

EDITORIAL

Human mast cell chemokines receptors: implications for mast cell tissue localization in asthma

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Mast cells

Mast cells are widely distributed throughout the body in both connective tissue and at mucosal surfaces, and form a heterogeneous population of cells with differences apparent in their mediator content, ultrastructure and functional behaviour. This applies both within the same tissue and between different tissues (reviewed in [1]). They arise from bone marrow-derived progenitor cells that circulate as undifferentiated CD34⁺ mononuclear cells in the peripheral circulation (reviewed in [2]). These progenitors subsequently migrate into tissue and mature under the influence of locally derived growth factors and cytokines, particularly stem cell factor. Mast cells are therefore found in significant numbers in normal airways, located predominantly in the sub-epithelial compartment, particularly adjacent to blood vessels.

Mast cells in asthma

Mast cells play a significant role in the pathophysiology of asthma through their ability to release a plethora of autacoid mediators, proteases and cytokines in response to their activation by both allergen and other secretory stimuli [3]. Secretion of histamine, PGD₂ and LTC₄ induces bronchoconstriction, mucus secretion and mucosal oedema, and therefore contribute to the symptoms of asthma. Mast cells also synthesize and secrete a number of cytokines including IL-4, IL-5 and IL-13, which regulate both IgE synthesis and the development of eosinophilic inflammation [4]. In addition, the mast cell produces several pro-fibrogenic cytokines and neutral proteases including transforming growth factor (TGF)- β , basic FGF, tryptase and chymase, which can interact with many cell types, and potentially contribute to airway wall remodelling. Importantly, mast cells are present in a chronically 'activated' secretory state within the asthmatic bronchial mucosa, with evidence of on-going mediator release and cytokine synthesis [5–8]. Furthermore, mast cells present in the bronchoalveolar lavage fluid (BALF) of asthmatic subjects exhibit increased spontaneous and IgE-dependent mediator release [7], and strong correlations have been observed between the severity of bronchial hyper-responsiveness (BHR) and mast cell numbers, histamine concentrations and constitutive histamine release in BALF [9–12].

Mast cells infiltrate three key structures in the asthmatic airway, namely the airway smooth muscle [13], the airway

submucosal glands [14] and the airway epithelium [15]. As mast cells in the asthmatic airway are activated and their mediators have profound effects on these structural elements, this redistribution of mast cells is likely to be of great importance for the development and propagation of this disease [16]. This concept is supported by the observations that the number and activation state of mast cells in the submucosal glands correlate strongly with the extent of mucus plugging [14], while the number of mast cells in the asthmatic airway smooth muscle correlates with the degree of BHR [13]. The mechanism for this relocation of mast cells within the asthmatic airway is therefore of great interest because if delineated then novel therapies that target this could be developed.

Chemokines and mast cell migration

The signals controlling mast cell recruitment and migration within tissue are poorly understood, but the C–C and C–X–C chemokines are particularly attractive candidates as mast cell chemoattractants. These ubiquitous structurally related peptides mediate the chemotaxis of many cell types (for a detailed review, see references [17, 18]). Chemokines are a superfamily of 8–15 kDa heparin-binding chemotactic cytokines that serve as potent chemoattractants for cells of the immune system. Although the overall sequence homology between them is poor, they have a similar three-dimensional structure. Chemokines are thought to derive from three or four ancestral genes and are categorized into four families, designated CXC, CC, C and CX3C, based on the number and spacing of cysteine residues found in the N-terminus of the polypeptides, and where X is any amino acid. The major families CC and CXC have more than 50 members between them; in comparison, the C and CX3C families are much smaller, with only one or a few members per family.

Chemokines exert their effects on cells through their interactions with a family of heterotrimeric, 7-transmembrane spanning, G protein-coupled receptors expressed on most cell types. Chemokine and chemokine receptor pairs vary widely in terms of selectivity. Certain chemokines bind only one receptor and vice versa, such as the exclusive interaction between CXCR4 and CXCL12 (SDF-1 α). Another pattern of pairing involves chemokine receptors that exclusively bind two or three chemokines as illustrated by CCR7-binding CCL19 (MIP-3 β , SLC) and CCL21 (ELC) [19]. Many other receptors and chemokines have many more partners such as CCR3 which binds CCL11 (eotaxin), CCL24 (eotaxin-2),

CCL26 (eotaxin-3), CCL8 (MCP-2), CCL7 (MCP-3), CCL13 (MCP-4), and CCL5 (RANTES).

Chemokines are typically expressed in one of two characteristic patterns. There are those such as CXCL12 and CXCL-13 (BCA-1) that are expressed constitutively by many cell types in tissue-specific sites and that contribute to homeostatic homing in these areas. The expression of inflammatory chemokines, in contrast, is induced only under specific conditions, typically in response to inflammatory signals. Generally speaking, lipopolysaccharide (LPS), IL-1 β , and TNF- α induce broad expression of inflammatory chemokines by a variety of cell types, compared with other inflammatory mediators, which induce more specific responses.

An increasing number of chemokines receptors are being identified on human mast cells cultured from various sources including the human lung, cord blood progenitors and bone

marrow progenitors (Table 1) [20–27]. Our own recent work has shown that CCR3, CXCR1, CXCR3 and CXCR4 are highly expressed by *ex vivo* human lung mast cells, and that the respective ligands for these receptors CCL11 (eotaxin), CXCL8 (IL-8), CXCL10 (IP-10) and CXCL12 (SDF-1 α) mediate lung mast cell chemotaxis [20]. Whether the mast cell infiltration of structural airway elements in asthma involves the recruitment of progenitors that then differentiate within the airway smooth muscle for example, or the local migration of resident differentiated cells is unknown. However, it is well recognized that mature differentiated cells have the ability to migrate within the airway wall [28–30]. In our own studies, it was interesting that both CXCR3 and CCR3 expression were increased on *ex vivo* human lung mast cells compared with bone marrow-derived mast cells, suggesting that they may be important for the migration of mature differentiated cells. Interestingly, CXCR3 was the most highly expressed chemo-

Table 1. Summary of chemokine receptors expressed by human mast cells from various origins

Chemokine receptor	Mast cell phenotypes expressing receptor	Agonist (Antagonist)	Further comments
CC Family			
CCR1	CBMC [22]	CCL3, CCL5*	CCL3 did not induce human lung mast cell migration [22]
	BMMC [20]	CCL7, CCL14	
	HLMC [20]	CCL15, CCL16	
CCR3		CCL23	CCL11 is chemotactic for 4-week-old CBMC progenitors and mature HLMC but not 9-week-old CBMC [20, 26, 24].
	HLMC [20, 26]	CCL5*, CCL7	
	CBMC [24]	CCL8, CCL11* CCL13, CCL15 CCL24, CCL26 CCL28 [CXCL9, -10, -11]	
CCR4	CBMC [22]	CCL5*	
	BMMC [20]	[CCL17]	
	HLMC [20]	[CCL22]	
CCR5	CBMC [24]	CCL3	
		CCL4	
		CCL5	
		CCL11	
		[CCL7]	
CCR7	BMMC [20]	CCL19, CCL21	
	HLMC [20]		
CXC Family			
CXCR1	HLMC [20]	CXCL6, CXCL8*	
	CBMC [25]		
CXCR2	CMMC [24]	CXCL8	
	CBMC [25]		
CXCR3	HLMC [20]	CXCL9	
	Synovial mast cells [23]	CXCL10*	
		CXCL11	
CXCR4	HLMC [20]	CXCL12*	CXCL12 is chemotactic for 4-week-old CBMC progenitors and mature HLMC but not 9-week-old CBMC [20, 24, 27]. It is also functional on a population of undifferentiated mast cell progenitors in adult peripheral blood [27]
CXCR6	BMMC [20]	CXCL16	
	CBMC [24, 27]		
	HLMC [20]		
	BMMC [20]		

*Ligands with proven chemotactic activity.

kine receptor on mast cells in both normal and asthmatic airways [20, 21]. In bronchial biopsies from patients with asthma approximately 50% of mast cells expressed CXCR3 compared with nearly 100% of mast cells within the asthmatic airway smooth muscle. In contrast CCR3 was almost absent on airway smooth muscle mast cells. We also found that there was increased expression of the CXCR3 ligand CXCL10 in asthmatic airway smooth muscle compared with that from normal subjects in both biopsies and when activated *in vitro*. Taken together these observations suggest that the CXCR3/CXCL10 axis may play a critical role in determining the distribution of mast cells within the lung, and in the recruitment of mast cells to the airway smooth muscle in particular.

The role of CCR3 in lung mast cell migration is less clear. An important role for CCR3 in mast cell homing was suggested by Romagnani et al. [26] who demonstrated that the vast majority of human CCR3-expressing mast cells were tryptase–chymase double-positive cells, which are known to predominate in connective tissues rather than the mucosa. Through chemotaxis assays, they showed that CCL11 (a selective agonist for CCR3) and CCL5 (a non-selective agonist for CCR3) mediated mast cell migration through CCR3, thereby suggesting that the CCR3/CCL11/CCL5 pathway may play an important role in mast cell migration in connective tissues. However, Humbles et al. [31] surprisingly found increased baseline numbers of submucosal mast cells in the trachea and large bronchi of CCR3 knockout mice. Furthermore, following antigen challenge, intraepithelial mast cell numbers increased significantly more in the CCR3-deficient mice compared with normal littermates. The

mechanism behind this is unclear but indicates a multifaceted role for CCR3 in mast cell migration in the airway.

Chemokine interactions

It is clear that there is an intricate network of interactions between chemokines and their receptors. Previous published work by Juremalm et al. [22] showed that CCL5, a recognized agonist for CCR1, also appeared to induce cord blood mast cell chemotaxis through CCR4, whereas the recognized ligands CCL17 and CCL22 did not. In this edition of *Clinical and Experimental Allergy*, these authors present further interesting observations relating to the function of CCR4 in human cord blood-derived mast cells [32]. Following pre-treatment with CCL17 and CCL22, an *in vitro* chemotaxis assay with CCL5 was performed. Both CCL17 and CCL22 significantly inhibited the migration towards CCL5 by 54% and 74%, respectively. In contrast, treatment with CCL2, CCL3, CXCL10 and CXCL12 did not affect CCL5-induced migration. The authors therefore conclude that CCL17 and CCL22 act as antagonists for CCL5-mediated migration induced through CCR4.

Their work also explored whether CCL17 and CCL22 mobilized intracellular calcium following stimulation with the CCR4 ligand. Sequential stimulation of CBMCs with CCL22 followed by CCL17 or vice versa revealed that CCR4 became desensitized in both cases. However pre-treatment with CCL5 did not desensitize either the CCL17- or the CCL22-induced calcium response. Finally, neither CCL5-, CCL17- nor

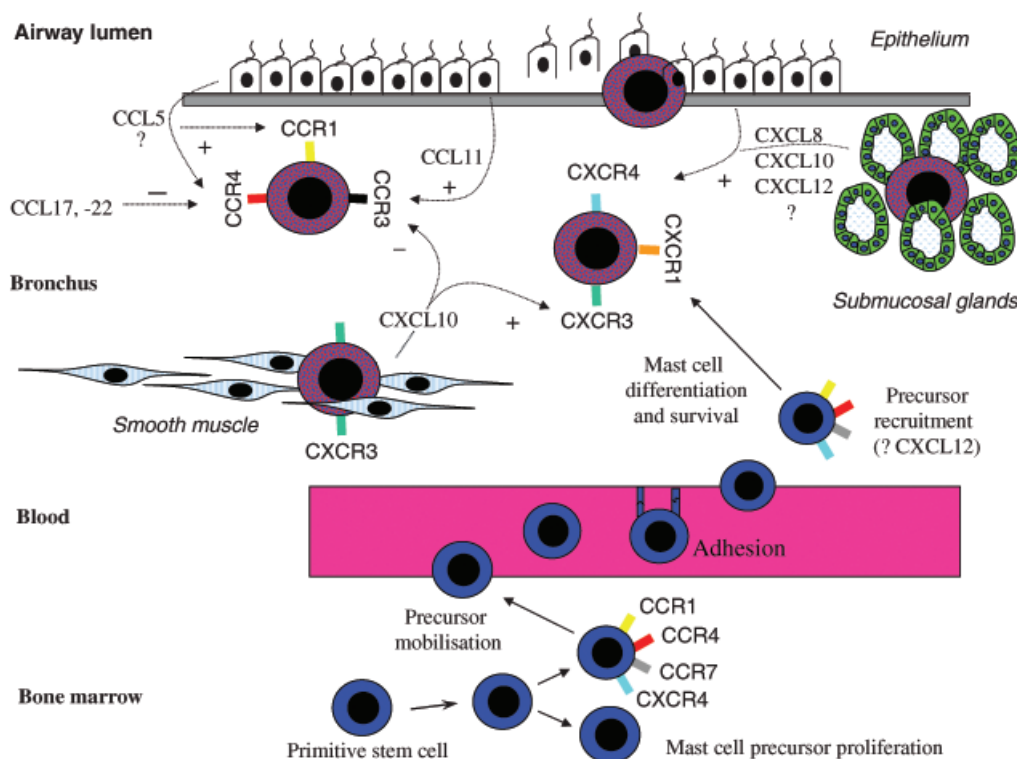


Fig. 1. Potential role of chemokines in the recruitment of mast cells to different sites within asthmatic airways.

CCL22-induced mast cell degranulation. The results taken together with their previous work conclude that CCL17 and CCL22 induce intracellular calcium mobilization without eliciting migration, and CCL5 can induce the migratory response of CBMCs in the absence of intracellular calcium mobilization. There are, however, some caveats required in their interpretation of the data. From the experiments performed, it is not proven that this CCL5, -17 and -22 interaction is occurring via CCR4. It is still possible that these effects are operating through alternative chemokines receptors, for example, CCL17 and CCL22 might be blocking CCR1 or 3, well-established receptors for CCL5. In fact, Romagnani et al. [26] demonstrated that CCL5-induced migration of human lung mast cells was inhibited by blocking CCR3. Careful experiments with CCR4 transfectants will be required to ascertain beyond doubt that the CCL5/CCL17/CCL22 interaction is operating on CCR4. Furthermore, the role that this has in determining the specificity of the mast cell migratory response in asthmatic airways requires further work, particularly as the expression of CCR4 on human lung mast cells is relatively low [20].

A potential mechanism eluded to by the authors for the varying effects of CCL5, -17 and -22 is chemokine receptor heterodimerization. This phenomenon has recently been described by Mellado et al. [33] and results in altered ligand specificity and activation of distinct intracellular signalling pathways. In addition, there is already a precedent for chemokines to act as antagonists as well as agonists (Table 1) [34], for example, CXCL10 is an agonist for CXCR3 but a potent antagonist of CCR3 activation by CCL11 [35].

The regulation of mast cell tissue migration by chemokines in health and disease is undoubtedly complex and we are only at the 'tip of the iceberg' with our understanding of the mechanisms involved. The recent observations by Juremalm clearly add a further level of sophistication to this interactive network of chemokines in biological responses, and suggest great scope for the fine tuning of mast cell migration in the airway. A simplified summary of potential chemokine interactions determining the distribution of mast cells in the asthmatic airway is depicted in Fig. 1.

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