Human mast cell and airway smooth muscle cell interactions: implications for asthma

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Page, Severine, Alaina J. Ammit, Judith L. Black, and Carol L. Armour. Human mast cell and airway smooth muscle cell interactions: implications for asthma. Am J Physiol Lung Cell Mol Physiol 281: L1313–L1323, 2001.—Asthma is characterized by inflammation, hyperresponsiveness, and remodeling of the airways. Human mast cells (HMCs) play a central role in all of these changes by releasing mediators that cause exaggerated bronchoconstriction, induce human airway smooth muscle (HASM) cell proliferation, and recruit and activate inflammatory cells. Moreover, the number of HMCs present on asthmatic HASM is increased compared with that on nonasthmatic HASM. HASM cells also have the potential to actively participate in the inflammatory process by synthesizing cytokines and chemokines and expressing surface molecules, which have the capacity to perpetuate the inflammatory mechanisms present in asthma. This review specifically examines how the mediators of HMCs have the capacity to modulate many functions of HASM; how the synthetic function of HASM, particularly through the release and expression of stem cell factor, has the potential to influence HMC number and activation in an extraordinarily potent and proinflammatory manner; and how these interactions between HMCs and HASM have potential consequences for airway structure and inflammation relevant to the disease process of asthma.

contraction; proliferation; remodeling; inflammation; cytokines

Asthma is characterized by inflammation, hyperresponsiveness, and remodeling of the airways. The main agents for the treatment of asthma are β2-agonists and corticosteroids. Although potentially life-saving during an attack, β2-agonists mainly provide symptomatic relief from airway narrowing. Corticosteroid therapy, on the other hand, aims to reduce the inflammatory process in the lung and to improve or reduce further deterioration of lung function. Despite these and other forms of treatment, asthma morbidity is on the increase (52, 56, 97) as is its worldwide prevalence (145a), with the highest prevalence being in English-speaking countries (10).

Several cells within the lungs are implicated in the disease and have been proposed as critical for the initiation and perpetuation of inflammation. These include mast cells (MCs), eosinophils, T lymphocytes, B lymphocytes, fibroblasts, and airway smooth muscle (ASM) cells. Which of these cells is (are) responsible for the initiation of the disease is not known. However, it is improbable that only one cell is responsible for both the initiation and the perpetuation of this chronic inflammatory disease. A more plausible scenario is one where the effector cells exert concerted, coordinated, and overlapping effects. The extent to which each effector cell contributes toward each phase of an inflammatory response remains to be fully elucidated.

The MC is one cell that appears to play an important role in the orchestration of inflammation in the airway (117). Human MCs (HMCs) release mediators that cause exaggerated bronchoconstriction, induce smooth muscle cell proliferation, and recruit and activate inflammatory cells (18) and could thus contribute to the characteristic features of asthma.

The human ASM (HASM) cell is another cell central to the disease. The HASM layer of an asthmatic pa-
tient is markedly increased (40, 59) and hyperresponsive, both of which contribute to airway narrowing (64, 77). An additional factor that makes HASM central to the process of asthma is its recently discovered synthetic function (70). The muscle has the potential to actively participate in the inflammatory process by synthesizing cytokines and chemokines and expressing surface molecules such as adhesion molecules (95), all of which have the capacity to promote and perpetuate the inflammatory mechanisms present in asthma (32).

Interestingly, the number of HMCs present on allergenic or asthmatic HASM cells is increased compared with that of nonallergic, nonasthmatic HASM cells (1, 30). Could there be a particular relationship between HMCs and HASM cells in asthma? Are there properties intrinsic to the asthmatic muscle that favor HMC accumulation, or does the increased number of HMCs present convert the muscle to an asthmatic phenotype?

Although HMCs and HASM cells interact with numerous other cells and play a role in many diseases of the lung, this review focuses solely on how the interactions between these two specific cells in an inflammatory milieu could contribute to the characteristic changes seen in asthma. Only studies with human cells will be cited, except in those instances where important data have been generated in animal models for which parallels do not exist in humans (see INVOLVEMENT OF THE MC IN THE EARLY- AND LATE-PHASE RESPONSES).

INVOLVEMENT OF THE MC IN THE EARLY- AND LATE-PHASE RESPONSES

The myriad of mediators synthesized and released by the MC and their involvement in IgE-mediated responses, leukocyte infiltration, and tissue remodeling has generated immense interest in the role that MCs may have in asthma in both the early- and late-phase responses.

The Early-Phase Response

The evidence for MCs being key players in acute allergic reactions is well documented (62, 93, 125, 139). MCs constitutively express the high-affinity receptor for IgE (FcεRI) in abundance (>130,000/cell), and on cross-linking with IgE, degranulation occurs rapidly. Other cells bearing FcεRI have been identified and have thus been postulated to participate in allergen-induced bronchospasm. Indeed, constitutive expression also occurs in basophils and dendritic cells (134), and FcεRI has been found on epithelial cells (27), monocytes and macrophages, and eosinophils in response to parasite infection (53). Basophils are circulating cells not normally present in the healthy lung and are only recruited within tissues during inflammatory responses; however, investigators (11, 80, 87, 138) have found few basophils in either bronchoalveolar lavage fluid (BALF) or biopsies of asthmatic patients. Dendritic cell FcεRI expression has been shown to be increased in asthmatic patients; however, dendritic cell numbers were ~6.5-fold lower than MC numbers (134). Furthermore, neither was the amount of FcεRI per dendritic cell determined nor was the functionality of the receptor assessed, although induction of a transduction signal would presumably result in facilitation of allergen capture and internalization. Similar to basophils, eosinophils are normally absent from the healthy lung but, unlike basophils, are a characteristic feature in the asthmatic lung. FcεRI expression by eosinophils was demonstrated in response to parasitic infection; however, Tunon-de-Lara et al. (134) were unable to demonstrate eosinophil FcεRI expression in asthmatic bronchial tissue. Thus, despite a large variability of FcεRI-bearing cells being found in the lung, the MC remains a strong candidate for initiating the IgE-mediated early-phase response based on the significant numbers found in critical locations in the lung as well as on its range of mediators that can induce bronchospasm.

The Late-Phase Response

In contrast to the widely accepted role of MCs in the early-phase response, the role of the MC in the late-phase response and its contributions to the features of chronic inflammation are controversial. This debate has been explored in mouse models in great depth and is discussed here. Results from studies with transgenic MC-deficient mice are conflicting. Some groups (26, 100, 130) showed that the involvement of MCs is unnecessary for the development of pulmonary eosinophilia after allergen challenge and airway hyperresponsiveness. Others demonstrated that MCs have some contribution to these features (28, 81, 89), notably at low levels of antigenic stimulation (90, 145). Yet another study (79) in MC-deficient mice came to the conclusion that MCs are essential to airway hyperactivity. Williams and Galli (144) suggested that the spectrum of results may be attributable to differences in the sensitization and challenge protocols; in the mouse models where MCs are not essential, sensitization agents producing strong nonspecific antibody responses were used, which may not be representative of “natural” sensitization where even low-dose exposure to an allergen can produce massive responses. In this natural model, it is proposed the MC would serve as a critical amplification mechanism for IgE-mediated responses.

In the human asthmatic lung, MC numbers are increased and bronchial hyperresponsiveness correlates with HMC number in both asthmatic adults and children (44, 138). In addition, in vivo evidence from endobronchial biopsies shows that HMC degranulation is greater in asthmatic subjects compared with nonasthmatic subjects (11, 38, 65, 112) and is ongoing (22, 112) and therefore not limited to the early-phase response. Moreover, metabolites of PGD₂, a MC-specific mediator, were found to be elevated in the urine of asthmatic patients after allergen challenge in both the early and late phases, providing direct evidence of in vivo MC involvement in the late-phase response in asthma (104). The same study (104) also provided evidence for MC involvement in non-IgE-mediated asthmatic re-
sponses such as exercise-induced asthma and aspirin-sensitive asthma.

Thus, despite the controversy over its role in the late-phase response and the features of chronic inflammation, there is growing evidence in both mice and humans that, if not crucial, MCs remain important contributors to certain aspects of the late or chronic phases of allergic as well as of nonallergic inflammation (47, 48, 103, 142, 145).

**HMC MEDIATORS: THEIR EFFECTS ON HASM CELLS**

HMCs are able to synthesize, store, and, on stimulation (with cytokines or FceRI cross-linking), release a plethora of mediators, most of proinflammatory consequence but some with anti-inflammatory actions. Data suggest that HMCs are present in an activated state in symptomatic asthma because analysis of the BALF from asthmatic patients shows increased levels of mediators capable of activating and degranulating HMCs (23). Of the mediators released by HMCs on stimulation, roughly three-fourths are known to have an effect on HASM cells (Table 1). Their effects can be divided into three broad categories related to the functions of the HASM and its altered characteristics in asthma.

**Proliferation and Remodeling**

The increased smooth muscle bulk in asthma that contributes to airway narrowing may be the result of an increased presence of mitogenic stimuli, an increased intrinsic capacity for mitogenesis (71), an impairment of apoptosis, or a combination of the above. The mediators of HMCs that influence proliferation of HASM cells are numerous and are summarized in Table 2.

**Proliferative effects and remodeling.** Those HMC mediators that are mitogenic for the HASM are tryptase (25) and platelet-derived growth factor (9). Tumor necrosis factor (TNF)-α at concentrations ranging from 0.3 to 30 pM can stimulate proliferation via the TNF-α p55 receptor (3). Low concentrations of PGE₂ (10⁻⁷ M) cause HASM cell proliferation (70). Leukotriene (LT) D₄ indirectly induces proliferation by stimulating an increase in matrix metalloproteinase (MMP)-1 that leads to the proteolysis of insulin growth factor binding protein, allowing more insulin growth factor to bind to its receptor and induce proliferation (106, 116).

HMCs may also participate in airway remodeling through the release of key factors involved in the control of fibrosis, migration, and differentiation. Tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) are important regulators of fibrinolysis; both convert plasminogen to plasmin, which regulates proteolytic degradation of the extracellular matrix and thus participates in fibrinolysis. Under unstimulated conditions, HMCs are a source of tPA (127) and therefore have fibrinolytic activity. HASM cells express the receptor for uPA (uPAR), and on association with the uPAR, uPA induces migration of HASM cells in culture (96). Whether HASM cells express receptors for tPA is unknown at present. The activity of both tPA and uPA is neutralized on binding with plasminogen activator inhibitors to promote fibrosis. Stimulated HMCs produce and release plasminogen activator inhibitor-1 (31), with two outcomes: 1) loss of HMC fibrinolytic activity to adopt a profibrotic profile and 2) inhibition of HASM migration via the prevention of the binding of uPA on to uPAR. How the latter would impact on airway remodeling has yet to be determined. Another activated HMC cytokine of importance in remodeling in the lung is transforming growth factor (TGF)-β1 (21). TGF-β1 is capable of inducing phenotypic modulation of human lung fibroblasts to myofibroblasts (57). This phenotypic modulation has been postulated as a possible explanation for the presence of fibrosis and increased bulk of muscle present in asthma (21). Others (52) have hypothesized that myofibroblasts originate from smooth muscle cells themselves after allergen challenge. Because HMCs are the predominant cells that are activated during an allergen challenge, they may produce the cytokines that are responsible for the differentiation of smooth muscle.

**Table 1. Mediators released from HMCs with effects on HASM**

<table>
<thead>
<tr>
<th>HMC Mediators</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM-CSF</td>
<td>55</td>
</tr>
<tr>
<td>Heparin</td>
<td>131</td>
</tr>
<tr>
<td>Histamine</td>
<td>123</td>
</tr>
<tr>
<td>IL-1</td>
<td>54</td>
</tr>
<tr>
<td>IL-4</td>
<td>19</td>
</tr>
<tr>
<td>IL-10</td>
<td>63</td>
</tr>
<tr>
<td>IL-13</td>
<td>78</td>
</tr>
<tr>
<td>Leukotrienes</td>
<td>88</td>
</tr>
<tr>
<td>PAF</td>
<td>123</td>
</tr>
<tr>
<td>PDGF</td>
<td>74</td>
</tr>
<tr>
<td>Prostaglandins</td>
<td>84, 101</td>
</tr>
<tr>
<td>TGF-β</td>
<td>73, 74</td>
</tr>
<tr>
<td>TNF-α</td>
<td>19</td>
</tr>
<tr>
<td>Tryptase</td>
<td>126</td>
</tr>
</tbody>
</table>

HMC, human mast cell; HASM, human airway smooth muscle; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; PAF, platelet-activating factor; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α.

**Table 2. HMC mediators that modulate HASM proliferation**

<table>
<thead>
<tr>
<th>Effect on HASM</th>
<th>HMC Mediator</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferative</td>
<td>Histamine</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>Tryptase</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>PDGF</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>LTDA (via MMP)</td>
<td>106, 116</td>
</tr>
<tr>
<td></td>
<td>Low concentrations of TNF-α</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>Low concentrations of PGE₂</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>TGF-β</td>
<td>36</td>
</tr>
<tr>
<td>Antiproliferative</td>
<td>Heparin</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>IL-4</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>High concentrations of TNF-α</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>High concentrations of PGE₂</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>TGF-β</td>
<td>35</td>
</tr>
<tr>
<td>LTD₄, leukotriene D₄, MMP, matrix metalloproteinase.</td>
<td></td>
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</tr>
</tbody>
</table>
cells to myofibroblasts, which are then recruited and accumulated beneath the basement membrane.

**Antiproliferative effects.** Some mediators of HMCs can inhibit proliferation of HASM cells. At a concentration of 300 pM, found in the BALF from asthmatic patients, TNF-α has indirect antiproliferative effects; it reduces the proliferative effects of mitogens acting on G protein-coupled receptors, such as thrombin, and on receptors having intrinsic tyrosine kinase activity, such as epidermal growth factor. This potentially protective inhibition of mitogenesis is abolished by corticosteroids (128). Heparin and PGE₂ at a high concentration (10⁻⁶ M) inhibit HASM cell proliferation (70). PGE₂ at high concentrations also inhibits platelet-derived growth factor-induced mitogenesis. Interleukin (IL)-4 inhibits HASM cell proliferation by inhibiting cyclin D1, an important protein for cell cycle progression into G₁ as well as inhibiting proliferation induced by mitogens such as thrombin (GTP-coupled receptor) or epidermal growth factor (intrinsic tyrosine kinase-coupled receptor) (58).

TGF-β has been shown to induce or inhibit cell proliferation depending on the cell type. In HASM cells, the evidence is conflicting in that TGF-β promotes HASM cell proliferation but inhibits mitogen-induced HASM cell proliferation acting downstream from GTP- and intrinsic tyrosine kinase-coupled receptors (35, 36).

Based on the evidence at this point, HMCs have the capacity, through the release of mediators, to modulate the growth of HASM and therefore to affect the remodeling process seen in asthma, with predominantly proinflammatory consequences.

**Contraction and Hyperresponsiveness**

Contraction of HASM leads to airway narrowing and dyspnea in the asthmatic patient. There is some evidence that HMCs may be involved in the process.

**Stimulatory effects.** Histamine and various LTs (LTC₄ and LTD₄) are the classic mediators of HMCs, causing contraction of HASM via their respective receptors (15). Platelet-activating factor and PGD₂ are also released and can cause contraction (16, 43, 69). The effect of PGE₂ is biphasic; at low concentrations, PGE₂ relaxes HASM, and at higher concentrations ranging from 10⁻⁶ to 3 × 10⁻⁵ M, PGE₂ causes contraction via the thromboxane A₂ receptor (5).

In addition to the direct contractile effects on the muscle, two HMC mediators, TNF-α [possibly acting via the TNF-α p55 receptor (3)] and tryptase, have both been shown to induce hyperresponsiveness of HASM (Table 3) (4, 68).

**Inhibitory effects.** In the lower concentration range of 10⁻⁹ to 10⁻⁶ M, PGE₂ causes relaxation of HASM cells (Table 3) (5).

The mediators of HMC degranulation all cause a contractile response or induce hyperresponsiveness in HASM, with only one mediator, PGE₂, capable of causing both contraction and relaxation of HASM depending on its concentration. Thus, in terms of effects on HASM contraction, HMC mediators may cause contraction or relaxation, but the evidence suggests that the majority of mediators are stimulatory.

**Inflammatory and Synthetic Functions**

HASM cells in culture have recently been shown to have the capacity to actively participate in the inflammatory process by synthesizing an array of cytokines and chemokines and expressing various surface molecules (72). Several HMC mediators are able to modulate the synthetic function of HASM (Table 4, Fig. 1).

**Proinflammatory effects.** IL-1β stimulates HASM cell production of IL-6 (41), IL-8 (66), IL-11 (41), granulocyte-macrophage colony-stimulating factor (GM-CSF) (55), PGE₂ (108), leukemia inhibitory factor (41), eotaxin (51), monocyte chemotactrant protein (MCP)-1 (115), and bradykinin B₂ receptors (124). TNF-α can increase IL-6 (2, 92), IL-8 (67, 140), regulated on activation normal T cell expressed and secreted (RANTES) (2, 67), GM-CSF (121), eotaxin (33, 51), and MCP-1 (115, 140) production from HASM. Low levels of PGE₂ increase TNF-α-induced IL-6 release from HASM cells (2). TNF-α can also stimulate the expression of the adhesion molecules intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 (105) as well as the membrane surface protein CD40 (83).

The ligand for CD40 (CD40L) is found on HMCs (50, 136), B cells, and activated T cells. Ligation of the pair plays a crucial role in B cell activation and isotype switching to IgE (17, 50). In HASM cells, CD40 ligation leads to increased IL-6 release (Fig. 1) as it does in activated B cells (34, 83). TGF-β can stimulate HASM cells to produce IL-6 (41), IL-8 (46), IL-11 (41), and leukemia inhibitory factor (41). PGE₂ (1 μM) and platelet-activating factor stimulate IL-8 (109) and RANTES (91) production, respectively, from HASM cells. LTD₄, as previously mentioned (106, 116), can increase MMP-1 production in HASM cells.

**Anti-inflammatory effects.** Only four HMC mediators have been reported to inhibit HASM cell mediator synthesis. Both IL-4 and IL-10 inhibit IL-8 and RANTES production from HASM cells (66, 67). IL-4 also reduces IL-1β- and TNF-α-induced MCP-1 (115) release from HASM. IL-13 reduces RANTES (67) production from HASM cells. PGE₂ inhibits TNF-α-induced ICAM-1 and VCAM-1 expression in HASM cells.

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### Table 3. HMC mediators that modulate HASM contractile properties

<table>
<thead>
<tr>
<th>Effect on HASM</th>
<th>HMC Mediator</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contraction</td>
<td>Histamine</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Leukotrienes</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>PAF</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>PGD₂</td>
<td>16, 43</td>
</tr>
<tr>
<td></td>
<td>High concentrations of PGE₂</td>
<td>5</td>
</tr>
<tr>
<td>Hyperresponsiveness</td>
<td>TNF-α</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Tryptase</td>
<td>68</td>
</tr>
<tr>
<td>Relaxation</td>
<td>Low concentrations of PGE₂</td>
<td>5</td>
</tr>
</tbody>
</table>
and TNF-a-induced RANTES production from HASM cells (2). HMC mediators can thus directly induce or modulate stimulated HASM cytokine synthesis and surface protein expression.

HASM SYNTHETIC PRODUCTS: THEIR EFFECTS ON THE INFLAMMATORY ENVIRONMENT IN THE LUNG

The release of HASM synthetic products (Table 4), modulated by HMC mediators, has important repercussions for the perpetuation of the inflammatory process in the asthmatic lung. IL-6 stimulates B cell maturation, IgE synthesis and secretion (129) (Fig. 1), and T cell growth, differentiation, and activation (Fig. 1). IL-8 is chemotactic for eosinophils, HMCs (86), and neutrophils and causes neutrophil activation (6, 42, 82) (Fig. 1). IL-11 has been shown to be elevated in asthmatic airways (94), but unlike in mice, it remains to be shown whether it increases fibrosis through its effects on fibroblasts in humans. RANTES is chemotactic and activating for T cells (122) as well as for eosinophils (118) (Fig. 1). Eotaxin is chemotactic for eosinophils (49) (Fig. 1), and GM-CSF is responsible for eosinophil chemotaxis, proliferation, activation, and survival (110). MCP-1, as its name implies, is chemotactic for monocytes but is also chemotactic for T lymphocytes (29) (Fig. 1) and is a potent basophil activator, causing histamine release (13). MMP-1 contributes to airway remodeling and obstruction through its capacity to induce HASM cell proliferation.

Thus HASM cell production of IL-6, IL-8, RANTES, GM-CSF, eotaxin, and MCP-1 and expression of CD40 and the adhesion molecules ICAM-1 and VCAM-1 result in the recruitment and activation of key inflammatory cells in asthma, namely the T and B lymphocytes, eosinophils, neutrophils, and fibroblasts. Moreover, MMP-1 release from HASM cells can contribute to remodeling by increasing muscle cell proliferation.

The above effects of HASM, which are initiated and modulated by HMCs, result in an inflammatory milieu that itself will have stimulatory effects on HMCs. This indirect interaction of HASM and HMCs is complemented not only by direct actions through specific HASM cytokines but also by surface protein contact with HMCs.

HASM CELL CYTOKINES AND SURFACE PROTEIN EXPRESSION: THEIR EFFECTS ON HUMAN MAST CELLS

Asthmatic HASM is exposed to and stimulated by many inflammatory cytokines. Stimulated HASM is capable of interacting with HMCs through the release of cytokines and surface protein expression.

Cytokines

The cytokines that HASM has the capacity to synthesize and that have an effect on HMC function are listed in Table 5. Some of these cytokines are constitutively expressed, e.g., GM-CSF or soluble stem cell factor (sSCF), whereas others are induced by HMC mediators (see HASM SYNTHETIC PRODUCTS: THEIR EFFECTS ON THE INFLAMMATORY ENVIRONMENT IN THE LUNG). Although stem cell factor (SCF) has defined proinflammatory actions, the effects of the other four cytokines, PGE2, IL-6, RANTES, and GM-CSF, on HMCs are not well defined.

PGE2 reduces cytokine release from HMCs (111, 113). IL-6 reduces c-kit (the receptor for SCF) expression on HMCs and thereby HMC proliferation (76, 114). It is well established that RANTES does not induce degranulation of HMC, although one study (99) found that RANTES weakly potentiates IgE-mediated degranulation. GM-CSF reduces histamine and tryptase contents, downregulates HMC FcεRI expression (45, 143), and downregulates SCF-driven HMC differentiation (39). The three cytokines PGE2, IL-6, and GM-CSF may serve as a negative feedback mechanism to suppress the activated HMCs in the HASM layer. Yet both PGE2 and IL-6 assist in vitro differen-

<table>
<thead>
<tr>
<th>HMC Mediator</th>
<th>Synthesis From HASM Cells</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>IL-6 66</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>IL-8 115</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>Leukemia inhibitory factor 41</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PGE2 105</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>GM-CSF 46</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>Eotaxin 41</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>MCP-1 115</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>TNF-α 115</td>
<td>66</td>
</tr>
<tr>
<td>TNF-α</td>
<td>IL-6 115</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>IL-8 115</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>Leukemia inhibitory factor 41</td>
<td></td>
</tr>
<tr>
<td>TGF-β</td>
<td>IL-6 115</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>IL-8 115</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>Leukemia inhibitory factor 41</td>
<td></td>
</tr>
<tr>
<td>PGE2</td>
<td>IL-8 115</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>PAF 115</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>LTD4 115</td>
<td>106, 116</td>
</tr>
</tbody>
</table>

MCP-1, monocyte chemoattractant protein-1; RANTES, regulated on activation normal T cell expressed and secreted; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1.
Differentiation of cord blood mononuclear cells into fully functional mature HMCs (119). IL-8 is chemotactic for HMCs (86). GM-CSF would appear to have potentially anti-inflammatory effects on HMCs; downregulation of FceRI expression and tryptase content could result in decreased degranulation on IgE binding.

SCF exists in two forms: a membrane-bound form (mSCF; see Surface Protein Expression) and a smaller soluble form (sSCF), which is thought to be the cleaved extracellular portion of the membrane-bound form (24). SCF is chemotactic and the primary regulating factor for HMC growth, function, and survival (12, 14, 24, 98, 135). SCF also enhances IgE-stimulated GM-CSF release from HMCs (102). Thus, by release of SCF (Fig. 1), the muscle has the ability to recruit, activate, and sustain HMCs within its microenvironment and thus promote inflammation.

Surface Protein Expression

Smooth muscle can also communicate with the inflammatory environment through the proteins expressed on its surface. Table 6 lists the surface proteins expressed on HASM that can interact with HMCs.

Table 5. HASM cytokines modulating HMC function

<table>
<thead>
<tr>
<th>HASM Cytokines</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>41</td>
</tr>
<tr>
<td>IL-8</td>
<td>86</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>55</td>
</tr>
<tr>
<td>PGE2</td>
<td>137</td>
</tr>
<tr>
<td>RANTES</td>
<td>2, 67</td>
</tr>
<tr>
<td>Soluble stem cell factor</td>
<td>75</td>
</tr>
</tbody>
</table>

Table 6. HASM surface protein expression

<table>
<thead>
<tr>
<th>HASM Surface Protein Expression</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradykinin B2 receptor</td>
<td>109</td>
</tr>
<tr>
<td>CD40</td>
<td>83</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>105</td>
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<tr>
<td>VCAM-1</td>
<td>105</td>
</tr>
<tr>
<td>Membrane-bound stem cell factor</td>
<td>75</td>
</tr>
</tbody>
</table>

IL-1β stimulates the expression of bradykinin B2 receptors on the surface of HASM cells. The docking of bradykinin on those newly expressed receptors stimulates PGE2 production from the muscle, which, in turn, stimulates IL-8 release from HASM (109). PGE2, as mentioned in Cytokines, assists HMC differentiation and maturation but also reduces cytokine release on degranulation.

CD40, a member of the TNF-α receptor family, was first discovered on the surface of B lymphocytes and was found to be crucial for B cell isotype switching on ligation with CD40L (8). CD40 is also expressed on HASM cells (Fig. 1) as well as on a number of other cells (leukocytes, dendritic cells, and mesenchymal cells) but not on HMCs (136). On the other hand, CD40L is expressed on HMCs (50) (Fig. 1). On HASM, ligation with HMC CD40L leads to IL-6 release from the muscle (83) (Fig. 1). As previously mentioned (2, 92), TNF-α can also induce IL-6 production in HASM. In combination, CD40 ligation and TNF-α further enhance IL-6 release from HASM (83). Again, although antiproliferative for HMCs, IL-6 assists HMC maturation.

The adhesion molecule ICAM-1 binds to its complementary adhesion molecule lymphocyte function-associated antigen-1.
ciated antigen-1 (LFA-1), and VCAM-1 binds to very late activating antigen-4. ICAM-1 and VCAM expression on HASM can be induced, as previously mentioned (105), by the HMC mediator TNF-α. HMCs express both very late activating antigen-4 and LFA-1 (141) (Fig. 1), and in an inflammatory milieu under the influence of IL-4, HMC expression of LFA-1 is upregulated (133). Binding of HASM ICAM-1 with HMC LFA-1 upregulates HMC degranulation and cytokine production. Moreover, this provides HASM with yet another means to promote and/or facilitate HMC infiltration into the local environment.

Finally, the mSCF not only retains the same crucial actions on HMCs as sSCF but may have even more profound effects on HMCs than the sSCF (20, 60). Moreover, although protein levels were not determined, Kassel et al. (75) found a higher proportion of mSCF mRNA than sSCF mRNA in HASM. The muscle, therefore, has the capacity through both sSCF and mSCF to recruit, activate, and prolong the survival of HMCs (Fig. 1). Furthermore, cell contact through mSCF not only maintains HMCs in close vicinity to the muscle but also allows potentiation of interactions between the two cell types.

SCF METABOLISM BY HUMAN MAST CELLS

Two distinct subtypes of HMCs have been identified according to their enzymatic content, tryptase positive, chymase negative (T) and tryptase positive, chymase positive (TC), and this may have implications for the degradation of SCF and modulation of its effects. The largely predominant subtype in the healthy human lung is the HMC-T, which represents ~90% of the total HMCs in the lung (61). This leaves 10% of HMCs in the healthy lung that contain chymase. Whether the ratio of HMC-T to HMC-TC changes in asthma is not known; however, there is evidence (61a) that this does occur in other disease states such as cystic fibrosis where the proportion of the MC-TC subtype is increased. What determines the differentiation of HMC precursors into HMCs-T or HMCs-TC remains to be fully elucidated. Nevertheless, what is known is that when HMC precursors from the bone marrow are stimulated in vitro with SCF alone or in combination with IL-6 and PGE2, HMCs-T only (85, 120) or HMCs-TC with low levels of chymase are generated. However, the addition of IL-4 promotes immature HMC differentiation to HMCs-TC containing high levels of chymase (132, 146).

Of the two enzymes, the one of relevance to SCF metabolism is chymase. Chymase has the capacity to cleave sSCF (37). Intact sSCF is made up of 166 amino acids that chymase rapidly and specifically cleaves at Phe159 to generate two metabolites: sSCF1–159 and sSCF160–166. Similarly, in the presence of activated lung HMCs, recombinant human SCF1–166 is metabolized, principally to sSCF1–159 and a shorter fragment, sSCF1–144. The small fragment, sSCF160–166, has no effect on HMCs. On the other hand, the larger fragment, sSCF1–159, retains activity on HMCs, causing histamine release, enhancement of histamine release after stimulation with anti-IgE, and chemotaxis.

Although Carroll et al. (30) were the first to notice an increased number of HMCs on the HASM layer of asthmatic patients, the subtype predominance, HMC-T or HM-TC, was not determined. A putative increased proportion of HMCs-TC could result in an attenuation in the intensity and duration of the effects of sSCF on surrounding HMCs. Conversely, a relative decrease in the number of HMCs-TC could result in a greater and longer-lasting effect of sSCF on HMCs. Alternatively, chymase released from HMCs may serve to cleave mSCF to its soluble form and thus cause further HMC chemotaxis and/or activation.

SUMMARY

HMC mediators can modulate many functions of HASM, leading to predominantly proasthmatic changes. HMC mediators can cause HASM proliferation, encourage fibrosis, and thereby contribute to airway remodeling and narrowing; HMC mediators can cause contraction and increase the responsiveness of HASM to contractile mediators, also contributing to airway narrowing; and last but perhaps most important, HMC mediators can increase proinflammatory cytokine release and surface protein expression on HASM, thus favoring inflammatory cell infiltration and activation (Fig. 1).

In comparison, HASM also interacts with HMCs; the cytokines and surface proteins of HASM affect HMCs in both excitatory and inhibitory manners. When released from HASM, PGE2 reduces HMC degranulation and IL-6 reduces HMC responsiveness to the effects of SCF by reducing the number of c-kit receptors expressed on the cell surface. However, both PGE2 and IL-6 play a role in HMC differentiation and maturation. GM-CSF also appears to be protective against HMC reactions to immunologic stimuli.

To complement the effects of stimulated HASM cytokines on HMC function, there are interactions after direct contact between HMCs and HASM cells. HMC CD40L ligation with HASM cell CD40 results in IL-6 release from the muscle. The two cells being “in contact” through the CD40/CD40L surface molecules means that the resultant IL-6 release from HASM is likely to have suppressing effects on the adjoining HMCs. IL-6 production from HASM may provide a way to downregulate the proinflammatory effects induced by HMC mediators in proximity to the muscle. However, the role of IL-6 in inducing maturation of human HMCs (114) needs to be considered. What seems to be evident and relevant to the disease process is the increased number of HMCs present in the HASM layer, possibly induced by SCF. sSCF release and mSCF expression by HASM will increase HMC number and activation in the HASM layer and have widespread consequences for both HASM (contraction, proliferation, and release of inflammatory cytokines) and other inflammatory cells (recruitment and activation of eosinophils, T cells, and B cells). These effects could be
potentiated or attenuated if normal SCF metabolism by HMCs was altered. Thus HASM cells, simply through the release and expression of SCF, have the capacity to influence HMCs in an extraordinarily potent and proinflammatory manner, which has consequences for airway structure and inflammation relevant to the disease process of asthma.

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