Mas-Related G Protein Coupled Receptor-X2: A Potential New Target for Modulating Mast Cell-Mediated Allergic and Inflammatory Diseases

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Abstract

Mast cells (MCs) are tissue resident immune cells that are best known for their roles in allergic and inflammatory diseases. In addition to the high affinity IgE receptor (FceRI), MCs express numerous G protein coupled receptors (GPCRs), which are the most common targets of drug therapy. Neurokinin 1 receptor (NK-1R) is expressed on MCs and contributes to IgE and non-IgE-mediated responses in mice. Although NK-1R antagonists are highly effective in modulating experimental allergic and inflammatory responses in mice they lack efficacy in humans. This article reviews recent findings that demonstrate that while neuropeptides (NPs) activate murine MCs via NK-1R and Mas related G protein coupled receptor B2 (MrgrpB2), they activate human MCs via Mas-related G protein coupled receptor X2 (MRGPRX2). Interestingly, conventional NK-1R antagonists have off-target activity against mouse MrgrpB2 but not human MRGPRX2. These findings suggest that the failure to translate studies with NK-1R antagonists from in vivo mouse studies to the clinic likely reflects their lack of effect on human MRGPRX2. A unique feature of MRGPRX2 that distinguishes it from other GPCRs is that it is activated by a diverse group of ligands that include; neuropeptides, cysteine proteases, antimicrobial peptides and cationic proteins released from activated eosinophils and neutrophils. Thus, the development of small molecule MRGPRX2-specific antagonists or neutralizing antibodies may provide new targets for the treatment of MC-mediated allergic and inflammatory diseases.

Keywords: Mast cells; NK-1R; MrgrpB2; MRGPRX2; Anaphylaxis; Asthma

Abbreviations FcεRI: High Affinity IgE Receptor; GPCR: G Protein Coupled Receptor; NK 1R: Neurokinin-1 Receptor; MrgrpB2: Mas-related G Protein Coupled Receptor-B2, MRGPRX2: Mas-Related G Protein Coupled Receptor-X2; MC: Mast Cell; NP: Neuropeptide; HK-1: Hemokinin-1; SP: Substance P

Introduction

Mast cells are multifunctional immune cells that are found in all vascularized tissues throughout the body and contribute to vascular homeostasis, innate/adaptive immunity and wound healing [1]. MCs are, however, best known for their roles in allergic and inflammatory diseases such as anaphylaxis, food allergy, rhinitis, itch, urticaria, atopic dermatitis and asthma. In these conditions, activation of MCs via the cross-linking of high affinity IgE receptors (FcεRI) results in the release of preformed and newly synthesized mediators that contribute to signs and symptoms associated with allergic diseases.

Mast cells are generally classified into two types based on the protease content of their secretory granules [2,3]. In human, most MCs that are found in connective tissues such as the skin contain tryptase, chymase, carboxypeptidase and cathepsin and are known as MC_{TC}. In contrast, majority of MCs that are found in lung and gut express only tryptase and are known as MC_{T}. MCs also differ in their responses to endogenous and exogenous stimuli that promote degranulation. Thus, while both human MC subtypes are activated via the aggregation of FcεRI, only MC_{TC} respond to the neuropeptide substance P (SP) [4,5]. In mice, connective tissue MCs (CTMCs) resembles MC_{TC} while mucosal MCs (MMC) resemble MC_{T} [2,6]. Thus, murine CTMCs are found in the skin and MMC mature in the mucosal tissues such as the lung and gut. In addition, CTMCs are responsive to SP for degranulation but MMCs are not [7,8].

Substance P activates a variety of cell types including MCs via NK-1R and induces cutaneous vasodilation, plasma extravasation, flare and itch [8-12]. Accordingly, NK-1R antagonists effectively modulate experimental allergic and inflammatory responses in mice but they lack efficacy in clinical trials [13-17]. This article reviews recent findings which indicate that the reason for this difference may reflect differences in GPCR utilization between mouse and human MCs.

Roles of NK-1R on Neuropeptide and IgE-mediated Responses in Mouse MCs In vitro and Allergic Responses In vivo

It is generally accepted that MMCs such as murine bone marrow-derived cells (BMMCs) do not express NK-1R [8]. However, BMMCs cultured in the presence of interleukin-4 (IL-4) and stem cell factor (SCF), which favor CTMC phenotype, results in the expression neurokinin 1 receptor (NK-1R). Furthermore, SP causes degranulation in these MCs, which is diminished by an NK-1R antagonist or in cells obtained from NK-1R⁻/⁻ mice [8]. Subcutaneous injection of SP in mice results in MC degranulation, which is followed by increased vascular permeability and marked infiltration of eosinophils and neutrophils [18,19]. These responses are absent in MC-deficient mice. However, when MC-deficiency is rescued with local engraftment of
cultured MCs, SP-induced responses are restored. These findings suggest that activation of NK-1R in murine MCs by SP results in increased vascular permeability and granulocyte recruitment in vivo (Figure 1A).

Substance P belongs to the tachykinin family of neurokinins and this family has recently been expanded to include hemokinin-1 (HK-1) [20]. Unlike SP, which is released from peripheral nerve endings of the sensory neurons, HK-1 is the only tachykinin peptide that is produced outside the neuronal tissue [20]. Sumpter et al. [21] recently demonstrated that FcεRI activation of murine BMMCs results in enhanced expression of both HK-1 and NK-1R without modifying SP levels. Furthermore, it was found that FcεRI-mediated MC degranulation and TNF/IL-6 production is substantially inhibited in NK-1R−/− BMMCs when compared to wild-type MCs. These findings suggest that HK-1, via its action on NK-1R, acts as an autocrine/paracrine factor for FcεRI-mediated MC mediator release. As described below, HK-1/NK-1R axis has a profound impact on IgE-mediated experimental anaphylaxis and MC-dependent model of chronic atopic airway inflammation, an important feature of asthma [21].

Passive cutaneous anaphylaxis (PCA) is often used as an experimental model to study the mechanism of IgE-mediated type 1 hypersensitivity reactions. In this model, FcεRI-mediated histamine release from dermal MCs leads to increased vascular permeability, resulting in the formation of edema. This is followed by the release of TNF from MCs, which promotes the recruitment of neutrophils to the site of MC activation. Using MC-deficient mice reconstituted with wild-type, Tac1−/− (HK-1) or NK-1R−/− BMMCs, Sumpter et al. [21] showed that HK-1 and NK-1R expressed in murine MCs contribute to both early (edema) and late (neutrophil recruitment) phases of IgE-mediated PCA. In a mouse model of chronic allergic asthma, TNF derived from MCs contribute to Th2/Th17 cytokine production, airway hyperresponsiveness and inflammation [22-24]. Given that FcεRI-mediated TNF production is substantially inhibited in NK-1R−/− BMMCs when compared to wild-type MCs, this raises the interesting possibility that this GPCR is important for the pathogenesis of allergic asthma. Indeed, absence of Tac1 or NK-1R in MCs leads to substantial decrease in eosinophil and neutrophil recruitment in a MC-dependent model of chronic asthma [21]. These findings suggest that HK-1 and its receptor NK-1R, which are upregulated following FcεRI activation, contribute to both anaphylaxis (Figure 1A) and chronic allergic asthma in mice (Figure 1B).

Role of MrgprB2 on SP-induced Degranulation of Murine MCs

In addition to NK-1R, murine CTMCs express Mas-related G protein coupled receptor B2 (MrgprB2) [25]. Generation of a transgenic mouse with td-Tomato reporter under the control of MrgprB2 promoter demonstrated that the expression of this receptor is restricted to CTMC in the skin, gut and trachea [25]. MrgprB2 mutant mice were generated by deleting a 4 base pair in the receptor's coding region, resulting in a frame shift mutation and early termination of the receptor (shortly after the first transmembrane domain). These mice have no defect in MC number and respond normally to IgE/FcεRI activation. However, peritoneal MCs from these mice show dramatic reduction in degranulation in response to SP in vitro and inflammation in vivo [25,26]. These findings suggest that NP-induced responses in murine MCs reflect the activation of twoGPCRs, NK-1R and MrgprB2.

Figure 1: Proposed model for the roles of NK-1R and MrgprB2 on experimental neuropeptide and IgE-mediated responses in mice. SP released from nerve endings and HK-1 generated from MCs activate NK-1R and MrgprB2 on MCs to induce degranulation and TNF production. These mediators contribute to increased vascular permeability and leukocyte recruitment in (A) anaphylaxis and (B) chronic asthma. It is proposed that conventional NK-1R antagonists modulate these responses in mice by inhibiting both NK-1R and MrgprB2.

Conventional NK-1R Antagonists Block MrgprB2-mediated Responses in Mice but Lack Efficacy in Humans

Although it is currently unknown if HK-1 level is increased following the activation of human MCs, the expression of SP and NK-1R is upregulated in human asthmatic but not in normal individuals [13,14,27,28]. These findings suggest that NK-1R not only participates in experimental MC-mediated allergic responses, it also plays an important role in the human disease. Not surprisingly, a number of NK-1R antagonists have been developed to modulate allergic responses in animal models and for clinical trials in humans [13-16]. These antagonists are highly effective in animal models of allergic asthma and inflammation but lack efficacy in humans. Azimi et al. [17] recently showed that conventional NK-1R antagonists have off-target effect on the mouse MrgprB2. This suggests that the effectiveness of NK-1R antagonists in mice reflect not only their ability to block NK-1R but also their action against MrgprB2 (Figure 1). The striking failure to translate findings from animal models to the clinic suggests that there are important differences in the mechanisms via which NPs activate murine and human MCs.

Substance P Activates Human MC\textsubscript{TC} via Mas-related G Protein Coupled Receptor-X2 (MRGPRX2) and Contributes to Chronic Urticaria

Similar to the situation in murine CTMCs, human skin MC\textsubscript{TC} and human MC line (LAD2) express NK-1R and respond to SP for degranulation [29,30]. However, unlike the case with murine CTMCs, NK-1R antagonists have little or no effect on SP-induced response in human MCs [30]. The recent demonstration that MRGPRX2 is the human counterpart of mouse MrgprB2 raises the possibility that SP activates human MCs via this receptor. Tatemoto et al. [31] provided
the first demonstration that SP activates human cord blood-derived MCs via MRGPRX2 and this finding was later confirmed in studies with transfected RBL-2H3 cells and primary human skin MCs [1,30,32]. Fujisawa et al. [30] showed that skin MCs of patients with severe chronic urticaria express MRGPRX2 at higher level than the healthy subjects. Furthermore, SP causes degranulation and prostaglandin D2 (PGD2) generation in human skin MCs via MRGPRX2. Eosinophils accumulate in chronic urticaria lesions and their granules proteins (major basic protein, MBP and eosinophil peroxidase, EPO) cause MC degranulation via MRGPRX2 [30,33]. These findings suggest that activation of human MCs by SP via MRGPRX2 serves to initiate the wheal response. Furthermore, recruitment of eosinophils and the subsequent activation of MRGPRX2 by MBP and EPO contribute to the late phase of chronic urticaria (Figure 2A).

Possible Roles of MRGPRX2 in Severe Asthma and Asthma Exacerbation

Mast cells are important effector cells that orchestrate the development of airway hyperresponsiveness and inflammation in asthma via their close interaction with airway smooth muscle cells, T cells and leukocytes [34-36]. Microarray and PCR analyses showed that while high level of MRGPRX2 transcript is expressed in human skin MCs, only low level is present in lung MCs [30,37]. Mild allergic asthma is generally correlated with an increase in MC\textsubscript{T} number in both submucosa and smooth muscle but severe asthma is dominated by the presence of MC\textsubscript{TC}, a MC subtype that expresses MRGPRX2 [30,38,39]. MC-derived mediators such as tryptase, histamine and cysteinyl leukotrienes interact with neurons to promote the release of neuropeptides such as SP [40]. The level of SP is elevated in the lung of asthmatics when compared normal lung [27,28]. Furthermore, SP causes degranulation of MCs obtained from bronchoalveolar lavage indicating a potential for MRGPRX2 in severe asthma [41]. Cathepsin S is a cysteine protease released from antigen-presenting cells and contributes to the pathogenesis of asthma [42-44]. Reddy et al. [45] recently showed that cathepsin S induces Ca\textsuperscript{2+} mobilization in HeLa cells expressing MRGPRX2. These findings suggest that both cathepsin S and SP contribute to the pathogenesis of severe asthma via the activation of MRGPRX2 in lung MC\textsubscript{TC} (Figure 2B).

A Tripeptide NK-1R Antagonist Inhibits Human MC Activation via MRGPRX2

A unique feature of MRGPRX2 that distinguishes it from other GPCRs is that it is activated by multiple cationic ligands and may play important role in a variety of MC-mediated diseases (Figure 2) [1,25,30]. Thus, the development of small molecule MRGPRX2-specific antagonists or neutralizing antibodies may provide potential new therapeutic target for modulating MC-mediated allergic and inflammatory diseases. Azimi et al. [17] showed that a while conventional NK-1R antagonists block both NK-1R and MrgprB2, a tripeptide (QWF) NK-1R antagonist also has activity against human MRGPRX2. Thus, QWF inhibits SP-induced responses in HEK-293 cells stably expressing MRGPRX2 and a human MC line (LAD2) naturally expressing the receptor. These findings suggest that while NPs activate murine MCs via NK-1R and MrgrpB2 they utilize MRGPRX2 for human MC activation. This difference likely explains why NK-1R antagonists modulate MC-mediated experimental allergic responses in mice but lack efficacy in humans. The tripeptide QWF is, however, unlikely to serve as an MRGPRX2-specific drug in the clinic because it lacks specificity for MRGPRX2 and it is rapidly metabolized in plasma [55]. Despite the potential limitations of QWF, its inhibitory effect on MRGPRX2-mediated MC degranulation provides a strong impetus for the development of specific small molecule MRGPRX2 antagonists or monoclonal antibodies for the treatment of MC-mediated diseases.

Conclusions and Future Direction

Neuropeptides activate murine MCs via NK-1R and MrgrpB2 and human MCs via MRGPRX2. Conventional NK-1R antagonists have off target activity against mouse MrgrpB2 but not human MRGPRX2. This
difference likely explains why conventional NK-1R antagonists modulate experimental allergic responses in mice but lack efficacy in humans [17]. It is noteworthy that MRGPRX2 displays only ~20 and ~50% sequence identity with NK-1R and MrgrpB2, respectively. Despite this difference, NK-1R, MrgrpB2 and MRGPRX2 are all responsive to SP and the tripeptide NK-1R antagonist (QWF) blocks SP responses to all three receptors [17]. These findings suggest that all three receptors possess specific binding sites for both SP and QWF. Site directed mutagenesis studies of NK-1R demonstrated that amino acid residues 23-25 (NQF) in the receptor's amino terminus along with H108 (third transmembrane domain), E268 (sixth transmembrane domain) and Y287 (seventh transmembrane domain) are important for binding substance P [56-58]. Furthermore, histidine at position 197 (H197) in the fifth transmembrane helix of human NK-1R binds specifically to the non-peptide receptor antagonist CP 96345 to block SP action [59]. Thus, identification of SP and QWF binding sites on MRGPRX2 may provide useful information for the development of MRGPRX2-specific antagonists.

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