



Exploring the origin and regulatory role of mast cells in asthma

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Purpose of review

Mast cells have previously been thought to function solely as effector cells in asthma but more recent studies have indicated that mast cells may play a more central role in propagating and regulating lower airway inflammation in asthma.

Recent findings

Initial studies have found increased numbers of mast cell progenitors (MCPs) in the peripheral blood of patients with asthma and these cells could contribute to the increased number of progenitors identified in the airways of patients with asthma. There are unique subpopulations of mast cells within the asthmatic airway, which are characterized by their physical location and distinguished by their expression profile of mast cell proteases. Intraepithelial mast cells are tightly associated with type-2 (T2) inflammation but additional studies have suggested a role for anti-mast cell therapies as a treatment for T2-low asthma. Mast cells have recently been shown to closely communicate with the airway epithelium and airway smooth muscle to regulate lower airway inflammation and airway hyperresponsiveness.

Summary

Recent studies have better illuminated the central role of mast cells in regulating lower airway inflammation and airway hyperresponsiveness.

Keywords

airway hyperresponsiveness, airway inflammation, asthma, mast cell, mast cell protease

INTRODUCTION

Asthma is a heterogeneous clinical syndrome with features of reversible airflow obstruction and airway hyperresponsiveness (AHR) that is driven by lower airway inflammation. Mast cells have long been implicated as key effector cells in asthma as they are increased in the airways of patients with asthma in comparison to healthy controls [1,2,3^{***}] and are known to generate key mediators that promote lower airway inflammation and bronchoconstriction, such as the cysteinyl leukotrienes (CysLTs), prostaglandin D₂ and other small molecules that are critical for manifestations of allergic airway inflammation [4]. However, recent studies have suggested that mast cells also play a unique role in regulating lower airway inflammation. Here we review the hematopoietic origin of mast cells and how they traffic to specific locations within the airways of patients with asthma. We also review the association between mast cells and specific inflammatory signatures in the airways with a focus on the role of mast cell proteases and mast cell–airway epithelial interactions in regulating lower airway inflammation.

ORIGIN AND LOCATION OF MAST CELLS IN THE AIRWAYS OF INDIVIDUALS WITH ASTHMA

Mast cells are enigmatic cells of hematopoietic origin that circulate in the peripheral blood as progenitor cells and mature in peripheral tissues where they develop unique expression patterns based on their tissue microenvironment [5,6]. Mast cell development has been predominantly studied in murine models where mast cell progenitors (MCPs) have been identified in peripheral blood [7] and peripheral tissues [8]. Murine models have also indicated

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KEY POINTS

- Mast cell progenitors (MCPs) are increased in the peripheral blood of patients with asthma and there is an association between these cells and reduced lung function in patients with asthma.
- There is a shift in mast cell location from the submucosa to the airway epithelium in asthma, which is tightly linked to airway hyperresponsiveness (AHR) and type-2 (T2) lower airway inflammation.
- There is a unique subpopulation of mast cells within the airways expressing tryptase and carboxypeptidase A3 (CPA3) but not chymase ($MC_{T/CPA3}$) in the airway epithelium of patients with asthma.
- Mast cells have been associated with both T2 and non-T2 inflammation in asthma suggesting a role for antimast cell therapies in both patient populations.
- Mast cells interact closely with multiple cell populations within the airways, most notably the airway epithelium and ASM, to regulate lower airway inflammation and AHR.

that allergic airway inflammation significantly increases the number of MCPs in the lungs because of recruitment rather than expansion of a local tissue-specific population [9] and that this process is dependent upon activation of the adaptive immune system [10]. Studies have also demonstrated that influenza infection can promote lung MCP recruitment [11] in *Rag2*^{-/-} mice, suggesting that the innate immune system also plays a key role in this process [12]. In humans, CD34⁺ cells have been isolated from peripheral blood that have the potential to develop into mature mast cells [13–15,16[¶]] and preliminary studies have suggested that this cell population is increased in patients with

allergic asthma and reduced lung function (Fig. 1) [15,16[¶]]. Two prior studies have revealed that the number of CD34⁺ cells is increased in the airways of patients with asthma [17,18]. Although some of these cells were identified as eosinophil progenitor cells, more than half of the total CD34⁺ cells were unclassified [18]. The portion of this population that represents MCPs is unknown and will be a key question moving forward in understanding the trafficking of MCPs from the peripheral blood to the lung. Additional studies with greater phenotypic detail are needed to gain a full understanding of the relationship between MCP trafficking and the features of asthma.

Mature mast cells are found throughout the lung [19–21] and are increased within the airway epithelium [3[¶],22,23], airway smooth muscle (ASM) [24], and alveolar parenchyma [25] of patients with asthma in comparison to nonasthmatic controls. There has been particular interest on the importance of intraepithelial mast cells as these cells have been closely linked with AHR [3[¶],23], type-2 (T2) lower airway inflammation [3[¶],22,23] and responsiveness to inhaled corticosteroids [22]. Our laboratory recently published a study using quantitative morphometry in the form of design-based stereology from patients with and without asthma who were not on controller asthma therapies and were extensively characterized for AHR in the form of a direct challenge with methacholine and with an indirect or endogenous stimulus in the form of exercise-induced bronchoconstriction (EIB). We discovered increased numbers of submucosal mast cells in healthy control patients in comparison to patients with asthma but increased intraepithelial mast cells in asthmatics compared with healthy controls suggesting that there is a shift in mast cells from the submucosa to the epithelial layer in asthma (Fig. 2a and b) [3[¶]].

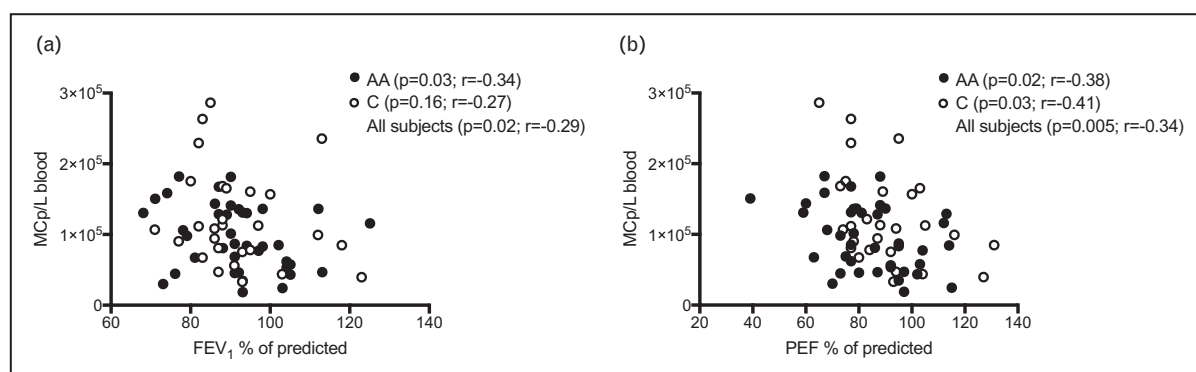


FIGURE 1. Individuals with reduced lung function have an increased number of circulating mast cell progenitors in their peripheral blood. Correlation analysis of mast cell progenitor (MCP)/l blood with FEV₁ (% predicted) (a) and peak expiratory flow (PEF) rate (% predicted) were performed for all participants, participants with allergic asthma (AA) and controls (C). Correlation analysis was tested using Spearman's correlation analysis [16[¶]]. Reproduced with permission from.

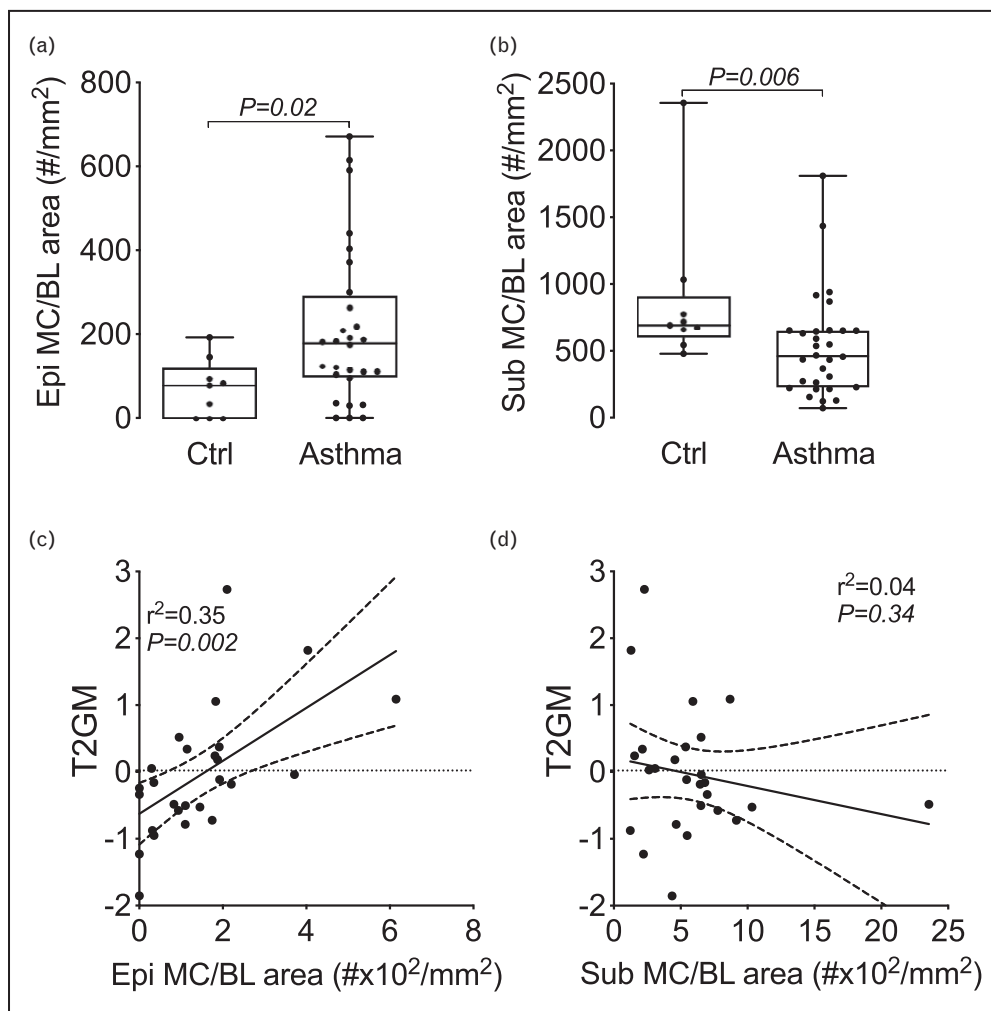


FIGURE 2. A shift in mast cells from the submucosa to the epithelium is present in asthma and correlates with sputum type 2 gene expression. Quantitative morphometry in the form of design-based stereology was used to provide an unbiased assessment of the density of mast cells, and specifically assessed the number of these cells relative to the surface area of the basal lamina in each compartment as well as the volume density of these cells in the epithelial and submucosal compartments [3^{***}]. (a and b) The number of intraepithelial mast cells relative to the area of the basal lamina (Epi MC/BL area) is increased in asthma compared with healthy controls (a). In contrast, the number of submucosal mast cells relative to the area of the basal lamina (Sub MC/BL area) is lower in asthma compared with healthy controls (b). Group comparisons are shown as box plots with median, interquartile range, minimum, and maximum. Significance was assessed by the Mann–Whitney *U* test (2-group). (c and d) The sputum type 2 gene mean (T2GM), which is a recently validated methodology for determining lower airway T2 inflammation [3^{***}], is correlated with the number of intraepithelial mast cells relative to the area of the basal lamina (Epi MC/BL area) (c), and not with the number of submucosal mast cells relative to the area of the basal lamina (Sub MC/BL area) (d). Associations are by linear regression; shown are regression lines and 95% confidence bounds. Reproduced with permission from.

FUNCTION OF MAST CELL PROTEASES AS REGULATORS OF LOWER AIRWAY INFLAMMATION IN ASTHMA

Mast cells vary not only by physical location in the airways but also by different expression patterns of mast cell proteases that identify unique subpopulations with differences in cellular function. Human mast cells express and produce a wide range of proteases but are classically defined by their expression of tryptase, carboxypeptidase A3 (CPA3) and

chymase. Mast cells located in the airway submucosa express both tryptase and chymase (MC_{TC}) whereas mast cells in the mucosal layer predominantly express tryptase and lack chymase expression (MC_T). Over the last decade, multiple translational studies evaluating epithelial brushings have identified a unique population of mast cells that are located in the airway epithelium and in the airway lumen of patients with asthma that are characterized by high expression of CPA3, persistent

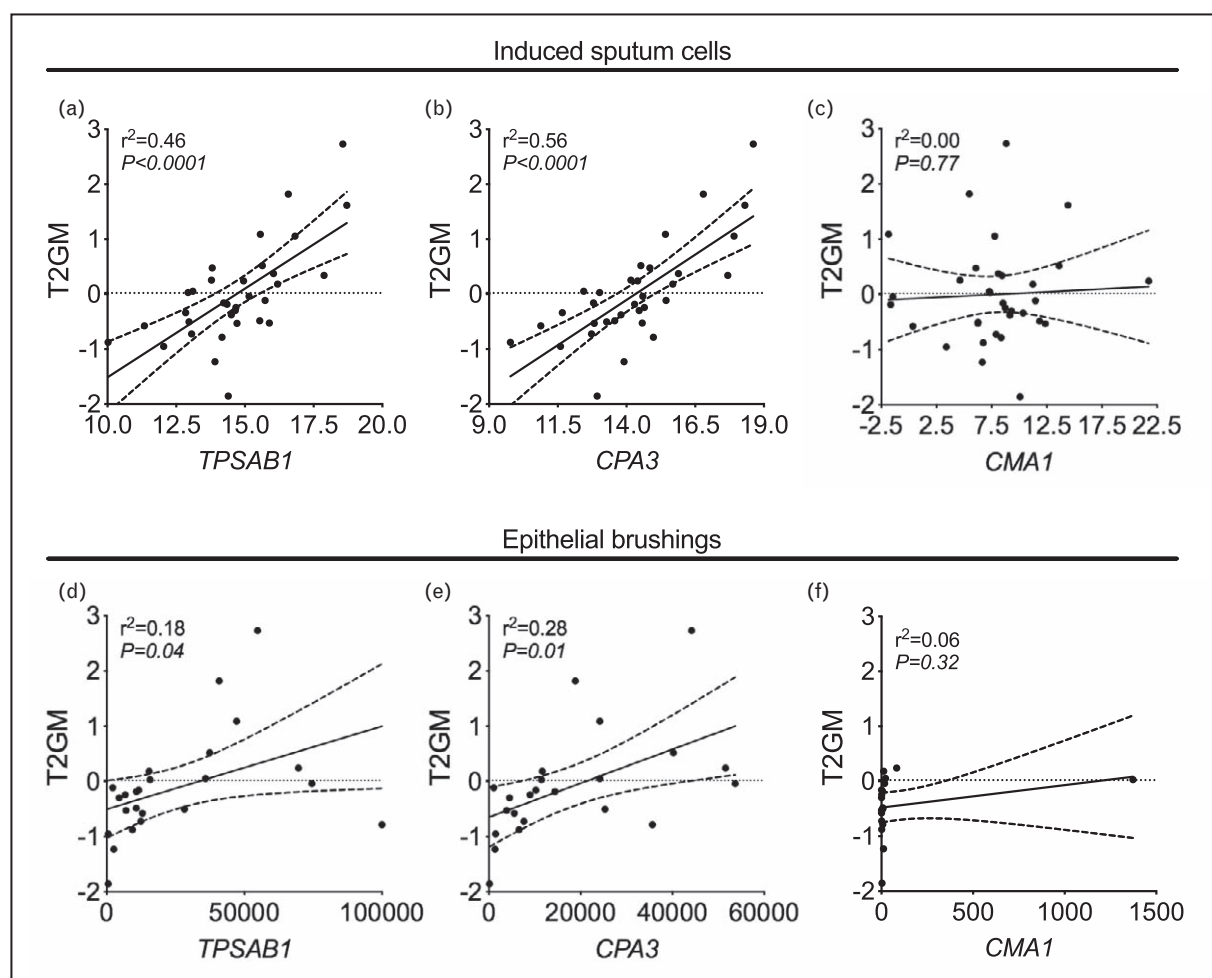


FIGURE 3. Sputum type 2 gene expression is associated with mast cell gene expression in sputum and bronchial brushings. The type 2 gene mean (T2GM) in sputum was significantly correlated with the expression of the mast cell genes *TPSAB1* and *CPA3*, but not *CMA1*, in induced sputum cells (a–c) and also in bronchial epithelial brushing (d–f) [3²²]. All associations are by linear regression; shown are regression lines and 95% confidence bounds. Reproduced with permission from.

expression of tryptase and the loss of chymase ($MC_T/CPA3$) [22,26,27]. This population of cells has been closely associated with indirect or endogenous AHR and the expression of T2 inflammatory genes in the airways (Fig. 3) [3²²,22]. This change in the individual mast cell protease expression profile may be especially critical for regulating T2 inflammation because of the differential effects of these proteases on interleukin (IL)-33 metabolism. IL-33 is a key airway epithelial-derived cytokine implicated in asthma pathogenesis and is known to promote T2 cytokine production in mast cells and other innate immune cells. IL-33 can be regulated by inflammatory proteases via cleavage from its full-length form to shorter length peptides with higher and lower potency [28]. Tryptase is known to metabolize IL-33 into a more active form whereas chymase proteolytically inactivates IL-33 [29–32]. Thus, the presence of tryptase but lack of chymase activity amongst the intraepithelial mast cell subpopulation found in

asthma may enhance the effects of IL-33 and further promote T2 inflammation.

RELATIONSHIP BETWEEN LOWER AIRWAY INFLAMMATION AND MAST CELLS

Airway inflammation in asthma has been dichotomized between type-2 (T2) and non-T2 inflammatory signatures [33] and studies have suggested that mast cells play a key role in driving both types of lower airway inflammation.

T2 inflammation is characterized by increased production of the T2 cytokines IL-4, IL-5, and IL-13 and is associated with allergic and eosinophilic inflammation. Eosinophilic inflammation is classically responsive to corticosteroids [34–38] but studies have identified persistent T2 inflammation in patients with asthma despite corticosteroid therapy [33,38–40], suggesting that another cell population

more resistant to corticosteroids contributes to ongoing T2 inflammation. Although the exact mechanisms that initiate and promote persistent T2 inflammation remain unclear, the airway epithelium is known to play a key role by secreting specific, epithelial-derived cytokines [e.g. IL-25, IL-33 and thymic stromal lymphopoietin (TSLP)] to signal innate and adaptive immune cells to produce T2 cytokines [41]. Early studies suggested that T2 cytokines were predominantly generated by helper T cells [33] and more recent studies have implicated IL-33-responsive innate cells, including type-2 innate lymphoid cells (ILC2s) and recruited macrophages as major sources of IL-13 [42–53]. Mast cells have received less attention but are known to produce T2 cytokines in response to airway epithelial-derived cytokines [3^{54,55}] and murine models of asthma have suggested that mast cells play a key role in the development of lower airway inflammation [56–58]. Prior translational studies have discovered increased numbers of intraepithelial mast cells in patients with T2-high asthma in comparison to patients with T2-low asthma and healthy controls [22] and, more recently, demonstrated a tight correlation between intraepithelial mast cells and expression of T2 inflammatory markers in the sputum (Fig. 2c and d) [3⁵⁹].

Several studies have also shown that mast cells are associated with features of asthma that appear separate from T2 inflammation. A recent study found increased numbers of mast cells in induced sputum samples from patients with asthma in comparison to healthy controls and, although there was correlation between sputum eosinophilia and sputum mast cells, there was no correlation between peripheral blood eosinophil counts and sputum mast cells; further, a subset of asthmatic patients had elevated sputum mast cells despite absence of airway eosinophilia [59⁶⁰]. Another preliminary study identified increased serum tryptase levels in patients with severe asthma, which did not correlate with peripheral blood eosinophils counts or serum periostin levels [60]. Additionally, a recent study demonstrated efficacy of an anti-mast cell therapy in treating patients without markers of T2 inflammation. Imatinib, a KIT proto-oncogene receptor tyrosine kinase inhibitor, was effective in reducing airway mast cell counts, decreasing AHR, and improving lung function in patients with severe asthma. Additional analyses demonstrated the effects of imatinib on AHR were negatively correlated with baseline peripheral blood eosinophil counts and improvement in lung function was positively correlated with baseline airway neutrophilia, suggesting imatinib predominantly suppressed inflammation in patients with noneosinophilic

inflammation [61]. It should be noted that measures of T2 gene expression in the lower airways were not assessed in this study.

INTERACTIONS BETWEEN MAST CELLS AND OTHER AIRWAY CELLS

With recognition of the importance of intraepithelial mast cells and their associations with lower airway inflammation and AHR in asthma, there has been increased interest in exploring the relationship between mast cells and other cell populations within the airway.

Airway epithelium

The relationship between intraepithelial mast cells and the airway epithelium has been of particular interest. Prior coculture modeling of airway epithelial cells with a human mast cell line demonstrated a significant increase in IL-6, CXCL8 and CXCL1 production by the airway epithelium upon exposure to mast cells [62]. More recent studies have focused on the effects of mast cells on the airway epithelial T2 inflammatory response. IL-33 is the key airway epithelial-derived cytokine for promoting T2 cytokine expression and production from mast cells [3⁶³] and a recent study discovered IL-33-stimulated mast cells can induce features of IL-13-mediated gene expression in airway epithelial cells [63]. Our laboratory recently expanded upon these findings by demonstrating that mast cells participate in a feed-forward loop in which activation of mast cells by IL-33 leads to an accentuation of epithelial *IL33* expression [3⁶⁴], indicating that mast cell–epithelial interactions play an important role in regulating the epithelial T2 inflammatory response. Interestingly, our studies clarified that the effects of IL-13 directly on the airway epithelium lead to a reduction in *IL33* gene expression.

Airway smooth muscle cells

Interactions between mast cells and ASM cells have been long considered a key feature of asthma pathobiology [64]. As previously discussed, mast cells are found in increased numbers within ASM bundles in patients with asthma, are associated with development of ASM hypertrophy and hyperplasia, and are known to promote ASM contraction and bronchoconstriction [65]. A prior study demonstrated that supernatant fluid and lysates of human lung mast cells modulated the secretory and proliferative functions of ASM cells [66]. A recently published study delineated a mechanism for CysLT-mediated ASM contraction by demonstrating that ASM-derived

ATP was necessary to trigger mast cell production of CysLTs [67]. There is also evidence that mast cells effect ASM contraction via phosphorylation of the β_2 -adrenoreceptor (β_2 -AR) [68–70]. Another study also suggested a role for tryptase in regulating ASM in asthma via metabolism of ASM-derived matrix metalloproteinase-1 to its active form, which has been shown to promote ASM proliferation, is increased in the airways of patients with asthma, and is associated with AHR and asthma severity [71].

Airway nerves

Afferent and efferent nerves are present throughout the airway and are known to play a key role in modulating AHR [72]. Prior studies have documented mast cells in close proximity to peripheral sensory nerves in other tissues [73–76] but, importantly, this co-localization has not been rigorously characterized in asthma. Studies have demonstrated that significant crosstalk between mast cells and peripheral nerves can occur via paracrine signaling, direct uptake of mast cell granules and direct contact between cells utilizing physical synapses via integrins [77]. Studies have also demonstrated that mast cell activation can be triggered by multiple neuropeptides, including substance P via neurokinin-1 receptor [78] and Mas-related G-protein-coupled receptor member X2 [79,80] as well as neuromedin U via neuromedin U receptor 1 [81,82], and direct binding to nerves via cell adhesion molecule 1 (CADM1) [83]. The regulatory effects of mast cells on airway nerve function are not well understood but preliminary studies suggest that allergen-induced bronchoconstriction is dependent on the presence of both mast cells and intact airway innervation [84], mast cell-derived neuropeptides promote increased innervation of ASM [85], and tryptase can induce peripheral nerves to produce inflammatory mediators by cleaving neuronal proteinase-activated receptor 2 (PAR2) [86].

Eosinophils

Similar to mast cells, eosinophils have long been recognized as key effector cells in asthma and are also increased within the airway epithelium of patients with asthma. However, despite their co-localization within the airway epithelium and well characterized effector functions in asthma, little is known about how mast cells and eosinophils interact [87]. Preliminary studies of co-cultured human cord-blood-derived mast cells (CBMCs) and peripheral blood eosinophils demonstrated reciprocal activation of each cell type via paracrine signaling as well as direct cell–cell contact [88–90]. CBMCs also

increased eosinophil survival and promoted eosinophil chemotaxis. Additionally, mast cell proteases may play a role in regulating eosinophils as tryptase has been shown to promote eosinophil activation and degranulation [91].

CONCLUSION

Although mast cells have been primarily known for their effector functions in asthma, recent work suggests that mast cells play a more central role in driving and regulating airway inflammation in asthma. Recent studies have indicated that mast cells are hemopoietic in origin, traffic to the airways in response to activation of both the innate and adaptive immune system and are found in increasing numbers within the peripheral blood that may contribute to the pool of progenitors in the airways of patients with asthma. Mast cells traffic to a specific location within the airway epithelium in asthma and undergo critical changes in their protease profile that may serve to regulate inflammation and can be therapeutically targeted. Mast cells have been associated with both T2 and non-T2 inflammation in asthma, suggesting that targeting these cells and their individual interactions with the airway epithelium, ASM, airway nerves, and other innate immune cells including eosinophils may be an effective treatment strategy for some patients. Ongoing studies will undoubtedly reveal more about the origin of this mast cell population, the regulation of trafficking of these cells to the airways, and the manner in which these cells interact with the other cell populations within the airways.

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Conflicts of interest

There are no conflicts of interest.

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- of special interest
- of outstanding interest

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