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G Protein-coupled receptors and the modification of FcεRI-mediated mast cell activation

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Abstract

By releasing multiple pro-inflammatory mediators upon activation, mast cells are critical effector cells in the pathogenesis of allergic inflammation. The traditional viewpoint of antigen-dependent mast cell activation is that of a Th_2-driven process whereby antigen-specific IgE molecules are produced by B cells followed by binding of the IgE to high affinity IgE receptors (FcεRI) expressed on mast cells. Subsequent antigen-dependent aggregation of the FcεRI initiates an intracellular signalling cascade that culminates in mediator release. Mast cell responses, including cell growth, survival, chemotaxis, and cell adhesion, however, can also be regulated by other receptors expressed on mast cells. Furthermore, FcεRI-mediated mast cell mediator release can be significantly modified by ligation of specific classes of these receptors. One such class of receptors is the G protein-coupled receptors (GPCR). In this review, we describe how sub-populations of GPCRs can either enhance or inhibit FcεRI-mediated mast cell activation depending on the particular G protein utilized for relaying signalling. Furthermore, we discuss the potential mechanisms whereby the signalling responses utilized by the FcεRI for mast cell activation are influenced by those initiated by GPCRs to produce these diverse responses.

Keywords

Mast cells; FcεRI; G protein-coupled receptors; IgE; antigen; signalling

Mast cell activation

Mast cells play a role in both the innate and adaptive immune responses which contribute to the body's defence mechanisms against invading pathogens and parasites [1–3]. However, when mast cells are inappropriately activated following antigen exposure, the resulting release of multiple pro-inflammatory mediators leads to the initiation of the clinical manifestations associated with allergic inflammation [4]. This response occurs as a consequence of the antigen binding to IgE molecules which occupy high affinity receptors for IgE (FcεRI) on the mast cell surface and resulting cross-linking of these receptors [5,6]. FcεRI aggregation induces a cascade of intracellular signalling events which lead to the rapid release of histamine and proteolytic enzymes, as a consequence of degranulation, and the release of leukotrienes (LTs) and prostaglandins (PGs), as a result of phospholipase A_2 (PLA_2)-mediated phospholipid hydrolysis [4,7]. Following enhanced gene expression, a variety of cytokines and chemokines

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are also released from the activated mast cells, however this is a delayed response observed several hours after degranulation and the release of phospholipid-derived mediators [4].

The FcεRI-mediated process described above is likely to be an over-simplification of what is actually occurring during an ongoing reaction *in vivo*. In this respect, the immediate microenvironment surrounding the cells in their resident tissues likely contains a multiplicity of factors which, under specific conditions, may modify antigen-dependent mast cell activation [6]. Support for this concept has come from studies conducted *in vivo* which have demonstrated that other receptors, apart from FcεRI, can quite significantly influence mast cell activation [6]. The most widely investigated of these interactions is the process by which the receptor for stem cell factor (SCF), Kit, modifies mast cell responses elicited by aggregated FcεRI [8–11]. For example, studies, conducted in both human and mouse mast cells, have revealed that concurrent administration of SCF with antigen produces a marked enhancement of both degranulation and cytokine production compared to that observed with antigen alone [9,11]. Although this interaction between SCF and antigen has yet to be demonstrated in a physiological setting, the critical role that SCF plays in mast cell growth, differentiation and survival [12,13] would suggest that such an integrated activation process may indeed occur *in vivo*. It has also recently been demonstrated that concurrent activation of toll like receptors (TLR) 2 and TLR4 with FcεRI aggregation also results in a marked enhancement of cytokine production [14]. However, this response is observed in the absence of a potentiation of degranulation and appears to involve a distinctly different integration process than that utilized by Kit.

In addition to the above examples, there is an increasing volume of literature describing how members of the G protein-coupled receptor (GPCR) superfamily can also impact mediator release. The responses elicited by these GPCR agonists, however, are quite divergent ranging from induction of chemotaxis and adhesion to potentiation of FcεRI-mediated mast cell activation [6,15]. Indeed multiple or even divergent responses in mast cells evoked by a single ligand have been reported [16–20]. To discuss how these responses may be regulated it is first necessary to briefly describe the properties of GPCRs.

GPCRs

GPCRS are a diverse group of receptors characterized by their 7 transmembrane spanning regions and their association with heterotrimeric GTP-binding proteins (G protein s) [21]. G proteins exist as a complex of Gα and Gβγ subunits. Upon GPCR ligation, GDP is displaced from the Gα subunit, thus permitting GTP to bind to the Gα subunit resulting in dissociation of this subunit from the Gβγ subunits [22,23]. This allows the free subunits to mediate downstream signalling. The G protein α subunit family is divided into four major groups based on sequence homologies and functional similarities ($G\alpha_s$, $G\alpha_i$, $G\alpha_q$, and $G\alpha_{12}$) [23,24]. $G\alpha_s$ stimulates adenylyl cyclase, Gαi inhibits adenylyl cyclase, $G\alpha_{12/13}$ stimulates the guanine nucleotide exchange activity of Rho, and $G\alpha_q$ activates phospholipase (PL)Cβ and Bruton's tyrosine kinase (Btk) (Fig. 1) [25–27]. The ability of G_i to relay signals is inhibited by pre-treatment with pertussis toxin, therefore this agent has been utilized to demonstrate the involvement of this particular G protein in cellular responses [28]. The dissociated Gβγ-dimer also regulates a variety of intracellular effectors, including PLCβ, phosphoinositide 3-kinase (PI3K), ion channels, and adenylyl cyclase [29]. Thus, the abilities of the individual G proteins to elicit specific responses are not only dependent on the cell types in which they are expressed but also the specific G proteins to which they are linked.

GPCRs and mast cell function

In the following sections we describe how mast cell function is modified by GPCRs, and discuss the potential mechanisms by which these processes may be regulated. We have not

endeavoured to comprehensively list all the GPCRs expressed on mast cells or their respective effects, but, rather, have focussed on several classes of GPCR which have been documented to modify FcεRI-mediated mast cell activation or by themselves to induce mast cell mediator release. These receptors include the receptor for the complement component C3a, receptors for the bioactive lipids, sphingosine-1-phosphate (S1P) and lysophosphatidic acid (LPA), receptors for adenosine and adenine nucleotides, receptors for chemokines such as MIP-1α and the β_2 adrenergic receptor. The majority of the studies described below have been conducted in either mouse or rat mast cells. When examined in human mast cells, the results have often been contrasting and the potential reasons for this are also discussed in the following sections. It should also be pointed out, that a number of other GPCRs, for example those for the chemokines [15], fractalkine [30], eotaxin [31], monocyte chemoattractant protein (MCP) [15], and the bioactive amine, 5-hydroxytryptamine [32], can induce chemotaxis and/or cell adhesion in the absence of an apparent ability to modify FcεRI-mediated responses or to induce mast cell activation in the absence of antigen. This category of GPCR is not further discussed in this review.

C3a

The anaphylatoxic peptide C3a is generated at the site of immune complex-dependent inflammation [33]. In addition to its ability to act as a mast cell chemoattractant [34], C3a also can modulate mast cell degranulation and the release of chemokines such as MCP-1 and Rantes [35,36]. The actions of C3a on degranulation, however, differ depending on the particular mast cell subtype. In the mucosal type of mast cells such as RBH-2H3 cells [37] or mouse bone marrow-derived mast cells (BMMCs) [38], C3a inhibits FcεRI-mediated degranulation, apparently independently of the C3a receptor. In these systems, C3a appears to inhibit degranulation through suppression of the phosphorylation of the FcεRI β subunit by an undefined mechanism. In contrast, C3a markedly induces degranulation and/or chemokine production in the HMC-1 and LAD-2 human mast cell lines [36], in primary cultures of human $CD34^+$-derived mast cells [39], and in rat peritoneal mast cells [40] by a C3a receptor-mediated mechanism. These responses are associated with an increase in intracellular calcium concentrations ($[Ca^{2+}]_i$). The effect of C3a on migration, calcium mobilization, and PLC activation in mast cells is blocked by pertussis toxin, indicating that the functional mast cell C3a receptor is coupled to G_i [34,40,41]. In addition to its ability to induce degranulation in the absence of antigen, C3a can act in an additive manner with antigen/IgG_1 via the FcγRI [39] and antigen/IgE via the FcεRI (Kuehn and Gilfillan, unpublished data) to enhance degranulation in human $CD34^+$-derived mast cells. Unlike with Kit and other GPCRs, however, there appears to be no synergy in these interactions (Kuehn and Gilfillan, unpublished data). The data, however, do suggest that the regulatory signalling pathways leading to degranulation elicited by the C3a receptor is different from that regulated by the Fc receptors [6].

Sphingosine-1-phosphate

In multiple cell types, the bioactive lysophospholipid, S1P, regulates quite diverse biological responses including chemotactic motility, calcium homeostasis, cell survival, and cell differentiation [42,43]. S1P is formed by the sphingosine kinase (SK) 1- and 2-dependent phosphorylation of sphingosine and several cell types of hematopoietic lineage, including mast cells, display enhanced S1P levels following activation of these enzymes as a consequence of immune stimulation [44,45]. Five GPCRs with alternative functions have been described for S1P and these have been designated $S1P_{1–5}$ [46]. In addition to acting through these cell surface receptors to modify cellular function, S1P may also act intracellularly to mobilize intracellular calcium and such a mechanism may contribute, in part, to the calcium signal observed in activated mast cell [47,48]. Mast cells express both the $S1P_1$ and $S1P_2$ receptors [43,45]. The $S1P_1$ receptor, which is linked to G_i, regulates mast cell migration in a pertussis toxin-sensitive manner [49] similar to that observed in T cells [50], whereas the SIP_2 receptor regulates

degranulation in a pertussis toxin insensitive manner [44,49]. Thus, although the $S1P_2$ receptor is linked to G_i, G_q and $G_{12/13}$, the ability of S1P to promote degranulation through the $S1P_2$ receptor is likely mediated via G_q and/or $G_{12/13}$, rather G_i. The concentrations of S1P required for degranulation are higher than those required for chemotaxis [16,45] and these higher concentrations of S1P also inhibits chemotaxis [45,49]. This has led to the suggestion that in allergic inflammatory reactions, mast cells would migrate to target tissues at low S1P concentration gradients, however once resident in the target tissues the higher S1P concentrations would prevent further migration, and mast cell degranulation would occur following ligation of the $S1P_2$ receptor [16].

Recent evidence has suggested that whereas intrinsic (mast cell) sources of S1P, released via the ATP binding cassette (ABC)C1 transporter [51], may act on the $S1P_1$ receptor to control chemotaxis, both intrinsic and extrinsic sources of S1P may act on the $S1P_2$ receptor to modify degranulation [52]. Experiments using $S1P_2$ knock out mice have also suggested that the S1P generated in mast cells following FcεRI aggregation, acts in an autocrine/paracrine manner to contribute to the degranulation observed following antigen challenge [45]. Our studies (Kuehn and Gilfillan, unpublished observations) do suggest that S1P can act in a synergistic manner with antigen to enhance degranulation in mouse BMMCs, however, whether this is due to an intracellular or extracellular response has not yet been determined. Despite the significant impairment of S1P-mediated degranulation in $S1P_2^{-/-}$ mouse BMMCs, the concentrations required to elicit degranulation (μM) are markedly higher than the K_d (20–27 nM) for the SIP_2 receptor. This, thus, raises the question as to whether the ability of S1P to promote degranulation of mast cells is entirely mediated by a cell surface receptor.

Lysophosphatidic acid

LPA is another lipid-derived molecule that can influence mast cell activation through a GPCR-mediated mechanism. LPA has been reported to both enhance the development of human mast cells from progenitor cells [53] and to promote chemokine generation in the mature cells [54]. Furthermore, LPA has also been reported to induce degranulation in mouse and rat mast cells [55,56]. All four GPCR receptors for LPA (LPA_1– LPA_4) are expressed in human mast cells [53] and the effects of LPA on mast cell proliferation appears to be mediated by either LPA_1 or LPA_3 receptors via G_i [53,55], whereas the effects of LPA on chemokine generation appear to be mediated via the LPA_2 receptor [54]. It is unclear, however, which LPA receptor may mediated the degranulation response observed in the mouse mast cells.

Prostaglandin E_2

The arachidonic acid metabolite PGE_2 is an important modulator in inflammatory responses which is produced in mast cells, dendritic cells, epithelial cells, fibroblast, and macrophage [57–59]. PGE_2 binds four receptor subtypes (EP1, EP2, EP3, and EP4) [60]. The EP2 and EP4 receptors are coupled with G_s which enhances adenylyl cyclase activity thus increases intracellular cyclic adenosine monophosphate (cAMP) levels, whereas the EP1 receptor is coupled with G_q and thus signals through intracellular calcium. The EP3 receptor is coupled with multiple G proteins (G_i, G_q, and G_s) [60]. PGE_2 modulates FcεRI-mediated mast cell activation in quite contrasting manners depending on the predominance of the receptor subtype ligated in the particular mast cell system. In this respect, PGE_2 has been demonstrated to enhance FcεRI-mediated degranulation and the production of cytokines, including IL-6 and GM-CSF, in mouse BMMCs [61,62]. These responses appear to be mediated via the EP3 receptor [62]. Similarly, it has been reported that PGE_2 also enhances antigen-dependent degranulation in cultures of peripheral blood-derived human mast cells through the EP1 and/or EP3 receptors [18]. In contrast, other studies conducted in human mast cells, have provided evidence that PGE_2 inhibits FcεRI-mediated histamine, PGD_2, LTC_4, and TNF-α production via the EP2 receptor which relays this inhibition through elevated cAMP levels [19,20]. Thus,

the relative balance between the expression of EP1/EP3 receptors and EP2 receptors appears to largely dictate the response in a particular model of mast cell function. Little information, however, exists concerning the relative expression of the EP receptors on human mast cells in a physiological setting and how the expression of these receptors may be regulated. Further studies are therefore required to establish how PGE_2 modifies mast cell activation in disease states *in vivo*.

Adenosine

Adenosine has been proposed to play an important role in asthma and chronic obstructive pulmonary disease , and the clinical treatment of asthmatic responses by methylxanithines may, in part, be linked to the ability of these compounds to act as adenosine receptor antagonists [63–65]. As with PGE_2, adenosine can produce quite contrasting responses on mast cell activation. For example, adenosine is reported to potentiate FcεRI-mediated degranulation and LT release in mouse BMMCs [66], RBL 2H3 [67,68], and human lung mast cells [17] in a pertussis toxin sensitive manner; indicating that these responses are mediated via G_i. In addition, in the HMC-1 human mast cell line, adenosine also stimulates the production of IL-4 [69] and IL-8 [70]. In contrast, adenosine has also been reported to inhibit FcεRI-mediated degranulation of human mast cells [17,71]. Furthermore, following pertussis toxin pre-treatment of RBL 2H3 cells, adenosine inhibits LT production in these cells [67].

These diverse responses produced by adenosine again are directly attributable to the multiple receptors for this ligand expressed on mast cells. Adenosine binds to P1 purinoceptors (purinergic receptors) and four P1 purinoceptors subtypes (A_1, A_{2A}, A_{2B}, and A_3) have been described. The A_{2A}, A_{2B}, and A_3 receptors have been reported to be expressed on mast cells [65,72]. The ability of adenosine to inhibit FcεRI-mediated degranulation is mimicked by the A_{2A}-specific agonist, CGS21680 [73], suggesting that it is this receptor that is responsible for down regulating mast cell responses, whereas the A_{2B} receptor upregulates mast cell activation [70,74]. Both the A_{2A} and A_{2B} receptors are linked to G_s, thus when ligated, will result in increased levels of cAMP following adenylyl cyclase activation. Increased levels of cAMP in mast cells would result in inhibition of FcεRI-mediated mast cell mediator release explaining the ability of tha A_{2A} receptor to down-regulate mast cell activation. The A_{2B} but not A_{2A} receptor is also linked to G_q, which regulates PLCβ activation [63,70]. Thus, the A_{2B} receptor appears to enhance mediator release through PLC activation and elevation of $[Ca^{2+}]_i$ generation. In the mouse and rat, it is the G_i-linked A_3 receptor that appears to be the major receptor involved in the ability of adenosine to enhance FcεRI-mediated degranulation [75]. However, in the human, the A_3 receptor does not appear to be involved in the regulation of degranulation [76], thus, the ability of adenosine to upregulate degranulation in the human appers to be primarily via the A_{2B} adenosine receptor.

Other purinoceptor agonisits

The bioactive purine and pyrimidine nucleotides, ADP, ATP, and UTP can influence cellular activation by binding to specific cell surface P2 purinoceptors [77]. P2 purinoceptors are subdivided into two major functional groups: P2X receptors, which form ligand-gated ion channels and pores, and P2Y receptors, which are coupled to G proteins [78]. Human mast cells have been documented to express $P2Y_1$, $P2Y_2$, $P2Y_{11}$, $P2Y_{12}$, and $P2Y_{13}$ purinoceptors in addition to several P2X subtypes [79]. As with PGE_2 and adenosine, adenine nucleotides produce contrasting responses in mast cells. In rat and mouse mast cells, ATP has been described to induce Ca^{2+}-dependent degranulation via P2X and P2Y receptors [80,81]. Furthermore, ATP has also been shown to potentiate FcεRI-mediated histamine release in rat mast cells [82]. Similarly, ATP and UTP have been reported to enhance FcεRI-mediated degranulation in human mast cells and this response appears to be mediated through P2Y but not P2X receptors [83]. However, although P2 purinoceptor agonists such as ADP produce an

increase in intracellular calcium levels and the activation of the MAP kinases ERK and p38 through $P2Y_1$ and/or $P2Y_{12}$, these agents appear to only minimally affect deganulation and eicosanoid production in the absence of antigen [79]. In contrast to their effects on FcεRI-mediated degranulation, ADP, ATP, and ATP analogues block cytokine production induced by both a TLR2 agonist and LTD_4 via the G_s-induced elevation of cAMP [79]. These observations suggest that, as with other GPCRs, multiple P2 purinoceptors with opposing function are expressed in mast cells.

Chemokines

Mast cells produce both chemokines and cytokines following cellular activation [84,85]. Thus, as with S1P, PGE_2 and adenosine, these represent compounds that are not only produced by mast cells, but which can also act upon mast cells to modify mast cell function. Unlike cytokines which utilize associating signalling subunits to relay their responses, receptors for chemokines are G protein-coupled. Chemokine receptors are divided into four different families (CXCR, CR, CX_3CR, and CCR) based on structural homology and the position of cystein residues [86]. CC-chemokine receptors, particularly the CCR3 receptors, have been proposed to play an important role in allergic diseases [87,88]. Mast cells express CCR3 and this receptor binds a variety of chemokines including eotaxin, RANTES, MCP [84,87,89]. Unlike eotaxin and MCP, which induce mast cell chemotaxis but not degranulation [15,31], RANTES has been demonstrated to induce mast cell degranulation [90] and PGD_2 generation [91], in addition to its chemotactic properties [92]. Similarly MIP-1α, which binds CCR1 and is produced in mast cells following FcεRI aggregation, also induces chemotaxis and enhances FcεRI-mediated calcium generation and degranulation in RBL-CCR1 cells (RBL 2H3 cells expressing human CCR1) [93] and mouse BMMCs [15,66]. Such synergy may have physiological relevance as antigen-induced mast cell responses *in vivo* were reported to be substantially reduced in MIP-1α–knock out mice [94], suggesting that MIP-1α is an important cofactor for FcεRI-mediated responses *in vivo*.

β_2-adrenergic agonists

The last category of GPCRs that modify antigen mediated mast cell activation, the β_2 adrenoceptor do so by inhibiting mast cell activation without having other apparent actions. β_2-adrenoceptor agonists are used as bronchodilators in the therapeutic management of asthma [95,96]. Both short-acting β_2-agonists, such as salbutamol and terbutaline, and longer-acting agonists such as salmeterol and formoterol, have also been demonstrated to inhibit the FcεRI-mediated degranulation and the release of PGD_2, and LTs from human mast cells [96–98]. Moreover, β_2 agonists have been shown to inhibit IgE-dependent cytokine production such as GM-CSF and TNF-α in these cells [98,99]. As with other GPCRs that downregulate FcεRI-mediated degranulation, the inhibitory properties of β_2 agonists are mediated by the G_s-dependent activation of adenylate cyclase, and subsequent increase in intracellular cAMP [100].

Integration of signalling

The complexity of the signalling cascade associated with the FcεRI has been extensively described in several recent reviews [6,85,101,102] and it is not the aim of this current review to revisit this theme. However, to understand how GPCRs may influence mast cell responses, it is necessary to provide a brief overview of these signalling processes.

FcεRI signalling

Although the basic principles of the receptor-distal signalling pathways utilized by the aggregated FcεRI for mediator release have been largely elucidated, the events that allow aggregated FcεRI to initiate these processes are still not fully understood. However, these initial

events appear to involve, and require, FcεRI translocation to glycolipid-enriched membrane domains within the plasma membrane termed lipid rafts [103,104]. Hence, the signalling FcεRIβ and γ chains are tyrosine phosphorylated by the action of the Src Kinase Lyn, which is constitutively activated within these domains [105–107]. The phosphorylation sites within the β and γ chains are contained within immunoreceptor tyrosine-based activation motifs (ITAMS) which provide docking sites for the src homology 2 (SH2) domains of associating signalling molecules [101]. In particular, the phosphorylated ITAMs of the γ chains recruit the tyrosine kinase, Syk [108] which, following its phosphorylation and activation, phosphorylates downstream substrates including the transmembrane adaptor molecules LAT and NTAL (also known as LAB and LAT2) [109,110].

The multiple tyrosines phosphorylated on LAT and NTAL allow the further recruitment of signalling molecules and by this means a multi-molecular receptor-signalling complex or signalosome is constructed [110,111]. The complex centred around LAT allows for the recruitment and, thus activation of $PLC_{\gamma 1}$ and $PLC_{\gamma 2}$ [6,109] both by direct binding and indirect binding via the cytosolic adaptor molecules Gads and SLP76 [112]. PLC catalyzes the hydrolysis of phosphatidylinosoitol (4,5)-bis phosphate (PIP_2) with resulting liberation of inositol (1,4,5)-trisphosphate (IP_3) and diacylglycerol (DAG) [113] which respectively liberate Ca^{2+} [114] from the endoplasmic reticulum and induce the activation of protein kinase C (PKC) [115,116]. Using knock out approaches [109], calcium buffering experiments [116] and pharmacological inhibitors [117], it has been demonstrated that these signalling processes are essential for the ability of antigen to elicit a degranulation response.

A parallel complementary pathway which is initiated following activation of the tyrosine kinase Fyn is also required for optimal degranulation [118]. This pathway, which follows phosphorylation of the cytosolic adaptor molecule Gab2, leads to the activation of class 1A PI3K [118]. In the mast cell, the p110δ PI3K subunit isoform appears to be the predominant type utilized by the FcεRI for deganulation and cytokine production [119]. PI3K catalyses the phosphorylation of plasma-membrane associated PIP_2 to form phosphatidylinositol (3,4,5)-triphosphate (PIP_3). This molecular configuration binds pleckstrin homology (PH) domains contained in a subset of critical signalling molecules allowing these molecules to be recruited into the signalosome. These signalling molecules include $PLC\gamma_1$ and $PLC\gamma_2$, the Btk, and the serine/threonine kinases PDK1 and AKT [120,121]. The use of knock out [122] and transgenic mice [119], and pharmacological inhibitors [117,119] have demonstrated a critical role for PI3K in mast cell activation, though mouse mast cell appear to show a greater dependency on PI3K mediated responses as do human mast cells. Regardless, it has been proposed that PI3K regulates an amplification pathway also involving NTAL and Btk, for ongoing signalling events initiated by the LAT-$PLC\gamma_1$ pathway [6].

Many of the same signalling events described above are also critical for eicosanoid production and cytokine gene expression [101,123], however, the terminal events diverge from those required for degranulation. The eicosanoids are generated as a consequence of MAP kinase-dependent activation of PLA_2 which liberates the precursor, arachidonic acid, from phospholipids [124]. The MAP kinase pathway also regulates cytokine gene expression by inducing the phosphorylation of transcription factors such as the AP1 components Fos and Jun [125]. Cytokine production is also regulated by other PI3K and calcium dependent process and the downstream activation of other transcription factors such as NF-κB and NFAT [119,125, 126].

GPCR-mediated signalling

The ability of GPCRs to regulate mast cell responses such as degranulation and cytokine production requires that the initial signals generated by these receptors ultimately impact on the terminal signalling processes described above for FcεRI. Unlike the FcεRI receptor, GPCRs

do not appear to require tyrosine kinases for the initiation of degranulation or cytokine generation but, as described earlier, GPCRs regulate their function through Gα subunits and βγ subunits [6,23,27]. Thus, how GPCRs influence mast cell function depends directly upon the class of G protein engaged and how the individual subunits regulate effector proteins. Although the initiating events utilized by the FcεRI and GPCRs may differ, there are common elements to these pathways at more receptor-distal stages. In this respect, as with the FcεRI, the signalling cascades which allow GPCRs to induce degranulation or potentiate FcεRI-mediated degranulation ultimately lead to the activation of the critical signalling enzymes PLC and PI3K [27,66]. However, unlike the FcεRI which utilizes PLCγ_1 and PLCγ_2 [6,109], and primarily the p110β subunit isoform of PI3K [119] for signalling, GPCRs activate PLCγ [27, 113] and the p110γ subunit isoform of PI3K [127]. Regardless, both PLCβ and PLCγ isoforms, when activated, will lead to the generation of IP_3 and DAG leading to calcium mobilization and PKC activation respectively. In addition, the p110β and γ subunit isoforms of PI3K will both induce the formation of membrane-associated PIP_3 allowing the membrane recruitment of PH domain-containing signalling molecules [121].

Transmission of signalling via G_i ($G\alpha_i$ and/or βγ) alone is sufficient for the GPCRs to initiate activation of both PLCβ and PI3Kγ [27] and indeed the majority of the GPCRs that promote degranulation of mast cells, whether in the presence or absence of antigen, are linked to G_i as evidenced by their sensitivity to pertussis toxin. The exception being the $S1P_2$ receptor which, although linked to G_i, likely mediates its effects via $G_{12/13}$ or G_q leading to the respective activation of either PLCβ, or of the GTP-binding proteins Rho [44,46]. The ability of specific GPCRs, e.g. the EP2, A_{2A}, and β_2 adrenergic receptor to down-regulate FcεRI-mediated mast cell activation is dependent on that fact that these receptors induce adenylyl cyclase-dependent cAMP production via G_s. cAMP has been demonstrated to negatively regulate mast cell function [100] although the precise mechanism controlling this response still remains unclear. However, conditions that elevate cAMP have been demonstrated to stimulated the phosphorylation of the transcription regulator CREB and to induce the expression of the CREB dependent inducible cAMP early repressor [79]. It is therefore possible that, at least the inhibitory effect of cAMP on cytokine production is linked to these events.

It is still unclear why the signals generated by adenosine and PGE_2 are insufficient by themselves to induce degranulation yet those induced by C3a and S1P are capable of inducing degranulation in the absence of antigen. One possible explanation is that adenosine and PGE_2 can simultaneously activate both inhibitory pathways, via G_s, and stimulatory pathways, via G_i, thus the ability to induce degranulation is kept in check by the inhibitory signals, whereas, both C3a and S1P appear to be only positively regulating mast cell function through G_i, and/or G_q. A further explanation may be that the signals produced by adenosine and PGE_2 are of sufficient strength and duration to allow degranulation to proceed. For example, the calcium signal produced by these agents is transient, and not of sufficient strength to effectively deplete the intracellular stores to trigger store-operated calcium entry necessary for degranulation [62,72]. C3a, in contrast, produces a maintained calcium signal [36] which appears to be of sufficient magnitude and duration to induce a degranulation response in the absence of antigen. S1P is unique in the sense that it does not require G_i to elicit its effects on degranulation [44,45]. As it is also linked to $G_{12/13}$, and G_q, it is possible that its ability to induce degranulation of mast cells is linked to PLCβ activation as mediated by G_q.

The activation of PLCs and PI3K by both G_i-coupled GPCRs and FcεRI provides a mechanism by which the signals indiuced by these receptors may be integrated for the synergistic enhancement of mast cell mediator release. Based on data obtained from BMMCs derived from the bone marrow of PI3Kγ knock out mice, it has been proposed that the ability of several GPCRs, including those for adenosine, MIP-1α, and Rantes, to synergistically enhance antigen-induced intracellular calcium modulation and degranulation, is regulated by Gi dependent

PI3Kγ activation [66]. However, Figure 2b of this study [66] suggest that although reduced, adenosine can still synergistically enhance FcεRI-mediated degranulation in the absence of PI3Kγ. Furthermore, our unpublished observations (Kuehn and Gilfillan) suggest that the ability of PGE_2 to synergistically enhance FcεRI-mediated degranulation in BMMCs is independent of PI3K but rather involves G_i-dependent trans-synergy in the activation of PLCγ and PLCβ leading to a marked enhancement of store operated calcium entry. Whether this applies for the other Gi-linked receptors which modify mast cell function remains to be established

Conclusions

In this review we have discussed how GPCRs can either induce mast cell activation or modify antigen-dependent mast cell activation. The majority of the studies described have been carried out in cultured mouse mast cell and it is apparent from the limited studies conducted in human mast cells that there may significant differences in the nature of the responses generated by GPCRs between mouse and human. These differences may be related to interspecies differences in the expression of subclasses of receptors for specific ligands and the nature of the G proteins which are coupled to these receptors. In this respect the balance between G_i- and G_s-coupled receptors (Fig. 2) appears to be critical, with the former G protein up-regulating mast cell responses likely via PLCβ and p110γ PI3K regulated pathways, and the latter G protein down-regulating mast cell responses following adenylyl cyclase-dependent enhanced cAMP levels. Further studies are thus required to determine how the relative expression of the GPCRs modifying mast cell responses are regulated and to determine to what extent G proteins contribute to mast cell reaction in normal and disease settings in the human.

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Abbreviations

BMMCs
: bone marrow-derived mast cells

cAMP
: cyclic adenosine monophosphate

EP
: E prostanoid receptor

FcεRI
: High affinity IgE-receptor

GPCR
G protein-coupled receptor

IgE
immunoglobulin E

LPA
lysophosphatidic acid

LT
leukotriene

MIP-1α
macrophage inflammatory protein-1α

MCP
monocyte chemoattractant protein

PI3K
phosphoinositide 3-kinase

PGE_2
prostaglandin E_2

PLC
phospholipase C

Rantes
regulated upon activation, normal T cell expressed and secreted

S1P
sphingosine-1-phosphate

TLR
Toll like receptor

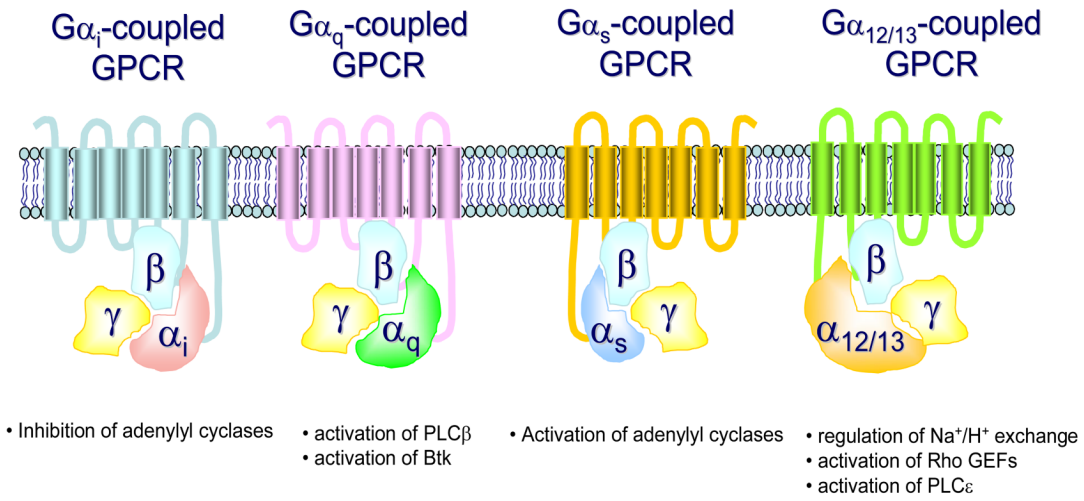

Fig 1.
Major classes of GPCRs and the signals relayed by the respective Gα subunits.

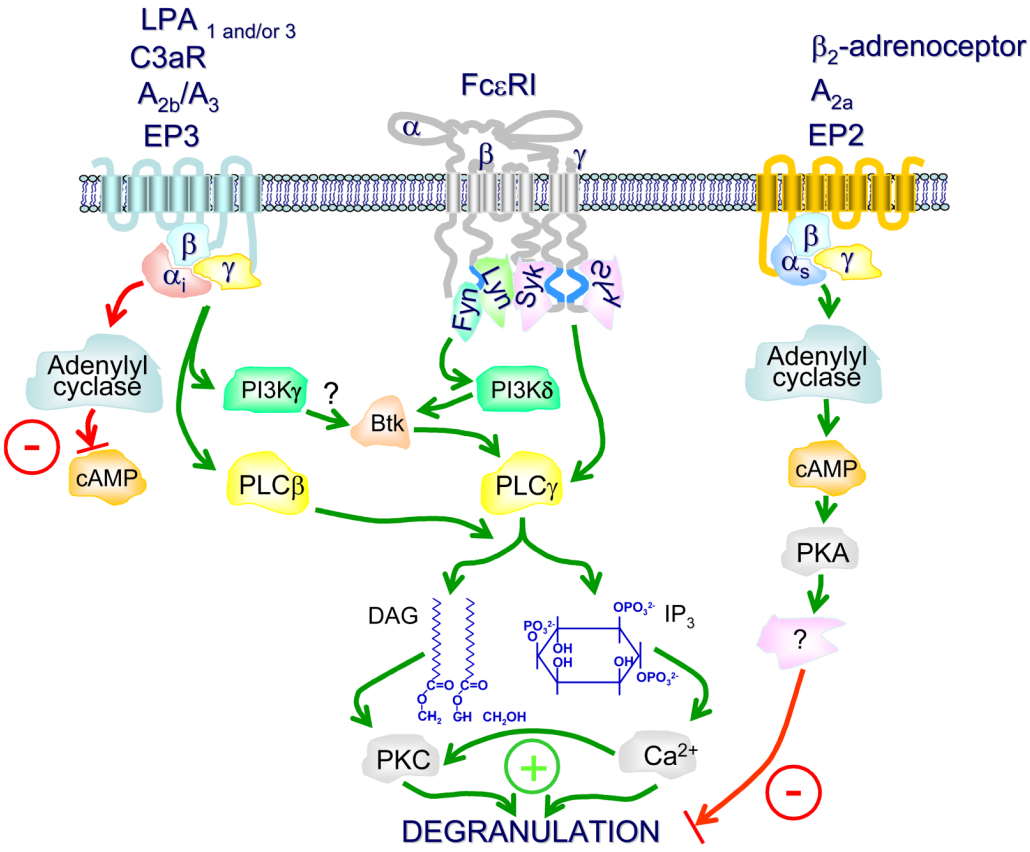

Fig 2.

The respective balance between G_i- and G_s-dependent signalling pathways and their roles in the modification of FcαRI-mediated mast cell degranulation. For clarity, many of the intermediary steps in the FcαRI-dependent signalling pathway have been omitted. For further details concerning this pathway and the pathways regulating cytokine production and eicosanoid release, the readers are referred to the text and references [6,101]. FcαRI aggregation leads to the phospholipase Cγ (PLCγ)-mediated production of diaclglycerol (DAG) and inositol trisphosphate (IP_3) which respectively activate protein kinase C (PKC) and mobilize calcium from intracellular stores, essential signals for degranulation. Phosphoinositide 3-kinase (PI3K), primarily the p110δ subunit, contributes to a maintained calcium signal partly through the Bruton's tyrosine kinase (Btk)-dependent enhancement of PLCγ activation. G_i-coupled receptors impact these events positively by downregulating the production of cyclic adenosine monophosphate (cAMP) which is a negative signal for mast cell activation and by promoting the activation of PI3Kγ and PLCβ. In contrast G_s-coupled receptors impact FcεRI-mediated mast cell activation by enhancing the production of cAMP which, via activation of protein kinase A (PKA), inhibits mast cell activation by an, as yet, unknown mechanism. $S1P_2$ and P2Y which are coupled to G_q-to induce calcium flux and result in degranulation (not shown in this figure). The green lines and positive symbols designate activating signalling pathways and the red lines and -symbols represent inhibiting signalling pathways.

Table 1

Ligands, and their GPCR expressed on mast cells that influence mast-cell activation. Although other GPCR ligands such as eotaxin and fractalkine etc. can induce mast cell chemotaxis, we have only listed the ligands that effect mast cell mediator release and/or cytokine or chemokine production.

Ligand	Receptor : G-protein	species	Mast cell response
C3a	C3aR : G_i	Human	• Induced degranulation [35,36], chemotaxis [34], and chemokine secretion [36] • enhanced FγRI-mediated degranulation [39]
S1P	$S1P_1$: G_i $S1P_2$: G_i,G_q,G_{12}	Mouse	• Induced degranulation [45] • Induced chemokine production and chemotaxis [44,45]
LPA	$LPA_{1\ \&\ 2}$:G_i,G_q,G_{12} LPA_3 : G_i,G_q LPA_4 : G_s	Human	• Induced chemokine release [54]
		Mouse	• Induced degranulation [56]
PGE_2	EP1 : G_q EP2 : G_s EP3 : G_i,G_q,G_s EP4 : G_s	Human	• Increased FcεRI-mediated degranulation [18] • Inhibited FcεRI-mediated degranulation [19] and TNF-α production [20]
		Mouse	• Increased FcεRI-mediated degranulation and IL-6, GM-CSF production [61,62]
Adenosine	A_{2A} : G_s A_{2B} : G_s, G_q A_3 : G_i, G_q	Human	• Inhibited and Increased FcεRI-mediated degranulation [17] • Induced IL-4 [69] and IL-8 [70] secretion
		Mouse	• Induced degranulation [72] • Increased FcεRI-mediated degranulation [66]
ATP, ADP UTP, UDP	$P2Y_1$: G_q $P2Y_2$: G_i,G_q $P2Y_{11}$: G_s, G_q $P2Y_{12\ \&\ 13}$: G_i	Human	• Increased FcεRI-mediated degranulation [83] • Inhibited TLR2 or LTD_4-induced cytokine production [79]
		Mouse	• Induced degranulation [81,128] • Induced cytokine production [129]
β_2-adrenergic agonists	β_2-adrenoceptor	Human	• Inhibited FcεRI-mediated degranulation and cytokine production [97–99]
MIP-1α	CCR1	Mouse	• Induced chemotaxis [15] • Increased FcεRI-mediated degranulation [66] and chemotaxis [94]
Rantes	CCR1,3,4	Mouse	• Induced chemotaxis [15] • Increased FcεRI-mediated degranulation [66]