

Targeting Human Mast Cells Expressing G-Protein-Coupled Receptors in Allergic Diseases

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ABSTRACT

The G-protein-coupled receptors (GPCRs) are the largest known group of integral membrane receptor proteins and are the most common targets of pharmacotherapy. Mast cells (MCs) have been reported to play an important role in allergic diseases, such as urticaria and bronchial asthma. There is an increasing body of clinical evidence that MCs are recruited into allergic reactions by non-IgE-dependent mechanisms. Human MCs are activated and secrete histamine in response to neuropeptides, such as substance P and somatostatin, mediated by a GPCR, MRGX2. The microenvironment surrounding MCs in their resident tissues is likely to contain multiple factors that modify antigen-dependent MC activation. MCs express various GPCRs, and since the function of human MCs is modulated by various GPCR ligands, such as adenosine and sphingosine-1-phosphate, which are present in high levels in the bronchial alveolar lavage fluid of asthmatic patients, the GPCRs expressed on MCs may play an important role in human allergic diseases. The GPCRs expressed on MCs may serve as drug targets for the treatment of allergic diseases.

KEY WORDS

allergic diseases, bronchial asthma, GPCRs, human mast cells, urticaria

INTRODUCTION

Mast cells (MCs) are known to be the primary responders in allergic reactions, most of which are triggered by cross-linking of a high-affinity IgE receptor, FcεRI. Upon activation, MCs exert their biological effects by releasing preformed and *de novo*-synthesized mediators, including histamine, leukotrienes, and various cytokines/chemokines. Biogenic amines and lipid mediators cause rapid plasma leakage from blood vessels, vasodilation, and bronchoconstriction, and cytokines mediate the late phase reaction characterized by an inflammatory infiltrate composed of eosinophils, basophils, neutrophils, and lymphocytes.¹ MCs have been reported to play an important role in allergic diseases such as urticaria and bronchial asthma. There is an increasing body of clinical evidence that MCs are recruited into allergic reactions by non-IgE-dependent mechanisms. Elevated serum neuropeptide levels have been observed in pa-

tients with urticaria, and skin MCs have been found to be activated and release histamine in response to the neuropeptides such as substance P and somatostatin.^{2,3} In this review article, we will describe the relationship between the clinical evidence of involvement of neuropeptides in urticaria and MC activation through GPCRs.

MCs have been reported to express various GPCRs on their surface, and a sub-population of GPCRs modulates FcεRI-mediated MC activation. A recent review by Kuehn and Gilfillan⁴ that thoroughly discusses the mechanisms whereby the signaling responses utilized by FcεRI for MC activation are influenced by those initiated by GPCRs to produce these diverse responses. High levels of several GPCR ligands, including adenosine and sphingosine-1-phosphate (S1P), are present in the bronchial alveolar lavage (BAL) fluid of asthmatic patients.⁵⁻⁷ In this review article, we will next describe the relationship between clinical evidence of involvement of GPCR

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ligands in bronchial asthma and modulation of FcεRI-mediated MCs activation through the GPCRs.

Smooth muscle (SM) hypertrophy and hyperplasia are now recognized as the most important factors related to airway hyperresponsiveness *in vitro* and *in vivo* and to the severity of asthma. Many inflammatory mediators and cytokines, including MC-derived mediators, contribute to airway smooth muscle (ASM) proliferation⁸ (see our review).⁹ Infiltration of ASM by MCs is reported to be associated with the impaired airway function in asthma, and the infiltration is mediated by various GPCRs expressed on MCs. We will describe the MC expressing GPCRs that are involved in the pathogenesis of airway remodeling.

The GPCRs belong to one of the largest known group of integral membrane receptor proteins have been exploited extensively as drug targets. For example, adrenergic β-agonists with selectivity for the β₂ adrenergic receptor, are the treatment of choice for reversal of acute bronchospasm in bronchial asthma. The adrenergic β-agonists inhibit FcεRI-mediated degranulation and cytokine production by human MCs.¹⁰⁻¹³ Increasing evidence indicates that GPCRs are capable of coupling to more than one G protein, raising the possibility that different agonists can direct signaling from the receptor to specific signaling cascades as a consequence of their relative affinities for different G-protein-coupled states of the same receptor.¹⁴ The potential for GPCRs to form complexes with signaling proteins other than G proteins also raises the possibility that agonists and antagonists can discriminate between these complexes in terms of both binding affinity and efficacy.¹⁵ Thus, each downstream signaling pathway evaluated in a particular cell may have its own unique pharmacology depending on the pathway that is stimulated by the unique ligand-receptor conformation or complex involved. In this paper, we review the involvement of GPCRs on human MCs, but not murine MCs, in the pathogenesis of allergic diseases.

GPCR-LIGANDS ACTIVATE HUMAN SKIN MCs AND ARE INVOLVED IN THE PATHOGENESIS OF URTICARIA

A great deal of clinical evidence suggests that neuropeptides play a very important role in the pathogenesis of urticaria, angioedema, and atopic dermatitis. Radioimmunoassay of vasoactive intestinal polypeptide (VIP), somatostatin, bombesin, neurotensin, and β-endorphin in plasma extracts from 20 acute idiopathic urticaria patients and 20 healthy subjects showed significantly lower VIP- and β-endorphin-like values in the patients, but significantly higher somatostatin- and bombesin-like values.² Serum substance P concentrations measured by enzyme immunoassay in 117 chronic ordinary urticaria patients, 40 atopic subjects, and 24 healthy subjects

showed no significant difference in mean concentration between the chronic urticaria patients and healthy subjects.¹⁶ However, some patients with chronic urticaria had a very high substance P level, suggesting that substance P does not play an important role as a histamine-releasing factor in chronic urticaria in general but does only in occasional patients in whom it may act as a trigger of urticaria symptoms.

The neuropeptides substance P, VIP, and somatostatin all induce similar concentration-related release of histamine by human skin MCs.³ The relative equipotency of these neuropeptides together with the observation that eledoisin, physalaemin, and neurokinins A and B (NKA and NKB) induce negligible histamine release strongly suggests that the activation site for neuropeptides on human skin MCs is not any of the classical high affinity tachykinin receptor subtypes identified on smooth muscle. This is supported by the observation that morphine, poly-L-lysine, and the synthetic histamine releaser compound 48/80 induce histamine release in a concentration-related manner, suggesting that the peptide sensitivity extends to other basic lipophilic substances. The peptide [D-Pro⁴, D-Trp^{7,9,10}] SP₄₋₁₁ inhibits not only the histamine release induced by substance P from human skin MCs in a competitive manner, but the histamine release induced by VIP, somatostatin, compound 48/80, morphine, and poly-L-lysine, suggesting that human skin MCs possess low affinity and low-specificity activation sites for neuropeptides and compounds with similar physicochemical properties. In 2006, the low affinity and low-specificity activation sites for neuropeptides and compounds were identified as a GPCR, MRG (mas-related gene) X2, although whether human skin MCs express MRGX2 has not been investigated.¹⁷ The gene product of MRG is a recently identified member of GPCRs family. MRG consists of 32 murine and 4–7 human genes (hMRGX1-hMRGX7).^{18,19} The MRG is expressed only in a specific subset of nociceptive neurons.^{18,20} Human cord-blood-derived MCs express MRGX1 and MRGX2.¹⁷ Human cord-blood-derived MCs undergo degranulation mediated by MRGX2 in response to substance P, VIP, somatostatin, cortistatin, indolicidin, platelet factor-4, and compound 48/80, however, there is still no direct evidence that these neuropeptides activate human skin MCs through MRGX2 and induce urticaria *in vivo*.

Several investigators have identified the complement anaphylatoxins C3a and C5a as potential effectors in Type 1 hypersensitivity reactions, including urticaria, rhinitis, and asthma (reviewed by Gerard and Gerard).²¹ A study of sera from chronic urticaria patients demonstrated that histamine release by basophils stimulated with patient IgG was significantly augmented by exposure to C5a. Furthermore, depletion of serum C5 or exposure to antisera against the C5a receptor clearly implicated C5a in this reaction.²²

Table 1 GPCRs expressed on human mast cells

Ligand	GPCR	Human MC subtype	Mast cell response	References
Adrenaline	Adrenergic beta2	Lung-, skin-, cord-blood-derived, peripheral-blood-derived	Inhibition of FcεRI-mediated degranulation and cytokine production	10,11,12,13
Adenosine	Adenosine A2a, A2b, A3	Lung-	Degranulation (low dose), inhibition of FcεRI-mediated degranulation and chemotaxis (high dose)	35,36,37,38,39,40,41,42,43
C3a	C3aR	Skin-, lung-, CD34 ⁺ -derived, LAD2	Degranulation, chemotaxis, chemokine secretion	23,25,26
C5a	C5aR	Skin-, cord-blood-derived,	Degranulation, chemotaxis	23,24
Fractalkine	CXCR1	Lung-, skin-	Induction of migration with VIP	56
CXCL10	CXCR3	Lung-	Migration toward airway smooth muscle cells	55
Lysophosphatidylserine	GPR34	LAD2 mast cell line	Degranulation	28
Histamine	Histamine H4	Mast cell precursors	Enhancement of CXCL12-induced chemotaxis	57
BAM	Mrgx1	Cord-blood-derived	Unknown	17
Somatostatin, platelet factor-4, substance P, indolicidin, VIP, compound 48/80	Mrgx2	Cord-blood-derived	Degranulation	17
ADP, ATP & UTP, ATP	Purinergic receptor P2Y1, P2Y12, P2Y13 (ADP), P2Y2 (ATP & UTP), P2Y11 (ATP)	Cord-blood-derived	ADP: calcium influx, eicosanoid production, exocytosis; ATP, ADP: inhibition of TLR2-mediated TNF-α, IL-8 and MIP-1α production	27
S1P	S1P1, S1P2	Cord-blood-derived, LAD2 mast cell line	Degranulation, chemotaxis	51
Leukotrienes	Cysteinyl leukotriene receptor 1 and 2	Cord-blood-derived	Induction of proliferation	58,59
Lysophosphatidic acid	LPA1, LPA3	Cord-blood-derived	Acceleration of development, chemokine secretion	60

C3a and/or C5a induce degranulation, chemotaxis, and cytokine secretion in MCs.²³⁻²⁶ Lysophosphatidylserine, and adenine nucleotides alone have also been reported to induce degranulation by human MCs.^{27,28} However, the relationship between these GPCR ligands and their involvement in allergic diseases is still unclear.

THE GPCRS EXPRESSED ON HUMAN MCs IN BRONCHIAL ASTHMA

Bronchial asthma is characterized by airway inflammation, airway hyperresponsiveness, and airway remodeling. MCs have been reported to play an important role in the pathogenesis of bronchial asthma upon IgE-dependent activation. The immediate microenvironment surrounding the MCs in their resident tissues is likely to contain multiple factors that, under certain specific conditions, modify antigen-dependent MC activation.^{1,4} Several GPCR-ligands, including adenosine and S1P, have been reported to play an important role in asthma. The MC-GPCRs that may be involved in the pathogenesis of allergic

diseases are summarized in Table 1. High levels of adenosine, a metabolic by-product of ATP, are present in exhaled breath condensates and BAL fluid from asthmatic patients.^{5,6} Adenosine binds to P1 purinoceptors (purinergic receptors) and P1 purinoceptor subtypes A₁, A_{2A}, A_{2B}, and A₃, and binding of adenosine to these receptors on ASM, goblet cells, MCs, and neurons has been reported to contribute to the pathogenesis of asthma. The MCs of allergic asthmatics are activated after an adenosine challenge,²⁹ and adenosine is thought to provoke bronchoconstriction indirectly via activation of A_{2B} adenosine receptors expressed on airway MCs.³⁰ This was confirmed by measuring the blood levels of a prostaglandin (PG) D₂ metabolite 9α, 11β-PGF₂, a sensitive marker of MC activation, before and after administering an adenosine inhalation challenge to 18 stable mild asthmatics.³¹ In patients with allergic asthma in response to adenosine challenge the bronchial reaction was followed by an early mild but significant ($P < 0.01$) mean 1.4-fold increase in plasma 9α, 11β-PGF₂ level, suggesting that MCs are involved

in adenosine-precipitated bronchoconstriction in allergic asthma but not in non-allergic asthma. However, it is still unclear whether the results reflect an increase in the number of MCs in the airway of bronchial asthma patients or an increase in sensitivity of the MCs of asthmatics to adenosine, compared to healthy controls. By contrast, MC mediators are released in healthy subjects after an endobronchial adenosine challenge, suggesting a dissociation between mediator release and bronchoconstriction in response to AMP.³²

A_{2A}, A_{2B} and A₃ receptors are expressed on the surface of murine MCs.³³ The A_{2A} receptor signals *via* adenylate cyclase involve G_{αs} coupling, and the A_{2B} signals *via* multiple mechanisms including adenylate cyclase, diacylglycerol, and inositol triphosphate, involve both G_{αs} and G_{αq} coupling.³⁴ A_{2B} signaling has also been shown to be mediated *via* PDZ-containing proteins independently of G proteins. The effects of adenosine on human lung MCs both *in vitro* and *in vivo* are complex. At relatively low concentrations *in vitro* (10⁻⁶ M), adenosine is said to potentiate IgE-dependent secretion, but the reported degree of potentiation has varied.³⁵⁻⁴⁰ Because adenosine has been found to induce cytokine secretion by a human MC line *via* the A_{2B} receptor,⁴¹ potentiation of secretion by human lung MCs is also thought to be mediated *via* the A_{2B} receptor, but that has not been demonstrated. At higher concentrations of adenosine (10⁻⁵ to 10⁻³ M), on the other hand, there is a more consistent dose-dependent and profound inhibition of secretion,^{35-37,42} mediated predominantly *via* the A_{2A} receptor.⁴⁰ Adenosine has recently been shown to dose-dependently and reversibly close K⁺ channel Kca3.1 at concentrations that inhibit human lung MC degranulation and migration (10⁻⁵ – 10⁻³M). The Kca3.1 suppression by adenosine is mediated by A_{2A} receptor.⁴³

The S1P level in the BAL fluid of asthmatics increases after antigen challenge, and the S1P levels in the BAL fluid of asthmatics correlate with the eosinophil numbers in the BAL fluid.⁷ Platelets, neutrophils, MCs, mononuclear cells, vascular endothelial cells, and erythrocytes are able to synthesize and secrete S1P.⁴⁴⁻⁴⁶ The cross-linking of FcεRI on rodent MCs activates sphingosine kinase (SphK), which in turn stimulates production of S1P.⁴⁷ S1P acts as a ligand for a family of five S1P receptors, S1P₁₋₅, all of which bind S1P with high affinity and specificity.⁴⁸ A variety of cells express S1P receptors, and S1P affects endothelial cell function and promotes adhesion molecule expression in endothelial cells, induces contraction and proliferation of ASM, and fibroblast proliferation, and shifts maturing dendritic cell-induced polarization of T cells toward a Th2 phenotype (see reviews by Oskeritzian⁴⁹ and Deshpande and Penn⁵⁰). Both S1P₁ and S1P₂ receptors are expressed on the surface of MCs and S1P₁ is pivotal for rodent MC chemo-

taxis. S1P₂, on the other hand, mediates MC activation and degranulation.⁴⁷ Since MCs secrete S1P, it is capable of acting on its own receptors expressed on their surface in a positive feedback loop and amplifying MC function (see reviews by Oskeritzian⁴⁹ and Kuehn and Gilfillan⁴). In contrast to its weak effect on degranulation of murine MCs, S1P potently induces degranulation by human LAD2 MC line and by human cord-blood-derived MCs.⁵¹

INVOLVEMENT OF MC EXPRESSING GPCRS IN AIRWAY REMODELING

Infiltration of ASM by MCs has been reported to be associated with the impaired airway function observed in asthma.⁵² Carroll *et al.*⁵³ studied the number of MCs and MC degranulation in tissue obtained at autopsies of patients who had died of asthma, patients who had asthma but died of other causes, and non-asthmatic controls. They found that the largest proportion of MCs was in the ASM and that the number of degranulated MCs was greatest in the patients who had died of asthma. Increased number of intact and degranulated MCs have been reported in ASM in the airways of patients who died of asthma and the increases were associated with a greater degree of ASM shortening.⁵⁴ These observations indicate a direct interaction between MCs and ASM cells.

It has been reported that human lung MCs migrate toward ASM in response to ASM-derived chemokines, and that CXCR3 is the most abundantly expressed chemokine receptor on human lung MCs in ASM in asthma and is expressed by 100% of such MCs, as compared to 47% of the MCs in the submucosa.⁵⁵ Human lung MC migration has been found to be induced by ASM cultures *in vitro*, predominantly through activation of CXCR3. Most importantly, CXCL10 has been shown to be preferentially expressed by ASM in bronchial biopsy specimens and *ex vivo* cells from asthmatics, compared with specimens and cells from healthy control subjects. Human ASM cells constitutively produce fractalkine, whose synthesis increased by proinflammatory stimulation.⁵⁶ Under basal experimental conditions, fractalkine production by human ASM cells is insufficient to induce MC chemotaxis, and exposure of MCs to the neuropeptide VIP increases fractalkine production to attract MCs.

It has recently been reported that histamine enhances CXCL12-induced chemotaxis by human MC precursors through histamine H4 receptors.⁵⁷ Since leukotrienes and lysophosphatidic acid induce human MC proliferation through their GPCRs,⁵⁸⁻⁶⁰ the GPCRs expressed on human MCs may modulate airway remodeling in bronchial asthma. Figure 1 shows a schematic representation of human MC-GPCR-mediated allergic inflammation of the airways.

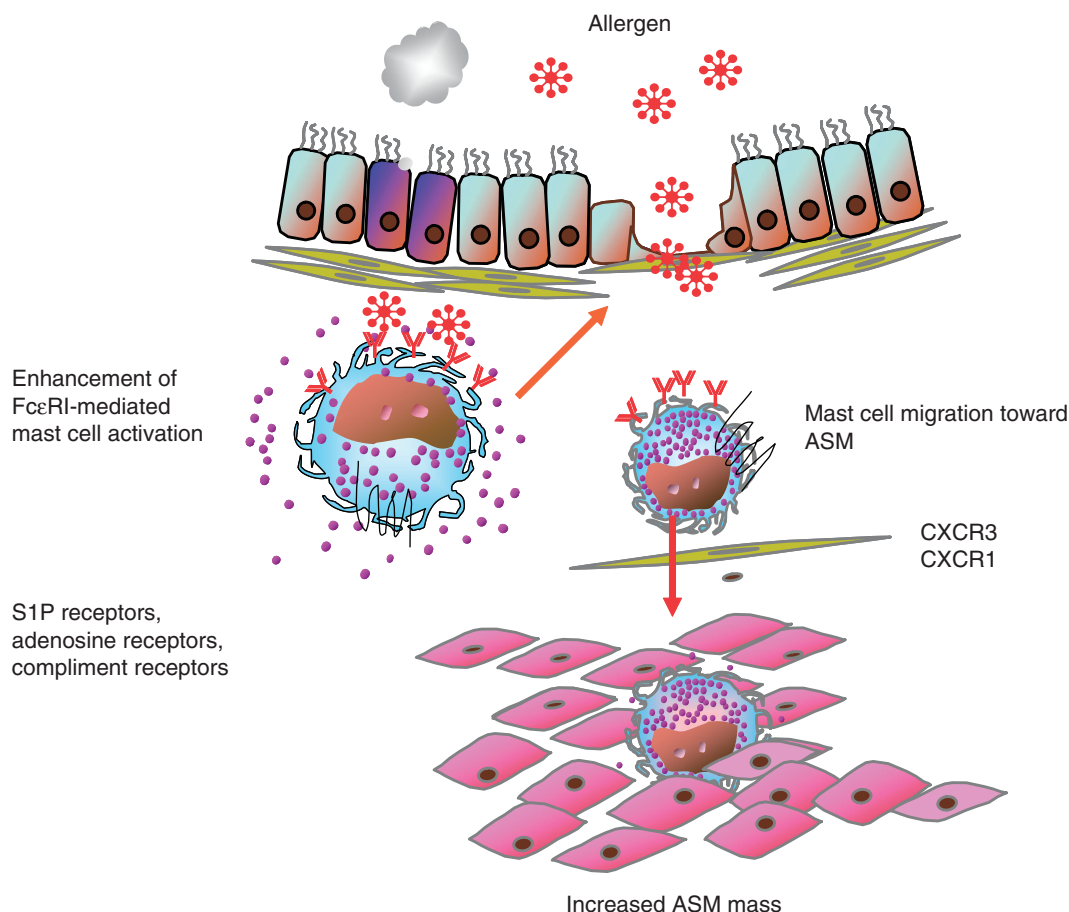


Fig. 1 Schematic representation of human MC-GPCR-mediated allergic inflammation of the airway. ASM, airway smooth muscle; red line, migration of human MCs toward ASM; orange line, migration of human MCs toward an allergen.

CONCLUSIONS

In this review we have discussed how GPCRs expressed on human MCs are involved in the pathogenesis of allergic diseases. The majority of studies of GPCRs on MCs have been carried out on rodent cells, but comparisons of the results obtained with human and rodent MCs have shown significant differences in the nature of the responses generated by GPCRs in mice and humans.⁴ As described above, every GPCR-downstream signaling pathway evaluated in a particular cell may have its own unique pharmacology, depending on the pathway stimulated by the unique ligand-receptor conformation or complex involved. Thus, extensive studies of human MCs will be required to exploit drugs for the treatment of human allergic diseases.

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