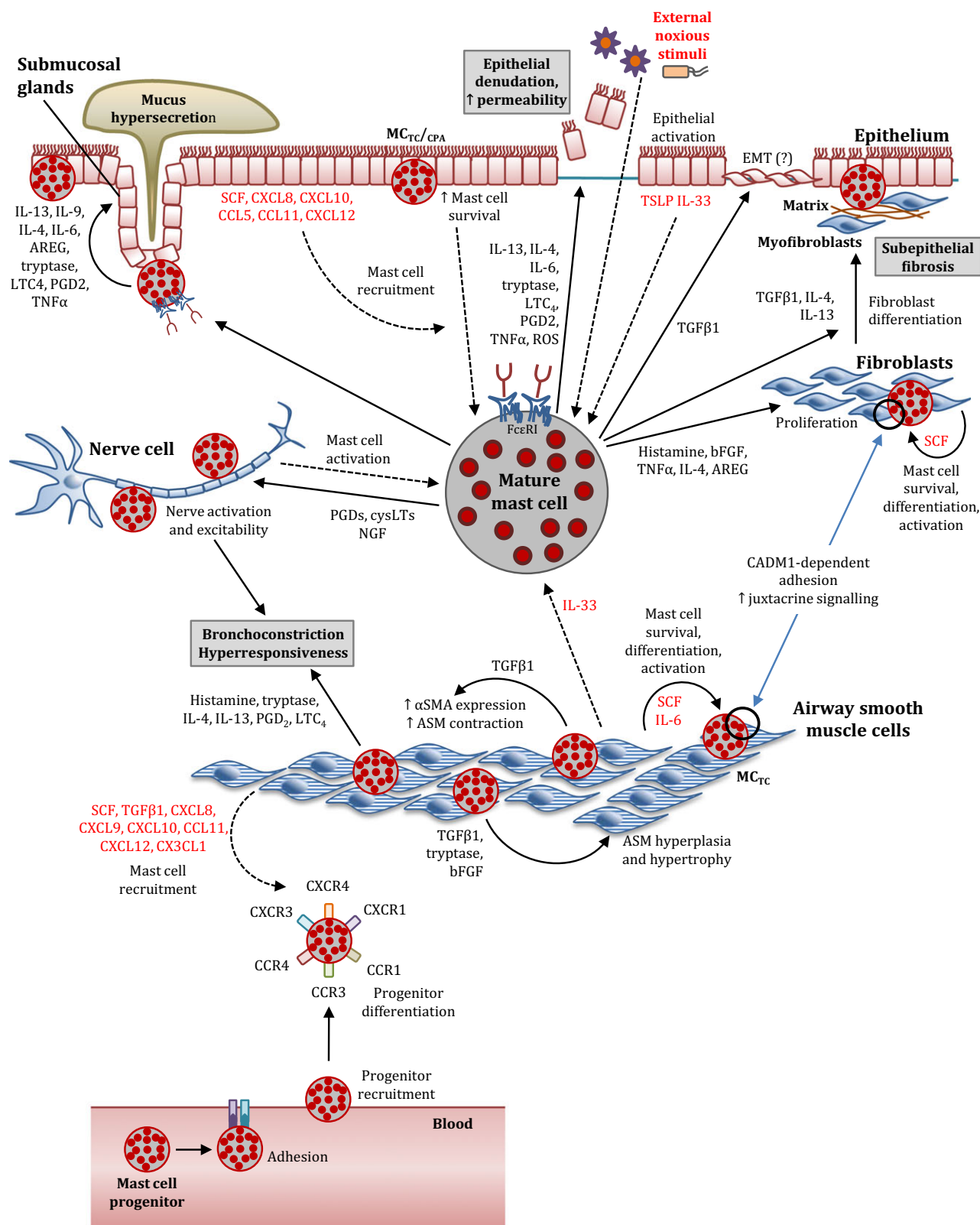


Fig. 12. Interactions between mast cells and structural cells within the airways. Potential MC interactions with submucosal glands, airway epithelial cells, fibroblasts and myofibroblasts, airway smooth muscle cells and nerve cells. Dotted arrows = stimuli acting on MCs; solid arrows = MC-derived stimuli or effects; red text = structural cell-derived cytokines; grey boxes = significant pathological events. ASM, airway smooth muscle; cysLTs, cysteinyl leukotrienes; PGDs, prostaglandins; EMT, epithelial mesenchymal transition; bFGF, basic fibroblast growth factor.

finding of a significant correlation between the number of ASM MCs and the severity of AHR in some but not all studies [108, 532], although the studies where there was no correlation are likely confounded by treatment effects including exposure to systemic corticosteroids [392].

MCs within asthmatic ASM in stable asthma demonstrate ultrastructural evidence of activation [136]. This is even more evident in cases of asthma death, where the number of degranulated MCs within the ASM is increased in fatal compared to nonfatal asthma [535]. The majority of MCs in the ASM are of the MC_{TC} subset and express both IL-4 and IL-13 but not IL-5 [108, 540]; both IL-4 and IL-13 are implicated in the enhancement of AHR in mice [542]. MC infiltration of the ASM is not linked to atopic status, is found in both eosinophilic and noneosinophilic subjects [533] and is present in both mild and severe asthma [392], and so it appears to be a common feature spanning different asthma phenotypes.

Another interesting feature relates to the bronchodilator response to deep inspiration. Normally, when a subject takes a deep inspiration this induces bronchodilation and therefore provides protection against airway narrowing. It has been established that in asthma this protective mechanism is impaired or lost altogether [543]. A recent cross-sectional study examining airway responses to methacholine challenge testing following deep inspiration [534] found that the reduction in expiratory resistance following deep inspiration was significantly impaired in asthmatic subjects compared to healthy controls and that this was related to MC density within the ASM bundles. MCs within the ASM are therefore also associated with the disturbance of a physiologically protective process.

Taken together these observations suggest that ASM infiltration by MCs is a key determinant of the asthmatic phenotype. There are also further implications. For example, it might clarify why many atopic patients do not have asthma and could explain why the presence of asthma is such a strong risk factor for death from anaphylaxis and allergen desensitization [544].

Interactions between mast cells and airway smooth muscle

The asthma-specific distribution of MCs within the ASM bundles is likely to promote specific interactions between these two cell types, through both soluble and adhesive signals, leading to important bi-directional crosstalk. We originally hypothesized that MCs promote ASM dysfunction and aggravate AHR, while the ASM provides chemoattractant signals and provides a permissive environment for MC survival and activation [545]. Research over recent years supports this hypothesis.

Recruitment of mast cells by asthmatic airway smooth muscle. Targeting the mechanism by which MCs are recruited to the ASM in asthma might offer a novel approach to treatment. The ASM secretes many chemokines and growth factors which exhibit MC chemotactic activity including CCL11, CXCL8, CXCL10, CXCL12, CX3CL1, SCF and TGF- β 1 [29, 531, 546–548] (Fig. 12), and it is likely that the ASM itself provides the primary stimulus for MC progenitor and/or mature tissue MC recruitment. Studies using human tissue, primary human ASM cells and primary human lung MCs suggest that the CXCR3-CXCL10 axis may be particularly important [20, 547]. CXCR3, the receptor for CXCL10, is the most highly expressed chemokine receptor on human lung MCs and is preferentially expressed on MCs within asthmatic ASM bundles, suggesting there may be preferential recruitment of CXCR3 positive cells [547]. Primary human ASM cells secrete the three CXCR3 ligands CXCL9, CXCL10 and CXCL11, but cultured human ASM cells from asthmatic subjects secrete significantly more CXCL10 after cytokine activation (TNF- α /IFN- γ /IL-1 β) than cells from healthy subjects, and this accounts for the enhanced human lung MC chemotaxis mediated by conditioned medium from asthmatic compared to healthy cultured ASM cells [547]. The relevance of this to MC recruitment by the asthmatic ASM *in vivo* is supported by the increased expression of CXCL10 by the ASM in bronchial biopsies from asthmatic compared to healthy subjects [547]. However, there is an added level of complexity in the regulation of MC migration by ASM in that healthy ASM *in vitro* secretes an inhibitor of HLMC migration [549]. A recent study suggests that this is mediated by CXCL1 [550]. Irrespective of the primary mechanism(s) promoting pathological MC recruitment to the ASM, both ASM-dependent and SCF-dependent human lung MC migration are inhibited by blocking the MC K_{Ca}3.1 ion channel [94], suggesting this could be a useful therapeutic strategy.

Mast cell adhesion to airway smooth muscle cells. Cell adhesion represents a crucial method of communication between neighbouring cells, enhancing the transduction of signals via both direct contact and soluble mediators [551]. Adhesion also retains cells within a particular location.

At rest, nonactivated human lung MCs adhere strongly to ASM cells *in vitro* [80]. This alone is interesting because T cells and eosinophils, which are not found in the asthmatic ASM bundles, adhere poorly to ASM cells unless activated [552, 553]. MC adhesion to ASM cells involves both Ca²⁺-dependent and -independent pathways [79, 80]. The Ca²⁺-independent mechanism involves CADM1, as described in greater detail in an earlier section of this review. As ASM cells barely

express CADM1, MC CADM1-dependent adhesion to ASM is likely to involve a heterophilic interaction [79, 80]. CADM1 counter-receptors expressed by human ASM cells include nectin-3, CADM3 and CRTAM, but their role in the MC-ASM adhesion is not yet defined [79]. Other Ca^{2+} -dependent mechanisms of MC adherence to ASM include the binding of cell surface receptors to extracellular matrix proteins. This has been demonstrated by blocking the vitronectin receptor CD51/61. However, this was not involved in constitutive adhesion, but contributed to modestly increased adhesion that was evident after activation of HLMCs and HASMCs with TGF- β 1 and TNF- α , respectively [554]. CADM1 also regulates integrin-dependent MC adhesion to ASM extracellular matrix indirectly [83].

Kit contributes to the adhesion of mouse MCs to mouse fibroblasts by binding to membrane-bound SCF [555], and CADM1 contributes through a heterophilic molecular interaction (CADM1 was not expressed on the fibroblasts used in this study) [82]. Human gut MCs adhere to human umbilical vein endothelial cells (HUVEC) via a Kit /SCF-dependent mechanism [14], which is attenuated by function-blocking antibodies to either Kit or SCF. However, similar function-blocking antibodies did not demonstrate a role for Kit/SCF in the adhesion of HLMCs or HMC-1 cells to human ASM cells or airway epithelium [80, 396, 554], or human skin MCs or HMC-1 cells to skin fibroblasts [556]. Nevertheless, using the technique of phage display, we have generated a short chain (sc)Fv antibody that recognizes Kit and which partially inhibits HMC-1 and human lung MC adhesion to human ASM cells, but which does not interfere with Kit signalling [557]. Because this antibody does not interfere with SCF-dependent Kit signalling, and because several studies have shown that function-blocking SCF/Kit antibodies do not inhibit HMC-1, HLMC or skin MC adhesion, it is possible that this antibody binds outside the SCF binding domain on Kit. If so, then this indicates the presence of a novel adhesion epitope on human Kit, which mediates adhesion via a counter-receptor which is not SCF.

The role of airway smooth muscle in mast cell differentiation, survival and activation. Human ASM cells not only recruit MCs but also enhance MC survival and activation (Fig. 12). In co-culture studies, human ASM cells promote human lung MC survival and proliferation in the absence of exogenous survival factors such as SCF, IL-6 and serum [81]. This is mediated through a co-operative interaction between human lung MC CADM1, membrane-bound SCF expressed on human ASM cells, and soluble IL-6, likely produced by both cell types [53, 81]. Co-culture of human lung MCs and human ASM cells also augments constitutive histamine release from MCs without affecting their ability to

respond to activation through Fc ϵ RI [81]. This human ASM cell-dependent human lung MC activation would explain the presence of chronically activated MCs within the ASM bundles in asthma [136].

MCs within the ASM bundle in asthma commonly express fibroblast markers when compared to those in the lamina propria, and the number of these fibroblastoid MCs correlates closely with the severity of AHR [103]. *In vitro*, human lung MCs and HMC-1 cells cultured with fibronectin or with primary human ASM cells acquire typical fibroblastic markers and morphology. This differentiation towards a fibroblastoid phenotype is mediated by ASM-derived extracellular matrix proteins and is attenuated by $\alpha_5\beta_1$ blockade. Fibroblastoid MCs demonstrated increased chymase expression and activation with exaggerated constitutive histamine release.

The effects of mast cell mediators on airway smooth muscle. The interaction of MCs with human ASM cells is undoubtedly bi-directional. MCs infiltrating the ASM bundles of asthmatic airways exhibit features of activation, shown by both immunohistochemistry and electron microscopy analyses [136] (Fig. 13). The plethora of cytokines, mediators and proteases expressed and released by MCs (Tables 3–5) and the immediacy of their proximity to ASM cells means that MC activation within the ASM bundles is likely to have profound implications on airway pathophysiology in asthma.

The well-described MC-derived autacoid mediators such as histamine, PGD₂ and LTC₄ are potent contractile agonists for ASM and drive the EAR following allergen challenge (see above). As ASM cells from patients with asthma exhibit enhanced contractile responses to

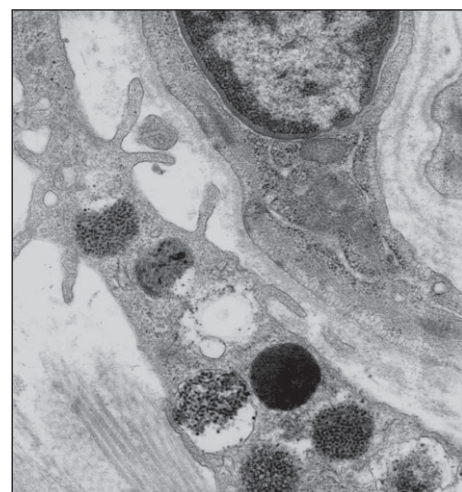


Fig. 13. Activation of a mast cell in close proximity to an airway smooth muscle cell. Scanning electron micrograph of a MC adjacent to an ASM cell in the airway of a patient with asthma, with the MC showing evidence of piecemeal degranulation. Image from Professor Patrick Berger, with permission.

histamine *in vitro* when compared to cells from healthy controls [558], ASM-dependent MC activation alone may aggravate background ASM lability in asthmatic airways.

MC tryptase may also mediate bronchoconstriction. *In vitro*, tryptase augments the contractile response of sensitized bronchi to histamine [248], and *in vivo*, exogenous tryptase induces bronchoconstriction and AHR in both dogs and sheep [246, 247]. Tryptase also stimulate cytokine release from human ASM cells and is also a potent ASM mitogen *in vitro* [548, 559–561]. The precise mechanism whereby tryptase interacts with human ASM cells is unclear because although several studies have suggested the need for an intact catalytic site [559, 562], one study reported that nonproteolytic actions may be involved in mitogenesis [560].

The transmembrane form of tryptase, but not the secreted form of tryptase which is ~50% identical, also induces AHR in mice, an effect which is dependent on expression of the IL-4R alpha subunit and STAT6 [563]. Transmembrane tryptase increases expression of IL-13 in the airways of these mice and also stimulates IL-13 release from T cells. It would be interesting to know if it has a similar effect on human lung MCs, leading to autocrine amplification of MC cytokine release and the subsequent development of AHR.

Chymase has been less extensively studied than tryptase, but is expressed by MCs infiltrating the ASM in asthma [108]. Chymase degrades human ASM pericellular matrix and inhibits T cell adhesion to ASM which might contribute to the paucity of T cells within the ASM bundles in asthma [564]. However, unlike tryptase, chymase inhibits human ASM proliferation. The effects of the joint expression of these proteases and other MC mediators *in vivo* therefore remains unclear, but it is noteworthy that ASM proliferation in asthma *in situ* has rarely been observed [565, 566].

MC-derived IL-4 and IL-13 may enhance AHR. For example, the instillation of IL-4, IL-13 or Th2 cell-conditioned medium into the airways of naïve mice induces AHR within 6 h via the IL-4 receptor α subunit and STAT6 [542]. *In vitro* studies have further suggested that IL-4 and IL-13 aggravate AHR by enhancing the magnitude of agonist-induced intracellular Ca^{2+} responses in human ASM cells [567–569], although there may be desensitization with chronic exposure [570]. IL-13 also reduces the response of human ASM cells to β_2 -adrenoceptor agonists [571]. Human MCs within the asthmatic ASM bundles express both IL-4 and IL-13 [540], providing a further mechanism through which MCs may contribute to the development of AHR in asthma.

In a co-culture system, Margulis et al. demonstrated that human peripheral blood-derived MCs induced human ASM contraction in collagen gels following

Fc ϵ RI-dependent activation [572]. In addition to roles for IL-13 and IL-6, a role for matrix metalloproteinases (MMPs) and serine proteases was also demonstrated. In fact, MMPs were critical mediators of the response, with both MMP-1 and MMP-2 strongly expressed in this system. Serine proteases were thought to contribute by mediating the proteolytic activation of MMPs.

TGF- β is released by both MCs and ASM cells. In co-culture, human lung MCs promoted the secretion of TGF- β 1 by human ASM cells through the release of tryptase [573]. ASM-derived TGF- β then up-regulated α smooth muscle actin (α -SMA) expression in an autocrine manner, leading to increased spontaneous and agonist-provoked ASM contraction (Fig. 12). The number of MCs within the ASM bundles correlated with the intensity of α -SMA expression staining, providing supporting evidence that MCs may drive the ASM towards a more contractile phenotype *in vivo* [573].

MCs may also contribute to the local production of angiotensin in lung tissue. MCs release and/or generate all components of the renin-angiotensin system, including angiotensinogen, renin (which cleaves angiotensinogen into Ang I), chymase (which further converts angiotensin I into angiotensin II) and angiotensin II [115, 574–577]. According to Veerappan et al. [577], MCs in rat and guinea-pig airways are essential for the local production of angiotensin II, which is a critical factor governing bronchoconstriction. However, an independent study was unable to confirm this [578]. In humans, angiotensin II levels are increased in acute severe asthma [579], but angiotensin II is only a weak bronchoconstrictor [579, 580]. Whether the components of this system are altered in asthmatic MCs and particularly those located within the ASM, and their contribution to ASM dysfunction remains to be determined.

Mast cell infiltration of the airway epithelium in asthma

Infiltration of the airway epithelium by MCs in asthma is likely to be significant in the pathogenesis of asthma for at least two reasons. Firstly, by residing within the superficial interface between the immune system and the environment, intraepithelial MCs are present at the point of entry of noxious stimuli and aeroallergens. This allows MCs to play effector roles in the ongoing immunological response such as antigen presentation [376, 384], Th2 cell differentiation [378], B cell class switching and IgE synthesis [386, 387]. Secondly, similarly to ASM cells, there is important crosstalk, which has implications for the function of both MCs and epithelial cells (Fig. 12).

Intraepithelial MCs in mild steroid-naïve asthma were initially shown to be predominantly of the classic MC_T phenotype, expressing tryptase but not chymase [106, 112]. However, in 2010 Dougherty et al. demonstrated

that intraepithelial MCs in asthma also express carboxypeptidase A3 (CPA3), which had previously been identified only in association with chymase [112]. We have called these MC_{T/CPA} in this article, but as discussed earlier, perhaps all MCs express CPA3. Mechanistically, IL-13-stimulated airway epithelial cells produced increased quantities of SCF and supported the survival of the MC_{T/CPA} phenotype, but suppressed chymase expression, providing a plausible explanation for these observations [112] (Fig. 1).

Overall, MC numbers are increased in the asthmatic airway epithelium of steroid-naïve subjects, but there is wide variation in the number of cells present, with a continuum rather than a dichotomy [112, 120, 530]. The number of epithelial MCs correlated strongly with the expression of IL-4 protein in the airway epithelium indicating a link between MC infiltration and Th2 cytokine expression [120]. This was confirmed more recently by Dougherty et al. where the number of intraepithelial MCs correlated with an epithelial Th2 gene signature [112]. In this latter study, the level of epithelial mRNA expression for both tryptase and CPA3 correlated strongly with the improvement in lung function in response to corticosteroid treatment [112]. This is in keeping with previous work suggesting that the response to corticosteroids in asthma is greatest in those with eosinophilic Th2-high disease [533, 581]. The presence of intra-epithelial MCs therefore predicts a steroid-responsive pathology in steroid-naïve subjects.

The presence of intraepithelial MC_{T/CPA} cells in asthma has been confirmed independently and is increased in patients with exercise-induced asthma compared to those without [582]. This would suggest that this should also apply to indirect bronchoconstriction to mannitol, but another study found that the response to mannitol was related to increased numbers of MC_{TC}s in the airway lamina propria [583]. While there is some inconsistency here, these data support a role for MCs in indirect AHR.

Both oral and inhaled corticosteroids reduce airway epithelial and submucosal MCs numbers in steroid-naïve asthma [584, 585], but only the MC_T (probably MC_{T/CPA}) phenotype [586]. However, one study looking at MC phenotype in severe refractory asthma found a persistence of MCs in the airway epithelium which was accounted for by a high proportion of MC_{TC} cells [587]. However, this is discrepant with another study demonstrating that the number of intra-epithelial MCs declines with increasing disease severity [392], which could be readily explained by the effect of increasing doses of inhaled or oral corticosteroids.

Recruitment of mast cells by the airway epithelium in asthma. The precise mechanisms of MC recruitment to the airway epithelium in asthma are not known.

CXCL12 is capable of inducing human lung and cord blood-derived MC migration *in vitro* [547], is expressed by airway epithelial cells, and its levels are significantly elevated in the BAL fluid of patients with asthma compared with healthy controls [588]. The airway epithelium in asthma also produces many other growth factors and chemokines for MCs including SCF, TGF- β 1, CXCL8, CXCL10, CCL5 and CCL11 (Fig. 12), which may contribute to MC recruitment.

The airway epithelium and mast cell adhesion, survival, differentiation and activation. Although there is limited information available about how MCs find their way into the airway epithelium in asthma, MCs adhere to the airway epithelium, although the adhesion pathway is poorly defined. Approximately 50% of human lung MCs and HMC-1 cells within any individual preparation adhere to airway epithelial cells, in part via a carbohydrate-dependent mechanism [396]. In addition, a non-function-blocking anti-Kit scFv antibody reduces human lung MC and HMC-1 adhesion to airway epithelial cells [557].

In marked contrast to the interaction between MCs and human ASM cells [81], co-culture of human lung MCs with primary human airway epithelial cells and the BEAS-2B cell line significantly reduces both constitutive and IgE-dependent histamine release [325, 589]. This effect is attenuated by epithelial wounding with a scratch. The mechanism by which epithelial cells inhibit human lung MC histamine release is not clear, but limited experiments suggest that a soluble lipid mediator acting via a Gi-coupled GPCR is involved [325]. At first these observations might appear to contradict the presence of degranulated MCs in the asthmatic airway epithelium. We have therefore proposed that the role of the healthy intact bronchial epithelium is to maintain MCs (and other cells) in a relatively quiescent state, but during tissue insults such as those caused by infective agents, pollution and allergens for example, epithelial damage and denudation removes this suppressive activity leading to MC priming and activation as part of the innate immune response [325, 589] (Fig. 14). If true, the unleashing of MC activity through the loss of an epithelial brake may be central to the pathogenesis of asthma.

The dysfunctional pro-inflammatory asthmatic airway epithelium may also activate MCs. Notably, SCF expression in the airway epithelium is elevated in asthma in comparison with health [262], providing a mechanism by which the airway epithelium promotes MC recruitment, survival and activation in asthma (Fig. 12). IL-13 triggers SCF production from human airway epithelial cells and IL-13 expression is increased in a proportion of subjects with asthma [590].

IL-13, IL-4 plus dsRNA and several innate stimuli including bacterial peptidoglycan, TLR3 agonists and

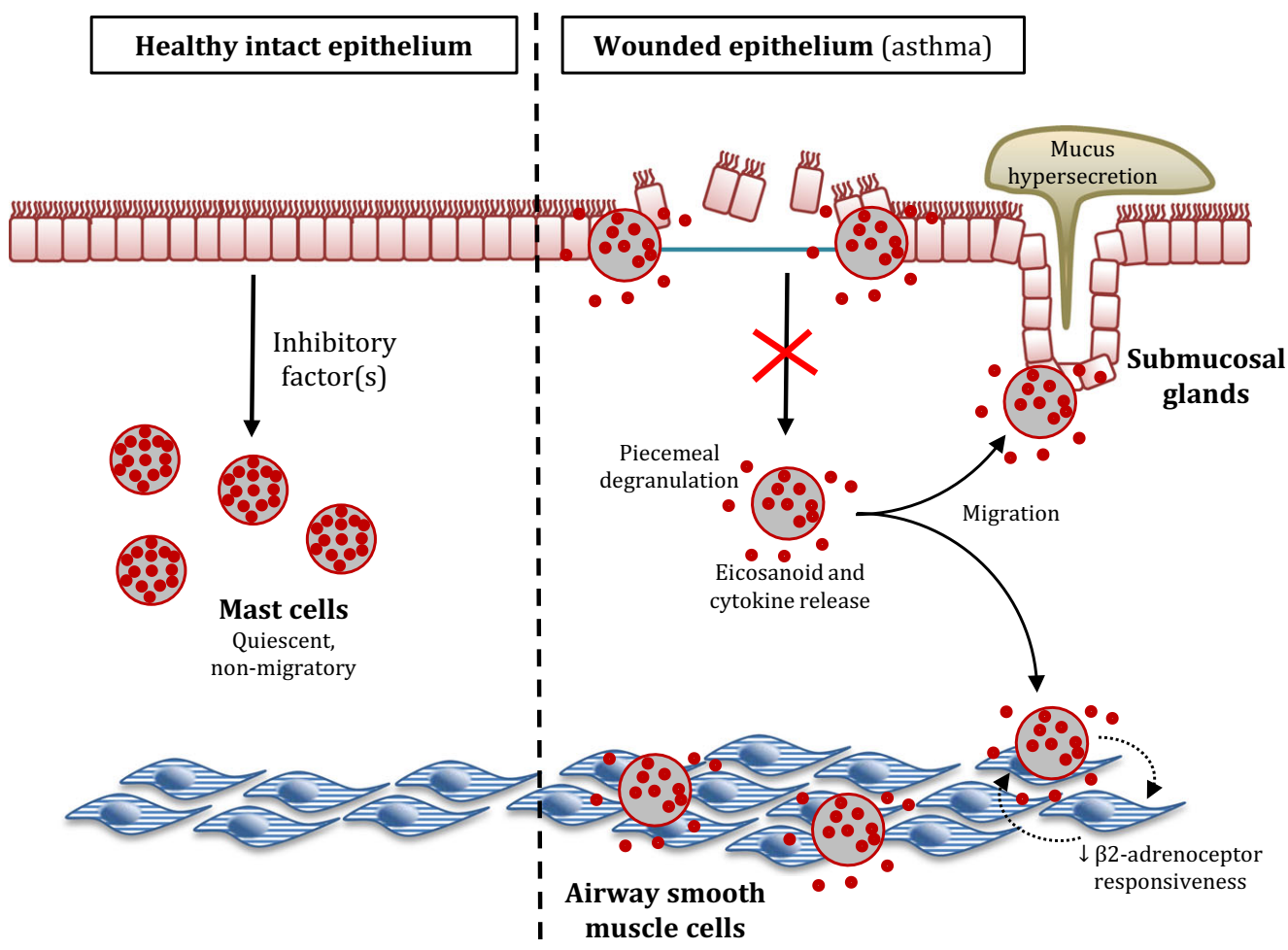


Fig. 14. The airway epithelial inhibitory hypothesis: inhibitory factor(s) in health maintain MCs in a quiescent and nonmigratory state. In asthma, these factors are lost, thus permitting piecemeal degranulation, mediator release and migration.

pro-inflammatory cytokines induce TSLP release from airway epithelium [233, 273, 591] (Fig. 12). TSLP is of great interest as a novel target for the treatment of asthma and is highly expressed in asthmatic airway epithelium across the spectrum of disease severity when compared to healthy controls [392]. TSLP derived from human airway epithelial cells induces the release of numerous cytokines and chemokines from human MCs, including IL-5 and IL-13 [233, 273], and when combined with IL-33, increases human cord blood-derived MC tryptase, carboxypeptidase A3 and cysLT production [582]. Furthermore, in a mouse model of rhinitis, MC activation was required for the increased TSLP production by airway epithelium [592]. There is therefore a potential positive feedback loop between MC-derived cytokines including IL-13, and the epithelial production of TSLP which in turn drives MC activation (Fig. 12). Human MCs are also able to produce TSLP and express TSLP in asthmatic airways [392, 452, 593], but human lung MC proteases rapidly

cleave TSLP [452], suggesting that they may regulate TSLP bioavailability tightly.

The biological effects of mast cells on the airway epithelium. Individual MC mediators have several important effects on airway epithelial cells, but dynamic co-culture experiments examining the epithelial response to MC activation are relatively lacking. However, bearing in mind the points discussed above, and the further biological activities listed in Tables 3–5, it seems likely that activated MCs within the airway epithelium in asthma will play an important role in epithelial biology.

For example, tryptase stimulates airway epithelial IL-8 release and up-regulates ICAM-1 expression [594]. This might increase susceptibility to rhinovirus infection and in turn, asthma exacerbations. Furthermore, as tryptase degrades respiratory allergens, there may be a MC-dependent negative feedback mechanism controlling the allergic inflammatory response [595, 596]. The airway epithelium is damaged in asthma and MCs may

contribute. Superoxide produced following MC degranulation [597] generates highly reactive oxygen species including hydrogen peroxide, the hydroxyl radical ($\text{OH}^\cdot$) and oxygen free radicals, which are able to damage cell membranes. Furthermore, canine chymase weakens intracellular bonds [598], so might release epithelial cells from the basal layer. As up to 20% of MCs infiltrating the bronchial epithelium in steroid-naïve asthma contain chymase [106], an effect of released chymase on bronchial epithelial integrity cannot be discounted.

Plasminogen activator inhibitor-1 (PAI-1) has been implicated in airway remodelling in asthma (reviewed in [599]) and is produced by both human MCs [600, 601] and airway epithelial cells [602]. Following $\text{Fc}\epsilon\text{RI}$ -dependent activation of the human LAD2 MC line, chymase activated latent $\text{TGF-}\beta 1$ secreted by MCs, which in turn induced the production of PAI-1 by bronchial epithelial cells. MCs may therefore promote airway epithelial-dependent airway remodelling through the stimulation of epithelial PAI production.

When human peripheral blood-derived MCs were cocultured with primary human bronchial epithelial cells, activation with exogenous IL-33 induced cytokine release from MCs, with MC IL-13 then initiating a transcriptional programme in epithelial cells that would be expected to exacerbate airway pathology and contribute to fibrosis, eosinophilia and mucous metaplasia [603]. It would be interesting to see if the effects of IL-33 on MCs are increased further when the epithelium is wounded.

Mast cell microlocalization within airway submucosal glands. Mucus hypersecretion by hyperplastic submucosal glands and epithelial goblet cells is a characteristic feature of asthma. It is an established feature of severe fatal asthma but is also present in milder disease [411, 412, 604]. An examination of the cartilaginous airways in post-mortem lung specimens suggested a strong association between MCs and mucus hypersecretion [411]. There was a significant increase in the number of MCs within the mucosal gland stroma in nonfatal asthma (patients with asthma who died from other causes), and a marked increase in the number of degranulated MCs in both fatal asthma and nonfatal asthma when compared to 'normal' controls. Additionally, the extent of mucus plugging of the airway lumen correlated with the total number of MCs and number of degranulated MCs within submucosal glands. These findings and those from the ASM strongly implicate a role for MCs in the series of events leading to asthma death.

The biological effects of mast cells on mucus secreting cells. Numerous MC products have the potential to con-

tribute to both mucous gland hyperplasia and mucus hypersecretion (Fig. 12). Among the autacoid mediators, histamine, prostanoids and leukotrienes (LTD_4 and LTC_4) are effective inducers of mucus secretion, with LTD_4 considered the most potent [476–478]. IL-13 is heavily implicated in goblet cell hyperplasia in both animal models and *in vitro* models of the human airway epithelium, as are the MC proteases chymase and trypsin [605–608]. Canine MC chymase is also a potent mucus secretagogue for bovine airway glands [609]. IL-6 and $\text{TNF-}\alpha$ induce mucous glycoprotein secretion and MUC-2 gene expression by both human bronchial organ explant cultures and airway epithelial cells obtained by bronchial brushing [610, 611]. IL-6 also induces expression of MUC5B and MUC5AC [612]. This is relevant because IL-6⁺ MCs are present within the bronchial mucous glands in asthma [120].

Amphiregulin, a member of the epidermal growth factor family, is a further molecule of interest in the context of mucus hypersecretion. *In vitro*, amphiregulin augments mucous metaplasia in bronchial epithelial cells [613]. Amphiregulin is produced by human cord and peripheral blood-derived MCs following activation through $\text{Fc}\epsilon\text{RI}$ [453, 614], and specifically, MC-derived amphiregulin increased mucin gene expression in the NCI-H292 epithelial cell line [453]. This effect was not suppressed by dexamethasone. Increased expression of amphiregulin in MCs correlated significantly with the extent of goblet cell hyperplasia in bronchial biopsies from patients with asthma [453], and amphiregulin concentrations were up-regulated in the sputa of patients with asthma during acute exacerbations [613]. Thus MC-derived amphiregulin may contribute to epithelial goblet cell metaplasia and mucus hypersecretion in asthma.

Mast cell interactions with airway fibroblasts

Deposition of type-III and type-V collagens in the lamina reticularis below the true epithelial basement membrane is a characteristic pathological feature of asthma [615]. This gives the appearance of a thickened basement membrane under the light microscope. The most likely origin for this collagen is proliferating myofibroblasts whose number correlates with the thickness of the lamina reticularis [616], although it is unclear whether this has any implications for airways function. Many decades of research have shown that MCs interact closely with fibroblasts [7, 40, 399, 617], and it is likely that they contribute to myofibroblast activity in asthma (Fig. 12).

In humans, histamine, basic FGF, $\text{TNF-}\alpha$ and IL-4 induce fibroblast proliferation [618–620]. In addition, $\text{TNF-}\alpha$ and intact MC granules stimulate fibroblasts to secrete collagenase [621, 622]. IL-4 is a chemoattractant

for human fibroblasts [623] and triggers the secretion of collagen types I and III and fibronectin from human fibroblasts [624]. IL-13 increases CCL11 release from human airway fibroblasts [625], and tryptase induces proliferation, chemotaxis, collagen synthesis and migration in human fibroblasts [626–628]. In addition, heparin indirectly potentiates fibroblast activation and proliferation by regulating basic FGF bioactivity: heparin provides structural stability for basic FGF and preserves its bioactivity by protecting it from degradation [629]. Furthermore, heparin and/or heparan sulphate facilitate the release of basic FGF from its basement membrane storage location and the binding of basic FGF to its receptors [630]. Similar to human ASM cells, Margulis et al. demonstrated that human peripheral blood-derived MCs induced human lung fibroblast collagen production and contraction in collagen gels following FcεRI-dependent activation [572]. They found that MMP-2 and -3 derived from MCs were important for these effects, likely via the activation of latent TGF-β1. Lung MC-derived TGF-β1 also has the potential to induce fibroblast differentiation into contractile myofibroblasts. Lastly, recombinant amphiregulin induces the proliferation of human airway fibroblasts but not ASM cells, suggesting a further mechanism whereby MCs might contribute to subepithelial fibrosis [613].

In addition to exerting effects on fibroblasts through a variety of mediators, MCs also interact with fibroblasts via direct contact. Cultured rat fibroblasts use long cytoplasmic processes to phagocytose and internalize rat MC granules, a process termed transgranulation by Greenberg and Woodstock [631]. This uptake of granules is followed by secretion of collagenase and β-hexosaminidase [632]. In co-culture, human MCs adhere avidly to human fibroblasts, in part via CADM1, and this promotes HLMC survival and proliferation [79]. Adhesion does not appear to involve known integrin or cadherin receptors [556]. Heterotypic cell–cell contact between either HMC-1 cells or human skin MCs with human skin fibroblasts augments fibroblast proliferation and requires expression of MC-associated IL-4 [620]. In this study, although IL-4 was present in the lysates of HMC-1 cells it could not be detected in the supernatant of the co-culture system. This suggests that IL-4 was secreted by MCs in low amounts and strictly limited to cell–cell contacts with fibroblasts. Interestingly, it has previously been proposed that MCs may present IL-4 on their surface to confer local cytokine specificity [120] (IL-4 is a heparin-binding cytokine is therefore likely to be retained on the MC surface bound to heparin sulphate proteoglycans [633]). This would be analogous to the recently described MC antibody-dependent degranulatory synapse discussed earlier in this article [315].

In summary, MC interactions with fibroblasts are likely to contribute to remodelling processes in asthmatic airways.

Mast cells and vascular remodelling in asthma

Neovascularization occurs in a number of physiological and pathological situations including wound healing and tumour growth, respectively. MCs are usually found at sites of neovascularization such as around the periphery of solid tumours [126, 127]. Vascular remodelling is a well-recognized features of asthma and is associated with the presence fixed airflow obstruction but not AHR [634]. It is associated with the expression of several angiogenic factors including basic FGF and VEGF. MCs, which are notably located adjacent to blood vessels, are therefore a potential source for these and other angiogenic factors including IL-6 and CXCL8 (Tables 4 and 5).

Rodent MCs induce endothelial cell migration and proliferation, and in the chick chorioallantoic membrane, MC activation leads to proliferation of microvessels [128]. Furthermore, following tumour cell implantation in MC-deficient mice, there are a decreased number of capillaries at the tumour periphery, reduced tumour size and fewer metastases than in normal mice [129]. Supernatants from unstimulated HMC-1 cells induce proliferation of human microvascular endothelial cells, an effect which inhibited 90% by neutralizing VEGF antibody [130]. In a system of vascular tube formation, human dermal microvascular endothelial cells exposed to HMC-1 cell supernatants or co-cultured with HMC-1 cells rapidly differentiated and matured into vascular tubes [131]. Tube growth was about 20-fold greater in HMC-1 supernatant-treated vascular cells than controls and was reproduced by exposure of vascular cells to purified human tryptase. Heparin alone also had a smaller but significant effect (25% enhancement over control). Human tryptase is therefore a further angiogenic factor.

Human MCs are therefore likely to play a critical role in angiogenesis, and this is highly relevant to the vascular changes evident in asthmatic airways.

Interactions between mast cells and airway nerves

The lung is innervated by the parasympathetic nervous system. This is responsible for regulating ASM tone via the release of acetylcholine, but also modulates features such as the cough reflex; the afferent component of the cough reflex is mediated by unmyelinated C fibres of airway sensory nerves situated within the airway epithelial layer. The ability of MCs and airway nerves to interact is implicated by the close proximity of MCs to nerve fibres within the lung; indeed MCs have been

anatomically associated with nerve cells in a plethora of organs, animal models and human tissue samples ([635–637]). In the airways, MC interactions with nerves may be particularly relevant to the pathogenesis of cough. MC-derived products such as histamine and PGD₂ are pro-tussive and are increased in the sputum of subjects with eosinophilic bronchitis, cough variant asthma and idiopathic chronic cough compared to healthy controls [638].

Similarly to many other MC interactions, communication between MCs and nerves is bi-directional; MC-derived cytokines and mediators can stimulate and influence nerve cell activity and growth, and likewise, there is evidence that neural transmitters such as neuropeptides can induce MC degranulation [639] (Fig. 12). Prostaglandins are capable of activating nociceptive neurons via TRPA1 channels [640] and nasal afferent C nerve fibres also express receptors for cysLTs which increase neuronal excitability to electrical and chemical stimuli [641]. A mouse model has suggested that cholinergic pathways leading to the lung are required for MC-mediated bronchoconstriction following allergen challenge [642]. This demonstrates the potential for neurogenic pathways to activate MCs independently of IgE and may contribute to the pathogenesis of allergic disease.

As an abundant source of ATP, MCs may communicate with nerve cells in the central and peripheral nervous systems via purinergic receptors expressed on neurons, astrocytes and microglia (for reviews see [643, 644]). In addition, direct cell–cell contact is again likely to facilitate MC–nerve interactions. Electron microscopy has shown that rodent intestinal mucosal MCs are capable of forming direct membrane–membrane contact with nerves or even associate with nerves by extending lamellipodia [635, 645]). CADM1 expressed on MCs mediates heterotypic MC adhesion to nerves, through either heterophilic (nectin-3) or homophilic (CADM1) molecular interactions [646, 647]. When the nerves were stimulated, then Ca²⁺ was mobilized in the adjacent MCs.

Mast cells in animal models of asthma

In vivo animal models have been developed in an attempt to recreate and discern the airway features of asthma and underlying pathological mechanisms. The dependency on MCs in these models with regard to the development of AHR and inflammatory changes in the airways is highly dependent on the model studied and the mode of antigen sensitization. The mouse model using intraperitoneal antigen sensitization followed by antigen challenge of the airways is the most widely reported. This most closely resembles the model of acute allergen challenge in the airways although the

route of sensitization is different. However, sensitization without adjuvant generates a MC-dependent model while sensitization with adjuvant creates a MC-independent model (reviewed in [648]). This immediately questions the relevance of these models as there is unequivocal evidence of MC-driven early and late responses in human asthma as discussed above. 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, and so, it is perhaps not surprising that short-term models using intra-peritoneal sensitization do not find a role for MCs in the outcomes commonly measured.

An alternative model uses airway sensitization without adjuvant from the outset and to some extent is more physiological. In this setting, MCs are an essential component required for the development of AHR, inflammation and remodelling (collagen deposition, goblet cell hyperplasia [649, 650]). However, some important features of asthma are not modelled. For example, 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 have been useful for generating hypotheses and investigating specific mechanistic pathways, their findings may also be potentially misleading.

Inhibiting human mast cell activation and its consequences in asthma

As can be seen from the above text, the factors contributing to MC activation in asthma are complex. There is a relatively poor understanding of the contribution many of these activation pathways make in disease, how they interact, and the signalling mechanisms involved.

It has often been proposed that MCs cannot be important in asthma pathophysiology because so-called MC stabilizing drugs are not very effective at preventing symptoms or attenuating the underlying inflammation. However, although several commonly used drugs are able to inhibit MC secretion, these either lack potency or are ineffective at inhibiting MC activation in chronic asthma. For example, disodium cromoglycate is only a weak inhibitor of IgE-dependent human lung MC secretion with maximal inhibition of histamine release *in vitro* of 10–20% when used in the high micromolar range [432] and it also exhibits rapid tachyphylaxis. It is now known that disodium cromoglycate and its derivative nedocromil target GPR35 [651]; it is possible that newer more potent agonists will demonstrate better efficacy than cromoglycate

itself. β_2 -adrenoceptor agonists such as salbutamol are more potent inhibitors of HLMC mediator release *in vitro* [432], but again there is rapid tachyphylaxis [652], so that with chronic administration the clinical evidence is that they do not attenuate MC secretion in the asthmatic airway and may even enhance it [137, 261, 653].

Targeting MCs effectively in diseased tissues therefore remains a significant challenge and an urgent goal. The overview of MC biology at the start of this article demonstrates that there are many check points in the biological activity of MCs that might be useful therapeutically, from inhibition of growth, survival and recruitment to tissue, to antagonism of pro-secretory molecules, signalling pathways and individual MC mediators (for recent review see [654]).

To-date there are not many examples of drugs targeting MC activation pathways that have reached man, and those newer drugs that have, are largely focussed on Fc ϵ RI-dependent MC activation. Omalizumab has been successful in treating IgE-dependent diseases such as urticaria, allergic rhinitis and to a degree, atopic asthma [217, 500, 655, 656]. In contrast, a Syk inhibitor R112 demonstrated modest short-term efficacy in patients with seasonal allergic rhinitis when administered intranasally [657], but with regular dosing for one week this was ineffective [658], and R112 did not attenuate the nasal response to allergen challenge [659]. The Kit/PDGF-R inhibitor masitinib showed some benefit in a small phase 2 over 16 weeks in severe corticosteroid-dependent asthma [660]; a phase 3 trial is currently underway.

In terms of targeting mediators, antihistamines are effective in allergic rhinoconjunctivitis and to an extent in urticaria, but of no benefit in asthma, although leukotriene receptor 1 antagonists are useful

in some patients with asthma. Regarding PGD₂, trials of thromboxane receptor antagonists in asthma met with disappointment, but unpublished data suggest there is hope for CRTh2 antagonists in patients with eosinophilic disease. Novel monoclonal antibodies targeting Th2 cytokines in asthma are showing promise when targeted to Th2-high patients [661, 662], but Th2 cytokines come from many sources of which MCs are one.

The vast majority of drugs being considered for clinical development aimed at MCs remain focussed on IgE-dependent signalling. However, the morphology of MC degranulation in chronic asthma is predominantly piecemeal [135–137], the mechanism of which is unknown, and this may simply not be susceptible to inhibition by drugs which attenuate classical IgE-dependent anaphylactic degranulation. It is our opinion that there is an urgent need to delineate the Fc ϵ RI-independent mechanisms and the associated signalling pathways that drive chronic MC activation in asthma if we are going to develop effective MC inhibitors for asthma, and in fact, other MC-mediated diseases.

Summary

In summary, there is extensive evidence implicating MCs as central players in the pathophysiology of asthma. Developing therapeutic strategies which dampen their pathological activity remains an important goal.

Conflict of interest

The authors declare no conflict of interest.

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