

Changing the threshold—Signals and mechanisms of mast cell priming

Ivana Halova¹  | Elin Rönnerberg² | Lubica Draberova¹  | Harissios Vliagoftis^{2,3}  |
Gunnar P. Nilsson^{2,4}  | Petr Draber¹ 

¹Department of Signal Transduction, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic

²Immunology and Allergy Unit, Department of Medicine, Karolinska Institutet and Clinical Immunology and Transfusion Medicine, Karolinska University Hospital, Stockholm, Sweden

³Alberta Respiratory Center and Department of Medicine, University of Alberta, Edmonton, AB, Canada

⁴Department of Medical Sciences, Uppsala University, Uppsala, Sweden

Correspondence

Dr. Petr Draber, Department of Signal Transduction, Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic.
Email: draberpe@img.cas.cz

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Summary

Mast cells play a key role in allergy and other inflammatory diseases involving engagement of multivalent antigen with IgE bound to high-affinity IgE receptors (FcεR1s). Aggregation of FcεR1s on mast cells initiates a cascade of signaling events that eventually lead to degranulation, secretion of leukotrienes and prostaglandins, and cytokine and chemokine production contributing to the inflammatory response. Exposure to pro-inflammatory cytokines, chemokines, bacterial and viral products, as well as some other biological products and drugs, induces mast cell transition from the basal state into a primed one, which leads to enhanced response to IgE-antigen complexes. Mast cell priming changes the threshold for antigen-mediated activation by various mechanisms, depending on the priming agent used, which alone usually do not induce mast cell degranulation. In this review, we describe the priming processes induced in mast cells by various cytokines (stem cell factor, interleukins-4, -6 and -33), chemokines, other agents acting through G protein-coupled receptors (adenosine, prostaglandin E₂, sphingosine-1-phosphate, and β-2-adrenergic receptor agonists), toll-like receptors, and various drugs affecting the cytoskeleton. We will review the current knowledge about the molecular mechanisms behind priming of mast cells leading to degranulation and cytokine production and discuss the biological effects of mast cell priming induced by several cytokines.

KEYWORDS

cell priming, chemokines, cytokine receptors, cytokines, high-affinity IgE receptor, mast cell

1 | INTRODUCTION

The mast cell might be considered to be a “less knowledgeable cell” that reacts easily and without much regulation when it encounters an antigen. The antigen binds to IgE, and aggregates the IgE-high-affinity IgE receptor (FcεR1) complexes on the cell surface initiating a down-stream signaling cascade, which results in degranulation and synthesis of lipid mediators, that leads to acute allergic symptoms, and cytokine and chemokine release leading to sustained allergic inflammation. That mast

cell reactivity would not be tightly regulated is of course a wrong notion about this highly powerful cell, which under the worst circumstances can cause severe, and even life threatening, reactions. Since this cell is so powerful and can release such a variety of mediators, it is crucial that its reactivity is highly regulated and that the functionality of the cell is adaptive to its location and circumstances that it is supposed to act in, eg, to react to trauma or to fight an infection.¹ Mast cells are highly sophisticated cells that sense the environment and react to changes by releasing potent mediators acting on both the vasculature and immune system.² Being such a multifunctional cell, the mast cell is consequently believed to be part of the pathogenesis of several diseases.³

This article is part of a series of reviews covering Mast cells and Basophils and their functions appearing in Volume 282 of *Immunological Reviews*.

The molecular mechanisms of regulation of mast cell activation through antigen-IgE-FcεRI complex-mediated signaling have been investigated extensively.^{4,5} Several types of receptors with immunoreceptor tyrosine-based inhibitory motifs (ITIMs) have been identified to be important inhibitory regulators of FcεRI-mediated mast cell reactivity, including FcγRIIB/CD32, CD200R, and several Ig-like receptors.⁶ Other receptor families, including tetraspanins (CD9, CD63, CD81, and CD151) and the CD300R-family, can mediate either activating or inhibitory signals.⁷ Thus, there is an intricate network of adapter proteins and receptors that can either increase or inhibit the FcεRI-mediated signaling and thereby regulate the release of mediators.

In this review, we will focus on the fine-tuning, ie, priming, of mast cell reactivity after FcεRI-mediated activation by soluble factors, such as growth factors, cytokines, G protein-coupled receptors (GPCRs), toll-like receptors (TLRs), and drugs affecting the cytoskeleton. Priming, which is characterized by transition of the cells to a state with enhanced responsiveness, is usually not an acute event, within minutes, but an effect that is induced over a longer time frame, which results in increased release of mediators after IgE-mediated activation, and thus exacerbation of the symptoms. An example is the increase in mast cell mediator release after prolonged exposure to a TLR ligand, lipopolysaccharide (LPS).⁸ The definition of priming is not absolute, since maturation of a cell might also change its reactivity and/or synthesis of mediators. An example is the long-term effect (4 weeks) of stem cell factor (SCF) on bone marrow cultured mast cells (BMMC) developed in the presence of interleukin (IL)-3, which changes the phenotype of the cells into more mature connective tissue-like mast cells containing increased levels of histamine and heparin.⁹ Nevertheless, here we will review and discuss the priming effect of several factors that have been demonstrated to increase the release of mast cell mediators, and hence have the possibility to increase the inflammatory response and exacerbate IgE-mediated mast cell disorders, such as allergy, anaphylaxis, and asthma.¹⁰

2 | PLASMA MEMBRANE RECEPTORS INVOLVED IN MAST CELL PRIMING

Studies dealing with the mechanisms of mast cell activation have been often performed in vitro using IgE-sensitized cells exposed to specific antigen. However, in tissues, mast cells are surrounded by other cells and compounds, which affect the threshold of mast cell activation. Among various compounds, cytokines, chemokines, viruses, and bacteria have been studied most extensively. Other substances such as adenosine, prostaglandins (PGs), cytokines, and sphingosine 1-phosphate (S1P) might also be produced by mast cells and influence the activation by an autocrine mechanism. Under certain conditions, these substances might induce mast cell priming. The molecular mechanism of priming is not completely understood, but is mostly related to a synergic effect of agents that on their own are unable to induce mast cell activation leading to degranulation. Many of the agents, such as SCF, IL-33, TLR ligands, PGE₂ and others, do not cause degranulation, but induce secretion of lipid mediators and/or cytokine production.

Priming could also be related to enhanced or modified expression of proteins involved in FcεRI signaling or to changes in plasma membrane and/or cytoskeleton properties.

3 | FCεRI SIGNALING

FcεRI expressed on mast cells is a tetrameric receptor composed of one IgE-binding α subunit, one membrane-spanning β subunit, and two γ subunits.¹¹ Multivalent antigen crosslinks IgE-FcεRI complexes on the cell surface and triggers a series of activation events. The process starts by engagement of the Src family protein tyrosine kinases LYN and FYN.¹¹⁻¹⁴ LYN phosphorylates ITAM motives of the adjacent β and γ chains, as well as the kinase SYK that is recruited to the phosphorylated FcεRI γ subunit. How LYN initiates the signaling cascade has not been completely elucidated and several models exist.¹⁵ Some observations indicate that LYN is located in plasma membrane microdomains and FcεRI aggregation brings the receptors into these microdomains, where phosphorylation occurs. Other data support a model suggesting that aggregation of FcεRI leads to a shift in the steady state between Src family kinases and phosphatases in favor of kinases.¹⁶ In both models, the actin cytoskeleton plays an important role. Activated SYK then propagates the signal by phosphorylation of many substrates including transmembrane adapter proteins, linker of activated T cells (LAT) and non-T-cell activation linker (NTAL).¹⁷⁻²⁰ Subsequently, FYN phosphorylates another adapter protein, GRB2-associated binding protein 2 (GAB2).²¹ The adapters act as scaffolds enabling assembly of large signaling complexes that are responsible for further propagation and branching of the activation signal. This leads to activation of phosphatidylinositol-3-kinases (PI3Ks), phospholipases C (PLCs), AKT, protein kinases C (PKCs) and other signal transduction molecules, whose precise regulation is crucial for setting the threshold of mast cell degranulation and cytokine production.²²⁻²⁵ Several surface receptors could modulate the FcεRI-mediated activation process. These include cytokine receptors, GPCRs, chemokine receptors, and TLRs (Table 1).

4 | THE PRIMING EFFECT OF STEM CELL FACTOR ON MAST CELL MEDIATOR RELEASE

SCF-mediated priming of mast cells has been primarily studied for anaphylactic release of mediators after FcεRI ligation, the most widely studied outcome in the priming studies. In 1992, Bischoff and Dahinden noticed that SCF could potentiate both preformed (histamine) and newly generated, leukotriene (LT)C₄, mediator release from human lung mast cells following FcεRI-mediated activation in a very quick time frame (maximal effect within 10 minutes) and in concentrations 10-100 fold lower than what had been previously shown to be required for mast cell proliferation.²⁶ A potentiating effect of short-term incubation with SCF on FcεRI-mediated mediator release was also shown for human skin mast cells around the same time.^{26,27} In the case of lung mast cells, SCF did not cause any mediator release on its

TABLE 1 Involvement of plasma membrane receptors in mast cell priming

Receptors	Common ligands (priming agents)	Mast cell type tested	Priming effect	
			Degranulation	Cytokine/chemokine production
Cytokine receptors				
c-KIT	SCF	hPBDMC	↑ ^{49,50}	↑ ^{49,50}
		hLMC	↑ ²⁶	
		hSMC	↑ ²⁷	
		rPMC	↑ ²⁶⁻²⁹	– ^{28,29}
		m,rBMMCs	↑ ^{30,32}	↑ ^{30,32}
IL4R	IL-4	hCBDMC	↑ ⁸⁴	
ST2	IL-33	hPBDMC	↑ ^{78,80}	
		hCBDMC		↑ ⁷⁶
		mBMMC	– ⁷⁹	↑ ⁷⁹
GPCR				
Adenosine receptors (A2A, A2B, A3)	Adenosine	BMMCs	↑ ^{105,106,109}	
		hLMCs	↑ ↓ ¹⁰²	
		hCBDMC	↓ ¹¹⁰	
Eicosanoid receptors (EP1, EP2, EP3)	PGE ₂	hPBDMC	↓ ¹¹³⁻¹¹⁵ ↑ ¹¹²	↓ ¹¹⁵
		mBMMCs	↑ ¹⁰⁴	↑ ^{101,104}
S1P1R, S1P2R	S1P	mBMMCs	↑ ¹⁰⁵	
β2AR	β2AR agonists	hICDMCs	↓ ¹²⁴	↓ ¹²⁴
Chemokine receptors (CCR1,3,4,5)	CCL3, CCL5, CCL7	mBMMCs	↑ ¹³¹	↑ ^{131,132}
TLRs				
TLR2	LTA, PGN	hpuMCs	↓ ¹⁷⁶	
	LPS, ****	BMMCs	↑ ^{143,144}	↑ ^{143,144}
TLR4	LPS, VSVG	mBMMC	– ¹⁴⁴ ↑ ⁸	↑ ^{8,144,146,147}
	ES-62	hBMMC	↓ ¹⁴⁸	↓ ¹⁴⁸

–, no effect; ↑, enhanced degranulation/cytokine production (priming effect); ↓, reduced degranulation/cytokine production; h, human; m, mouse; r, rat; PBDMC, peripheral blood-derived mast cell; LMC, lung mast cell; SMC, skin mast cell; PMC, peritoneal mast cell; CBDMC, cord blood-derived mast cell; ICDMC, intestinal cell-derived mast cell; puMC, pulmonary mast cell.

own but could activate human skin mast cells to induce the release of histamine and PGD₂. Although these studies did not show any data on FcεRI expression, the short time needed for the SCF potentiating effect suggested that this effect consisted in priming the cells to respond to FcεRI-mediated activation, possibly through alterations in signaling pathways.

SCF also potentiated histamine release from rat peritoneal mast cells in a similar time frame.²⁶⁻²⁹ In that case, 18-24 hours incubation with SCF also potentiated the FcεRI-mediated histamine release. Although the short- and long-term effects were similar, there were different effects on Ag-induced Ca²⁺ level increases in mast cells. The short-term effect was shown to be pertussis toxin sensitive, while the long-term effect was cycloheximide sensitive.²⁸ These data suggest that multiple mechanisms may mediate the SCF enhancing effects on FcεRI-mediated mast cell activation. SCF priming of mast cells, however, had no effect on the release of tumor necrosis factor (TNF)^{28,29} or nitric oxide (NO)²⁹ from these mast cell preparations.

A recent study also compared acute with chronic effects of SCF exposure on FcεRI-mediated degranulation and cytokine production in mouse BMMC.³⁰ Although it is not addressed directly in the paper, it appears that acute exposure to SCF still has a potentiating effect on FcεRI-mediated degranulation and cytokine production, even in cells that had been pre-exposed to SCF in the long term. This further supports the idea that the short- and long-term effects of SCF on degranulation occur through different mechanisms. The long-term effect of SCF has been suggested to be the result of downregulation of a signaling molecule, Hck.³⁰ This observation brings up the issue of maturation and/or phenotypic changes in mast cells when cultured in different conditions, and how much those conditions may affect their responsive phenotype.

Studies described so far have been performed with a variety of mast cells, either primary cells or cell lines from humans, rats, and mice. Interestingly, the SCF effect on FcεRI-mediated activation was different in mouse peritoneal mast cells,³¹ where SCF induced the mediator release on its own and had an additive, not synergistic, effect

with FcεRI-mediated activation. Why SCF would prime certain mast cells and not others has not been studied. Whether it is the species, the maturation level, or the anatomical site of mast cell that is important for this effect has not been addressed, either.

Potential of FcεRI-induced mediator release by SCF was also shown for human bronchoalveolar lavage (BAL) mast cells.³¹ In that study, a significant percentage of BAL preparations studied were unresponsive to FcεRI-mediated mediator release, even though they contained similar numbers of mast cells in the preparations. SCF pre-incubation could render many of these preparations responsive to FcεRI-mediated activation. Following on the idea of SCF incubation being permissive for FcεRI-mediated activation, Hill et al, using rat BMMCs, showed that SCF could not only increase the “% of total mast cell content” of mediators released by mast cells, but could also increase the number of mast cells responding to the FcεRI-activating stimulus, whether this stimulus was represented by anti-IgE antibodies or antigen-specific IgE cross-linked by antigen.³²

Although studies using rat peritoneal mast cell showed no potentiation of FcεRI-mediated TNF release by SCF,^{28,29} a different situation was observed in the MC/9 mast cell line,³³ where co-incubation with IgE/antigen and SCF had a synergistic effect on the transcriptional activity of the TNF gene. Activation through FcεRI in the presence of SCF also showed a synergistic effect between the two signals in the release of IL-13 from mouse BMMC.³⁴ Understanding the role of priming on cytokine production requires a different setup than in degranulation since cytokines are produced hours and not minutes after activation. More studies on this intermediate time range (6–12 hours) are needed to better understand this effect. It will also be important for such studies to be performed with human mast cells.

It is interesting that in contrast to the effect seen with degranulation, both mouse BMMC and human cord blood mast cells pre-incubated with SCF for as little as 1 hour showed decreased chemotaxis following FcεRI-mediated activation, even though SCF pre-incubation increased chemotaxis toward nerve growth factor.³⁵ There have been no attempts to understand the role of SCF priming on other FcεRI-mediated mast cell functions.

Although FcεRI-mediated activation is the best studied, SCF may also alter the responsiveness of mast cells to other stimuli, some of them being important in allergic airway inflammation. Mouse BMMC upregulated PG-H synthase I (PGHS-I), but not PGHS-II, after incubation with SCF and prednisone in a synergistic fashion reminiscent of priming of cells.³⁶ This co-incubation with the two factors led to the release of very high levels of PGD₂, while there was no synergy in the release of thromboxane B₂ or LTB₄. Whether the enhanced PGD₂ release following activation of human skin mast cells with IgE/antigen and SCF²⁷ occurs through a similar mechanism is not known. PGD₂ is a ligand for CRTh2, a receptor expressed on most of the cells important for the pathogenesis of allergic diseases such as Th2 CD4⁺ lymphocytes,³⁷ eosinophils and basophils,³⁸ ILC2 cells,³⁹ and mast cells.⁴⁰

The phenomenon of priming is very interesting, but its biological significance is not clear. What could be the *in vivo* stimuli that induce a rapid increase in SCF to alter mast cell responsiveness to the anaphylactic mediator release? We know little about alterations in the levels

of SCF *in vivo*, especially when it comes to rapid changes in the tissue microenvironment. There are only a few studies trying to address the issue of the priming effects *in vivo*. To our knowledge, there are no other studies on the priming role of compounds for FcεRI-mediated release *in vivo*. There are, however, studies suggesting that various factors generate a “permissive phenotype” for mast cells to be able to become activated following FcεRI ligation *in vivo*. A number of these studies further show that the same factor exhibits synergistic effects with FcεRI activation on the histamine release *in vitro*. Using an *in vivo* mouse model of allergic conjunctivitis, it was shown that FcεRI-mediated mast cell activation requires the presence of macrophage inflammatory protein (MIP)-1α (CCL3).⁴¹ A similar requirement for corticotropin-releasing hormone receptor-1 (CRHR1) was shown in a model of passive systemic anaphylaxis.⁴² In addition, *in vitro* stimulation with MIP-1α⁴¹ or CRHR1 agonists⁴² prime mast cells for degranulation following FcεRI-mediated activation. A number of other factors have been shown to enhance FcεRI-mediated mast cell activation in various *in vivo* systems but also directly on mast cells *in vitro*.^{43–45}

In the majority of the studies reviewed, the main finding of priming is an effect seen after short-term (in the range of minutes) incubation with SCF. In some of these studies, there is also a comparison with long-term effects, which in some cases gives concordant and in others discordant results of short- and long-term incubation. Long-term culture with SCF may alter the mast cell phenotype, and what we see may not be priming through an alteration in signaling pathways by the priming agent, but an altered repertoire of receptors or other molecules required for the reactivity of mast cells against the activating stimulus.

This is evident in the case of mast cell activation with eosinophil granule major basic protein (MBP). In the case of mouse mast cells, BMMC grown in the presence of SCF respond to MBP with release of 5-HT, arachidonic acid and TNF, while they do not respond if they are cultured in the presence of mouse spleen conditioned medium.⁴⁶ In the case of human mast cells, soluble SCF was not adequate for this effect. Human lung mast cells had to be co-cultured with human lung fibroblasts for 4 days to become responsive to MBP.⁴⁷ Human cord blood-derived mast cells were responsive when cultured in the presence of SCF, but this was synergistically increased following culture in the presence of fibroblasts⁴⁷ or even just fibroblast membranes.⁴⁷ This effect is mediated through membrane-bound SCF that can activate β1 integrin via inside-out signaling. All these effects were studied in a timeline of 24–96 hours and there is no information on the potential for faster effects. If we expand the meaning of long-term priming, this does not only need to refer to the ability of mast cells to increase the mediator release, but also to prepare cells for other functions. Under this definition, SCF-mediated activation may also prepare mast cells for apoptosis through the TNF-related apoptosis-inducing ligand pathway.⁴⁸

Although the signaling pathways induced by activation of KIT (CD117) and FcεRI share many signaling molecules, key differences could also be found. In contrast to activation of mast cells by antigen, activation by SCF does not lead to degranulation or cytokine production. These differences could be caused by the inability of

transmembrane kinase KIT to activate SYK kinase and therefore the inability to induce LAT adapter phosphorylation,⁴⁹ as well as inability to induce activation and translocation of PKC.⁵⁰ It has been shown that LAT activation is crucial for proper mast cell activation, as indicated by the fact that LAT-deficient mast cells display reduced degranulation, calcium response, as well as cytokine release.¹⁸ On the other hand, both activation pathways lead to phosphorylation of the NTAL adapter protein.^{19,20,49} KIT phosphorylates NTAL directly and at different tyrosines when compared to NTAL tyrosines phosphorylated by SYK after FcεRI triggering.⁵¹ When activated simultaneously by engagement of both KIT and FcεRI, enhanced phosphorylation of NTAL is observed, resulting in synergistically enhanced degranulation and cytokine production (Figure 1). Burton tyrosine kinase (BTK), PI3K, and PLCγ are also important not only for each pathway involved, but also for synergistic enhancement when FcεRI and KIT are engaged simultaneously.^{23,52,53} These synergistic signaling pathways may mediate priming of mast cells, as discussed above, although this has not been clearly shown in all cases.

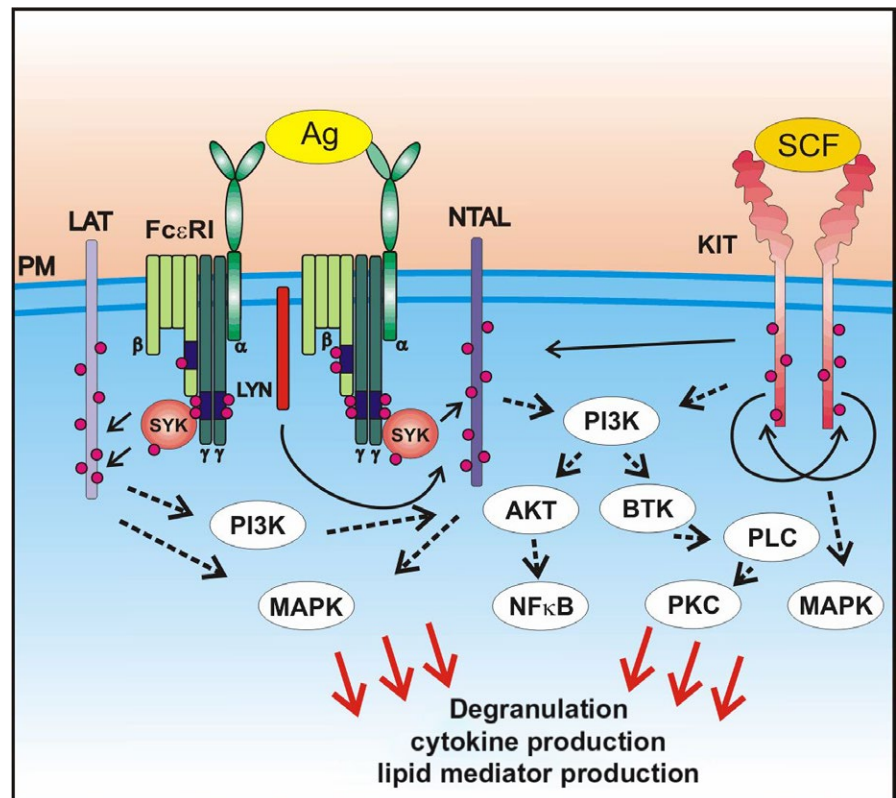
5 | MULTIFUNCTIONAL ROLES OF IL-33 IN MAST CELL SIGNALING

IL-33 is a potent activator of immune cells, including mast cells, and IL-33 and its receptor ST2 are strongly associated to allergy and atopy.⁵⁴⁻⁵⁹ IL-33 is constitutively expressed in the nucleus of several cell types involved in maintaining mechanical barriers, primarily epithelial cells, endothelial cells, fibroblasts, and smooth muscle cells.⁶⁰⁻⁶² IL-33

expression can also be induced in leukocytes, including macrophages, dendritic cells, monocytes, and mast cells, under inflammatory conditions.⁶²⁻⁶⁴ However, their relative importance as an IL-33 source is not clear, as its expression in epithelial cells is 10-fold higher than in leukocytes. IL-33 lacks a signal sequence that enables classical protein secretion and is released during cell death by injury or necrosis, and in this context is considered to be an alarmin.^{65,66} Hence, mast cells sense cell injury/trauma through the recognition of IL-33 and rapidly initiate an acute inflammatory response.^{67,68} There is, however, evidence that IL-33 can also be released from activated living cells through an unknown mechanism.⁶⁹⁻⁷² IL-33 acts through the ST2 receptor in association with co-receptor IL-RACp and triggers TRAF6, MyD88, and MAPK signaling pathways in the responding cells⁷³⁻⁷⁵ (Figure 2).

IL-33 activates mast cells, leading to secretion of cytokines and lipid mediators,^{76,77} and potentiates degranulation in response to FcεRI triggering.⁷⁷⁻⁸⁰ Not only does IL-33 increase the percentage of mast cells that degranulate, but also the magnitude of degranulation at a single cell level.⁸¹ Antigen/IgE-mediated degranulation is also increased by IL-6, but to a lesser extent than by IL-33, and combining IL-33 and IL-6 has a synergistic effect.⁸⁰ IL-33 has no effect on the calcium response elicited by FcεRI engagement, and the mechanism by which IL-33 primes mast cells to be more prone to degranulation remains to be elucidated.^{79,81} The synergistic effect of FcεRI and IL-33 receptor engagement was most apparent at the level of phosphorylated transforming growth factor-β-activated kinase (TAK)1. Although antigen/IgE activation was able to induce only weak phosphorylation of TAK1 and activation by IL-33 led to

FIGURE 1 Mast cell priming by KIT. Aggregation of FcεRIs by IgE-antigen (Ag) complexes leads to receptor phosphorylation by the LYN kinase and subsequent recruitment and activation of the SYK kinase. Activated SYK is able to phosphorylate NTAL and LAT adapter proteins to further propagate the signal. Different tyrosines of NTAL could be directly phosphorylated by SCF-activated KIT. The obtained signals could synergize in activation of PI3K, mitogen-activated family kinases (MAPKs), AKT, BTK, PLC, PKC and transcription factors such as NFκB. This leads to enhanced degranulation and cytokine and lipid mediator production. Many intermediate steps are omitted for the clarity of the figure



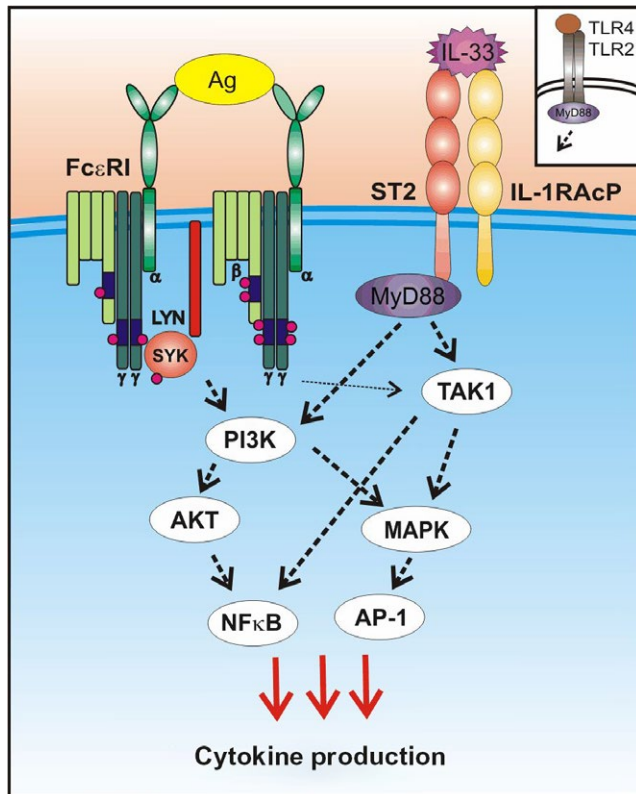


FIGURE 2 Mast cell priming by IL-33R. The initial step after aggregation of FcεRI by IgE-antigen complexes is the receptor phosphorylation by the LYN kinase and subsequent recruitment and activation of the SYK kinase. Propagation of the signal results in activation of PI3Ks, MAPKs, and AKT, resulting in activation of transcription factors NF-κB and AP-1. Activated IL-33R consisting of ST2 and IL-1RAcP (IL-1R3) subunits recruits adapter MyD88, whose activation leads to activation of TAK1 as well as PI3K and MAPKs. Priming effect of IL-33 on FcεRI-activated cells first appears at the level of TAK1 activation that is synergistically upregulated when antigen and IL-33 are added simultaneously, although antigen alone causes no or just low activation of TAK1. The synergistic activation of transcription factors such as NFκB and AP-1 results in increased cytokine production. As activation of TLRs by LPS or PNG also leads to the recruitment of adapter MyD88, the synergistic activation of FcεRI and TLRs is similar to that of FcεRI and IL-33R. Many intermediated steps are omitted for better clarity of the picture

medium activation of TAK1, simultaneous addition of antigen and IL-33 resulted in a synergistic enhancement of TAK1 phosphorylation. This process led to increased activation of transcription factors—nuclear factor (NF)κB, nuclear factor of activated T cells (NFAT), and activator protein (AP)-1.⁷⁹

In contrast to IgE-mediated degranulation, IL-33 has no priming effect on degranulation induced by substance P through the MRGX2 receptor.⁸⁰ Consistent with the fact that IL-33 primes mast cells, mice treated with anti-IL-33 and anti-ST2 antibodies show reduced ear swelling in the passive cutaneous anaphylaxis model induced by FcεRI aggregation.⁸² IL-33 also amplifies IgE synthesis via IL-4-mediated pathway in mice,⁸³ and IgE can in turn increase the expression of FcεRI.⁸⁴⁻⁸⁶ In this way, IL-33 can indirectly affect mast cell

degranulation. On the other hand, prolonged exposure to IL-33 has been shown to induce a hypo-responsive phenotype in mast cells,⁸⁷ suggesting that IL-33 exerts different effects on mast cells in a chronic condition compared to an acute allergic phase.

IL-33 can also stimulate the synthesis and storage of mediators in the granules and thereby increase their amount released upon FcεRI-mediated activation. Culturing BMMC on a cell layer of synovial fibroblasts was shown to increase the tryptase levels in the cells, an effect mediated by IL-33.⁸⁸ Similarly, it was shown that IL-33 increases expression of tryptophan hydroxylase 1, the rate limiting enzyme in the synthesis of serotonin, and thereby increases the levels of serotonin in the cells, which leads to exacerbation of bronchoconstriction in a murine asthma model.⁸⁹

6 | IL-4 ENHANCES IGE RECEPTOR EXPRESSION

IL-4 is a pleiotropic cytokine that plays a prominent role in stimulating and maintaining the allergic response. It is produced mainly by Th2 lymphocytes, basophils, and mast cells.⁹⁰ Typically, IL-4 drives CD4⁺ T cell polarization in the Th2 phenotype, suppresses Th1 cells, and supports the growth and differentiation of B lymphocytes.^{91,92} IL-4 activates cells through the IL-4Rα subunit paired with IL-13Rα or the common γ chain.⁹³ In addition to producing IL-4, mast cells can also react to IL-4.⁹³ IL-4 increases the expression of FcεRI at the mRNA level⁹⁴ and enhances the mediator release upon antigen-IgE-mediated aggregation, in the absence of a significant effect on FcεRI surface expression.⁸⁴ IL-4 can also enhance the production of IgE by B cells,^{95,96} and in this way, increase the FcεRI surface expression on mast cells.⁸⁴⁻⁸⁶ IL-4, therefore, has both a direct and indirect effect, through IgE, on FcεRI expression.⁹⁷

7 | GPCRS AND FCεRI SIGNALING

GPCRs are seven-transmembrane domain surface receptors that regulate and transmit extracellular signals to divergent intracellular signaling pathways. In contrast to FcεRI-mediated activation, activation through the GPCRs is not dependent on initial activation of tyrosine kinases, but the signal is transduced through activation of trimeric G-proteins consisting of Gα and Gβγ subunits.⁹⁸⁻¹⁰⁰ Receptors for different ligands, and even the different subtypes of the receptor for one ligand, can be coupled to four different Gα-subunits: Gα_s (stimulates adenylyl cyclase), Gα_q (activates PLC), Gα_i (inhibits adenylyl cyclase), or Gα_{12/13} (activates small GTPases). One ligand can thus regulate mast cell activities both positively and negatively depending on the receptor subtype expressed.¹⁰⁰ A number of GPCR agonists were found to promote FcεRI-induced degranulation and cytokine production, and the majority of them are coupled to the Gα_i subunit.¹⁰¹⁻¹⁰⁴ The Gα_i subunit can be selectively inhibited by pertussis toxin, and this inhibitor is thus widely used for confirming the role of GPCRs in mast cell activation. Activation of

$G\alpha_i$ leads to inhibition of cAMP production and subsequent inhibition of PKA, whereas activation of $G\beta\gamma$ subunits leads to activation of PLCs and PI3Ks and subsequent activation and translocation of PKC to the plasma membrane. The activation of PLC and subsequent translocation of PKC to the plasma membrane induced by co-stimulation of Fc ϵ RI and prostaglandin EP3 receptor was proposed to be responsible for enhanced BMMC degranulation and cytokine production.¹⁰⁵ This process was found to be independent of PI3Ks. On the other hand, co-stimulation of Fc ϵ RI and adenosine receptor A3AR led to synergistic activation of PI3Ks that was responsible for enhanced activation.¹⁰⁶ Recently, it has been shown that the p110 γ subunit of PI3K that previously was thought to be connected only to GPCR signaling, can be phosphorylated by PKC β upon Fc ϵ RI stimulation and thereby be disconnected from $G\beta\gamma$ signaling.¹⁰⁷ In contrast, activation of GPCRs connected to $G\alpha_s$ leads to enhanced production of cAMP through activated adenylyl cyclase, resulting in an increased activity of PKA.^{98–100} PKA is a well-known activator of C-terminal Src kinase (CSK), which upon activation can inhibit the Src family tyrosine kinases LYN and FYN¹⁰⁸ and thus inhibit the activation caused by the Fc ϵ RI aggregation. The two activation pathways (via GPCR and Fc ϵ RI) that are initiated by independent signaling mechanisms merge at later stages in signaling pathways utilizing the same signal transduction molecules, which could form the basis of the synergy of these two pathways (Figure 3). Despite intensive studies, the mechanism how engagement of GPCRs affects the Fc ϵ RI signaling remains incompletely understood. The role of individual GPCRs in Fc ϵ RI activation is discussed below.

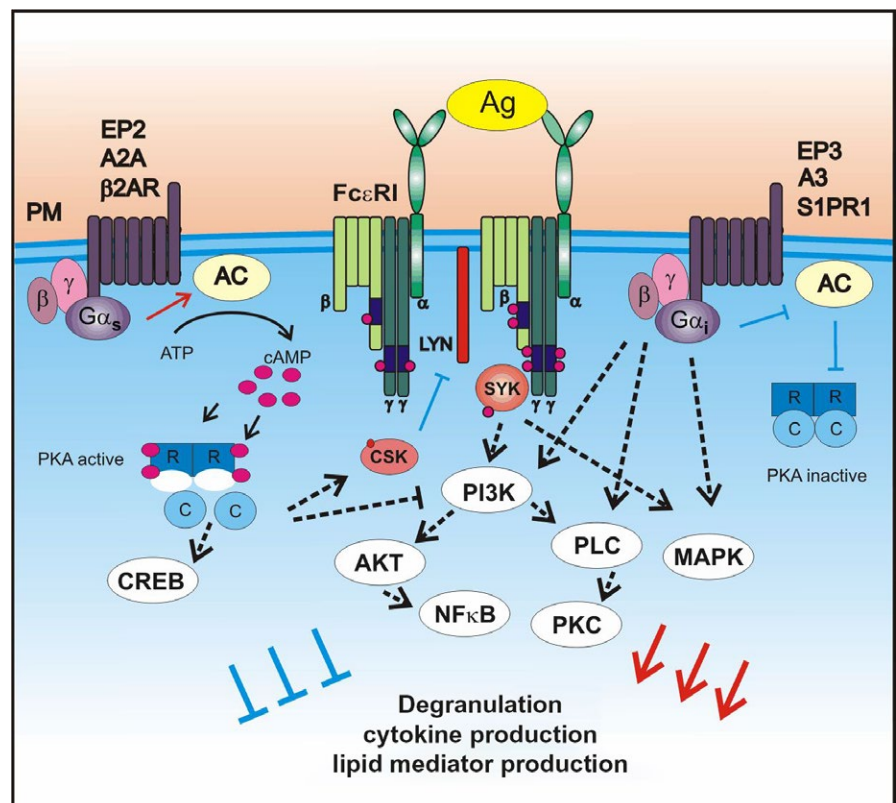
7.1 | Adenosine receptors

Three types of adenosine receptors have been reported to be expressed on mast cells, A2A receptor coupled to $G\alpha_s$, A2B bound to $G\alpha_s$ or $G\alpha_q$, and A3 connected to $G\alpha_i$ or $G\alpha_q$. Through these interactions, adenosine could regulate mast cell activity in a positive or negative way.¹⁰⁰ In IgE-sensitized BMMC, simultaneous addition of adenosine and antigen led to increased degranulation in a pertussis toxin-dependent manner, suggesting that A3R is involved.^{103,106} The suggestion that A3R is engaged in Fc ϵ RI priming was supported by the finding that genetic removal of A3AR ablated the ability of A3AR specific agonist to increase antigen-induced degranulation.¹⁰⁹ Further studies showed that the impact of adenosine co-stimulation could be dependent on the time interval at which adenosine is added to the cells. Addition of adenosine to human lung mast cells up to 5 minutes before Fc ϵ RI engagement inhibited histamine release, whereas adding adenosine 5 minutes after Fc ϵ RI engagement potentiated the histamine release, coincidental with the end of the rapid phase of histamine release. This process was regulated by A2 receptors.¹⁰² Furthermore, inhibition of tryptase release from human mast cells upon IgE-antigen stimulation was inhibited by adenosine through A2A receptor.¹¹⁰

7.2 | PGE₂ receptors

PGE₂ binds to four types of GPCRs, EP1–4. The EP1 receptor is coupled to PLC and its stimulation leads to an increase in free intracellular Ca²⁺ concentration. The engagement of EP2 and EP4 receptors

FIGURE 3 Fc ϵ RI-mediated activation is differentially affected by GPCRs connected to $G\alpha_i$ and $G\alpha_s$ trimeric G-proteins. Aggregation of Fc ϵ RI leads to its phosphorylation by the LYN kinase and subsequent activation of the SYK kinase. Propagation of the signal results in activation of PI3Ks, PLCs, AKT, and PKC. Activation of GPCRs coupled to $G\alpha_i$ inhibits adenylyl cyclase (AC) and inactivates PKA. Activation of β and γ subunits results in PI3K, PLC and PKC activation. Synergistic activation of Fc ϵ RI and GPCRs connected to $G\alpha_i$ leads to increased degranulation and cytokine production. On the other hand, activation of GPCR connected to $G\alpha_s$ increases production of cAMP and subsequently activates PKA. This could result in activation of CSK, followed by inhibition of the LYN kinase and decreased activation of Fc ϵ RI. Many intermediated steps are omitted for better clarity of the picture



induces an increase in the cAMP level via G_{α} -subunit stimulation of adenylate cyclase. The EP3 receptor exists in several isoforms generated by alternative splicing and these isoforms are coupled with different G-proteins (G_i , G_q , and G_s). The major signaling pathways described for the EP3 receptor lead to a decrease in intracellular cAMP levels and increase in free intracellular Ca^{2+} .^{100,111} Similar to adenosine, PGE_2 regulates Fc ϵ RI-mediated activation of mast cells in a positive or negative way, depending on the EP receptors engaged. Enhancement of Fc ϵ RI-mediated degranulation and cytokine production was described in mouse BMMC exposed to PGE_2 , which binds to the EP3 receptor^{101,104} or in human mast cells treated with PGE_2 bound to EP1 and/or EP3 receptors.¹¹² It should also be mentioned that there are other studies describing inhibition of histamine, eicosanoids, and TNF α production in Fc ϵ RI-activated human mast cells caused by binding of PGE_2 to the EP2 receptor, followed by inhibition through elevated cAMP levels.^{113–115}

7.3 | S1P receptors

Mast cells express two receptors for S1P, S1PR1 and S1PR2. S1PR1 interacts with G_{α_i} , which is involved in mast cell migration regulation, and S1PR2 is coupled to G_{α_i} , G_{α_q} , and $G_{\alpha_{12/13}}$, with the latter two being involved in the regulation of mast cell degranulation.^{116–119} In contrast to other GPCR agonists PGE_2 and adenosine, S1P is able to induce mast cell degranulation even without stimulation of Fc ϵ RI,^{105,116} but when added together with antigen to IgE-sensitized cells, the extent of degranulation is increased, even though to a lower level when compared to the synergistic effect of PGE_2 or adenosine and antigen.¹⁰⁵ As S1P is produced in IgE-antigen-activated mast cells, the synergistic effect probably reflects an autocrine type of regulation of mast cell activation.

7.4 | β 2-adrenoceptor (AR) function

Human and mouse mast cells as well as numerous other cell types express membrane-spanning β 2-AR.¹²⁰ Signaling from the β 2-AR is prototypically initiated by coupling of the receptor to G_s , resulting in activation of adenylyl cyclase, followed by activation of PKA. Several studies indicate that β 2-adrenergic agonists are the most potent agents clinically used in inhibiting mast cell degranulation.^{121–124} However, chronic β 2-AR agonist administration in asthmatic patients could result in loss of the protective effects of β 2-AR agonists on mast cells and may increase mediator release from the lung mast cells.¹²⁵ Further studies showed that lung mast cells cultured in the presence of SCF lost their sensitivity to β 2-AR agonists and in fact exhibited enhanced IgE-induced histamine release when compared to cells cultured in the absence of β 2-AR agonists.^{126,127} This was related to SCF-dependent phosphorylation of the β 2-AR on Tyr350, which is involved in counterregulation and internalization of this receptor by insulin tyrosine kinase receptor. The phosphorylation of Tyr350 was paralleled by rapid attenuation of β 2-agonist-induced PKA-dependent phosphorylation of vasodilator-stimulated phosphoprotein, KCa3.1 channel closure, and inhibition of histamine release. The ability of β 2-AR activation

to enhance IgE-dependent histamine release could be related to the functioning of Tyr350-phosphorylated β 2-AR as an activator of ERK1/2 through the Ras-Raf-mitogen-activated protein kinase pathway, which is a positive regulator of mast cell degranulation.

7.5 | Chemokine receptors

Chemokine receptors belong to the family of GPCRs, and generally almost each of them is capable of binding more than one chemokine. Considerable differences in the expression of chemokine receptors between mature and immature mast cells and among individual mast cell subtypes have been described.^{128–130} The role of chemokine receptors in mast cell biology is usually studied in association with mast cell chemotaxis, but their influence on IgE-antigen-induced degranulation and cytokine production was also recognized. Similar to stimulation with other GPCRs, ligation of CCR1 with CCL3 is unable to cause mast cell degranulation or cytokine production, but its co-stimulation with Fc ϵ RI enhances the antigen-induced activation of BMMC.¹³¹ Co-stimulation of Fc ϵ RI and CCR1 led to increased expression of phosphorylated disassembled vimentin, and prevention of vimentin disassembly by a specific inhibitor was able to reduce chemokine production.¹³² CCR1 is expressed by conjunctival mast cells, and subconjunctival injection of CCL3 increased conjunctival mast cell number and degranulation in vivo.⁴¹ Chemokine CCL7, which interacts with CCR1 and CCR3 receptors is upregulated in conjunctival tissue during an OVA-induced allergic response, and co-stimulation of Fc ϵ RI with CCL7 led to enhanced degranulation.¹³³

8 | TOLL-LIKE RECEPTORS

TLRs belong to a family of pattern recognition plasma membrane receptors known to play important roles in the host defense against viruses, bacteria, and bacterial components such as LPS, peptidoglycan (PGN), and lipoproteins.¹³⁴ Several TLRs have been found to be expressed in different types of murine as well as human mast cells. The most extensively studied TLRs involved in mast cell signaling are TLR2 and TLR4.^{135–137} Due to the sequence similarity between TLRs and the IL-1 receptor family, activation of these two types of receptors mediates the downstream signals through common components. These include adapter protein MyD88 that links the receptor to IL-1R-associated protein kinase (IRAK). At initial stages, TLR-mediated responses differ from those utilized by Fc ϵ RI.^{134,137} However, both signal transduction pathways lead to activation of the mitogen-activated protein kinase (MAPK) family, causing activation of both AP-1 transcription factor and I κ B kinase, resulting in the activation of transcription factor NF- κ B. The engagement of the TLR-gene expression regulating pathway in Fc ϵ RI-activated cells could lead to enhanced cytokine and chemokine production (Figure 2).

Stimulation of mast cells by TLR2 and TLR4 ligands cause production of different chemokines and cytokines, and their pattern could differ depending on the mast cell type, mast cell culture conditions, and the ligand used.^{138–142} The ability of TLR2 agonists to induce mast

cell degranulation is controversial because in some studies they were able to induce release of histamine from murine as well as human mast cell whereas in others not.¹⁴¹⁻¹⁴⁴ On the other hand, stimulation of TLR4 alone did not lead to degranulation.^{138,141-144} It should also be mentioned that activation of TLRs by LPS requires co-expression of CD14. In cells lacking membrane expression of CD14 such as in mast cells, soluble CD14 has to be present for efficient activation.^{142,145} Both TLR2 and TLR4 activation events were found to synergize with FcεRI-induced signaling, resulting in enhanced cytokine and/or chemokine production.^{8,141,143,144,146,147} In contrast, ES-62, a product of parasitic filarial nematodes, forms a complex with TLR4 and subsequently inhibits FcεRI-induced degranulation and cytokine production, but in this case a different mechanism seems to be involved.¹⁴⁸

9 | CYTOSKELETON AND MAST CELL PRIMING

Cellular and extracellular matrix tissue microenvironment where mast cells are located alters mast cell responsiveness through effects on the cells by adhesive interactions.¹⁴⁹⁻¹⁵¹ These interactions point out to biological significance of priming through cytoskeletal reorganization. The involvement of the actin cytoskeleton in FcεRI-mediated mast cell signaling was deduced from numerous experiments *in vitro* using various mast cell types. It has been shown that adhesion of rat basophilic leukemia (RBL) cells, which is dependent on the presence of F-actin, leads to a complex series of events including tyrosine phosphorylation of FcεRI,¹⁵² focal adhesion-associated tyrosine kinase pp125FAK,¹⁵³ and other proteins.^{154,155} This was accompanied by enhanced antigen-stimulated degranulation and calcium response in adherent RBL cells when compared to nonadherent cells.^{156,157}

Furthermore, inhibitors that interfere with actin cytoskeleton dynamics, such as latrunculin, and cytochalasin D, which do not cause any degranulation on their own, enhance FcεRI-mediated degranulation.^{158,159} The authors found that there is a good correlation between inhibition of actin polymerization and increased degranulation in antigen-activated RBL cells. When pervanadate was used, which inhibits the phosphatases and thus bypassing the cross-linking of the receptor, no effect of latrunculin alone on degranulation was observed. The cells activated through FcεRI in the presence of latrunculin exhibited increased tyrosine phosphorylation of the FcεRI β subunit by tyrosine kinase LYN, as well as increased tyrosine phosphorylation of SYK, PLCγ, GAB2, and LAT. The enzymatic activities of PI3K, PLCγ, and phosphatase SHP-2 were also upregulated, but tyrosine phosphorylation of ezrin was inhibited. All these findings indicated that the very earliest signaling events after FcεRI crosslinking are affected by the actin cytoskeleton. Interestingly, significant changes in the cellular distribution of surface molecules, tyrosine phosphorylation of signaling proteins, and enzymatic activities were observed in latrunculin-treated cells before the cell triggering. These results suggested that actin microfilaments may interact, either directly or indirectly, with the receptor itself and that they may regulate the signaling process at the level of receptor phosphorylation. The authors suggested that

microfilaments may act by uncoupling LYN from the aggregated FcεRI, thus increasing the threshold of FcεRI activation.

This conclusion was confirmed by super resolution fluorescence localization microscopy (FLM) to image the colocalization of LYN and FcεRI with nanoscale resolution.¹⁶⁰ Pair cross-correlation analysis of FLM images showed that colocalization of IgE-FcεRI complexes with LYN and phosphotyrosines are enhanced by both cytochalasin and latrunculin. Because the total specific activity of plasma membrane LYN was not affected by inhibition of actin polymerization,¹⁵⁹ the data suggest that actin destabilization leads to enhanced association of LYN with FcεRI, resulting in enhanced tyrosine phosphorylation of the FcεRI β subunit. A similar conclusion was obtained by observations of quantum dot-labeled FcεRI motion and GFP-tagged actin dynamics.^{161,162} In these experiments, the authors concluded that actin contributes to membrane partitioning not only by restriction of diffusion of the membrane proteins, but also by influencing their long-range mobility, sequestration, and response to ligand binding.

Previous studies showed that FcεRI and other plasma membrane components are compartmentalized in various plasma membrane microdomains.¹⁶³⁻¹⁶⁶ These and other data pointed to engagement of the actin cytoskeleton in physically restricting the entry of cross-linked receptor into plasma membrane microdomains containing the LYN kinases. The reduced extent of actin polymerization would increase the likelihood of the cross-linked receptor entering the domains and becoming phosphorylated by the LYN kinase, and in this way setting the threshold of mast cell activation.

Another piece of evidence that actin filaments are involved in mast cell activation came from experiments in which BMMC derived from NTAL-deficient mice¹⁹ were analyzed.¹⁶⁷ These cells showed reduced spreading on fibronectin, reduced filamentous (F)-actin polymerization, and enhanced degranulation. NTAL-deficient cells also exhibited more reduced RhoA activity in antigen-stimulated cells. RhoA is a small GTPase, which is an important regulator of actin polymerization. In these experiments it was also noticed that in control NTAL-positive cells, there is a transient decrease in actin polymerization, followed by enhanced F-actin polymerization at later stages after antigen triggering. The results suggested that NTAL links FcεRI signaling to actin cytoskeleton reorganization in mast cells. They also raised the possibility that NTAL may play a more direct role in the granule movement and fusion with the plasma membrane. Thus, FcεRI aggregation resulted in rapid changes in the F-actin content. The initial decrease in the F-actin content was followed by a subsequent gradual increase in F-actin.¹⁶⁸ These results were in line with data obtained with permeabilized mast cells, which showed that in the course of degranulation, actin is rapidly depolymerized.^{169,170} The finding that inhibition of actin polymerization increases the readiness of cells to become activated support the concept that actin filaments contribute to the setting of the dynamic threshold for mast cell signaling. The FcεRI signaling threshold could be regulated by the changing availability of kinases and/or phosphatases for their substrates. In this context, it should be mentioned that most of membrane-bound oxidized phosphatases were associated with the actin cytoskeleton.^{16,169}

Hematopoietic cells express a set of unique actin regulators, which when mutated lead to altered dynamics of the actin cytoskeleton and cause diseases. A well-known immunodeficiency of this type is the Wiskott-Aldrich syndrome, which is caused by mutation in the WAS gene encoding the Wiskott-Aldrich syndrome protein (WASp).¹⁷¹⁻¹⁷³ WASp possesses a number of different motifs that interact with multiple proteins and/or are involved in regulation by different stimuli. WASp directly or indirectly interacts with cytoskeletal organizing complex Arp2/3 and small GTPase CDC42, known to regulate the formation of actin filaments. Studies in mouse B cells showed that loss of WASp resulted in reduction in the amount of filamentous actin in resting B cells. In response to antigen, WASp-deficient mouse B cells exhibited increased signaling and antibody production, and in these properties resembled the B cells in patients with the Wiskott-Aldrich syndrome. These data indicated that in B cells, WASP is involved in setting the BCR threshold. When BMMC derived from WASP-deficient mice were analyzed, a different phenotype was found.¹⁷⁴ Although the cells exhibited reduced amounts of filamentous actin, their exposure to antigen-IgE complexes resulted in reduced degranulation and cytokine secretion. However, early activation events that include tyrosine phosphorylation of the FcεRI β subunit and SYK were not affected by the WASP deficiency, although tyrosine phosphorylation of PLCγ and Ca²⁺ mobilization were diminished. This phenotype is different from properties of NTAL-deficient cells, which after FcεRI triggering exhibited enhanced filamentous actin depolymerization, SYK, LAT and PLCγ tyrosine phosphorylation, calcium response and degranulation.¹⁹ The differences in phenotype in WAS-deficient B cells and BMDCs deficient in WAS or NTAL are examples of the complexity of signaling events that are dependent on numerous factors.

10 | CONCLUSION

Mast cell priming has a key role in proper functioning of the cells under physiological conditions by setting a threshold of mast cell activation. Detailed understanding of the molecular mechanisms of mast cell priming has important clinical significance for the treatment of mast cell-mediated diseases. This is exemplified by the above-described studies indicating attenuation of the effect of β2-AR agonists in human lung mast cells in the presence of SCF. It is very likely that other compounds involved in mast cell priming will be discovered. For example, it has been shown recently that drugs used for the treatment of cardiovascular diseases, metoprolol (β-blocker) and ramipril (angiotensin-converting enzyme), display modest but discernible anaphylaxis-promoting effects when used in patients individually and more pronounced effects when combined.¹⁷⁵ These data were confirmed using a mouse model system and it was found that direct targets for the drugs are mast cells, which are primed for enhanced response to antigen-induced IgE-FcεRI aggregation. The observed effects were manifested namely when the degree of FcεRI aggregation was in the suboptimal range, a scenario most commonly encountered in clinical settings.

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CONFLICTS OF INTERESTS

The authors report no conflicting financial interests.

ORCID

Ivana Halova  <http://orcid.org/0000-0003-3078-7986>

Lubica Draberova  <http://orcid.org/0000-0002-2459-4435>

Harissios Vliagoftis  <http://orcid.org/0000-0002-5029-855X>

Gunnar P. Nilsson  <http://orcid.org/0000-0001-6795-5512>

Petr Draber  <http://orcid.org/0000-0002-3986-2025>

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