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The high affinity IgE receptor: a signaling update

by

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Highlights

- Studies with different antigens for the FcεRI-bound IgE reveal that differences in affinity, valency and antigen concentration are translated to intracellular signaling events and different outcomes of cell activation.
- Analysis of the secretory machinery of FcεRI-induced mast cell degranulation reveal a highly complex machinery regulating involved cytoskeletal reorganization, secretory granule transport mechanisms and fusion.
- Advances made in signaling-dependent cytokine and chemokine gene transcription in response to antigenic stimulation

Abstract

Here we update receptor proximal and distant signaling events of the mast cell high affinity IgE receptor (FcεRI) launching immediate type I hypersensitivity and an inflammatory cytokine-chemokine cascade. Different physiologic antigen concentrations, their affinity, and valency for the IgE ligand produce distinct intracellular signaling events with different outcomes. Investigating mast cell degranulation has revealed a complex molecular machinery that relays proximal signaling to cytoskeletal reorganization, granule transport and membrane fusion. Several new phosphorylation- and calcium-responsive effectors have been described. FcεRI signaling also promotes *de novo* gene transcription. Recent progress has identified enhancers at genes that are upregulated in mast cells after stimulation through FcεRI using next generation sequencing methods. Enhancers at genes that respond to antigenic stimulation in human mast cells revealed Ca²⁺-dependency. Stimulation-responsive super enhancers in mouse mast cells have also been identified. Mast cell lineage-dependent transcription factor GATA2 primes these enhancers to respond to antigenic stimulation.

1. Introduction

The antigen-dependent aggregation of IgE bound to mast cell (MC) high affinity IgE receptors (FcεRI) launches the immediate type I hypersensitivity response and an inflammatory cascade characterized by the exocytosis of secretory granule (SG) content, the *de novo* synthesis and secretion of prostaglandins/leukotrienes and a diverse set of cytokines/chemokines. Despite unquestionable physiological roles, FcεRI-mediated signaling has been largely studied for its implication in allergies. Initially, research focused on receptor-proximal events but recent studies also investigate further downstream pathways. In this review we update the accumulated knowledge on receptor proximal and distant signaling.

2. Receptor proximal events

FcεRI expressed on MC and basophils consists of αβγ₂ heterotetramers of three subunits [1]: The α chain is a type I transmembrane protein that binds the IgE Fc portion at a 1:1 ratio, the β chain is a receptor-stabilizing and signal-amplifying subunit with four transmembrane domains, and the disulfide-linked dimeric γ chains are signal-triggering subunits. β and γ subunits have an immunoreceptor tyrosine-based activation motif (ITAM). Upon engagement of receptor-occupied IgE by multivalent antigen, tyrosine residues in the ITAMs are phosphorylated by β chain-bound Src family protein tyrosine kinase (SFK) Lyn [2]. Phosphorylation of Tyr47/58 residues within γ-ITAMs recruits another tyrosine kinase Syk via SH2 domain-phosphotyrosine interactions [3]. This activates Syk through conformational changes and tyrosine phosphorylation at multiple sites by autophosphorylation and SFKs to phosphorylate numerous signaling enzymes and adaptors [4]. Syk is essential for most if not all activation events leading to degranulation and *de novo* synthesis and secretion of lipid mediators, cytokines and chemokines [5,6] (**Fig. 1**). For example, Syk (and another tyrosine kinase Btk) phosphorylate phospholipase C (PLC)-γ1 and -γ2 that generate inositol 1,4,5-trisphosphate (IP₃) and

diacylglycerol (DAG) from phosphatidylinositol 4,5-bisphosphate (PIP₂). Diacylglycerol activates protein kinase Cs (PKCs) and IP₃ is critically important for Ca²⁺-release from ER storage sites; Ca²⁺-depletion from the ER sites is detected by ER Ca²⁺-sensors STIM-1 and STIM-2, and STIM-1/2 recruitment to plasma membrane Ca²⁺ channel Orai-1 (calcium release-activated channel (CRAC)) leads to activation of the Orai1 channel and consequent Ca²⁺ influx into MC [7]. Syk also phosphorylates adaptor proteins, LAT1 and SLP-76 both being required for PLC- γ 1/2 activation (**Fig. 1**). Another important enzyme is a small family of phosphatidylinositol 3-kinase (PI3K). For activation of PI3K, p85 adaptor subunit of PI3K interacts with the cytosolic adaptor Gab2 after Gab2 phosphorylation by Fyn or Syk [8]. p85-bound catalytic subunit p110 δ is essential for antigen-induced anaphylaxis, degranulation and cytokine production [9] (**Fig. 1**). Analysis of Fyn-deficient MCs also led to the proposal of a Lyn-independent complementary pathway for MC activation [10]. Dynamic complex formation by these adaptors and associated signaling molecules have been covered by excellent reviews [4,11-13].

These signaling events were revealed mostly by using optimal concentration of a high-affinity multivalent antigen in combination with a hapten-specific IgE (e.g., DNP₂₀₋₄₀-HSA with anti-DNP IgE). However, different antigen concentrations result in subtle differences in receptor usage of Lyn and other SFKs [14] as well as MAP kinases (MAPKs), with p38 phosphorylation requiring lower receptor occupancy than JNK1 and ERK2 phosphorylation. MAPKs phosphorylation differences are correlated with differential requirement for mRNA expression of cytokines/chemokines [15]. Mobility of IgE-Fc ϵ RI complexes are slowed to a greater extent by high concentrations than low concentrations of DNP₂₅-BSA and by higher-valency than lower-valency antigen [16]. Interestingly, stimulation with low-affinity antigen (2NP₄-Fab) of IgE anti-DNP sensitized MCs induces less degranulation, leukotriene and cytokine production, but enhanced chemokine production, compared with high-affinity antigen

(DNP₄-Fab) stimulation [17]. Receptor clusters induced by the low-affinity antigen are slower moving and larger in size than those induced by high-affinity antigen. Low-affinity stimulation induces increased Fgr (another SFK) association with FcεRI and shifts the signaling from LAT1 to LAT2. Therefore, differences in affinity, valency and concentration of antigen are translated to intracellular signaling events and different outcomes of cell activation [13].

3. Regulation of receptor-distal events in exocytosis

Cytoskeletal reorganisation

FcεRI stimulation leads to compound exocytosis releasing virtually all granular content. Such degranulation requires exquisite signal coordination between associated cytoskeletal reorganization and the membrane fusion machinery enabling lipid bilayer mixing. Visible changes in the actin cytoskeleton include cell spreading, lamellar actin ruffling and loss of cortical actin liberating the physical barrier for SG access. Actin reorganization requires Ca²⁺-influx and is coordinated by the GTPases Rac, RhoA and cdc42 [18,19], downstream targets Wiskott-Aldrich syndrome protein (WASP) promoting actin branching, WASP interacting protein (WIP) and Drebrin (DBN) all of which positively regulate degranulation [19-22]. Experiments with knockout MC indicate a negative regulatory role for the F-actin binding protein Coronin1A in degranulation. Upon FcεRI-stimulation Coronin1A becomes phosphorylated on Ser2, promoting its cytoplasmic relocation and cortex disassembly [23]. Cortical actin also represents a carrier for myosinV actin motors to capture and transport vesicles to fusion sites [24]. These differential functions are explained by a tight coupling to Ca²⁺ oscillations with SGs being captured at low Ca²⁺ and high cortical F-actin, followed by vesicle passage through the cortex when Ca²⁺ levels start to increase and SG fusion occurring at peak levels [25]. Imaging studies showed that SGs fuse at sites where actin dynamics generates circular openings to extrude granular content [26].

Long-range SG movement involves a Ca^{2+} -independent Fyn/Gab2/RhoA signaling pathway with microtubule formation being blocked in Fyn- and Gab2-deficient cells [18]. Others showed Ca^{2+} -dependency of microtubule nucleation regulated by the association of nucleating γ -tubulin with p21-activated kinase interacting exchange factor β (β PIX), and G protein-coupled receptor kinase-interacting protein (GIT) 1 affecting, respectively, negatively or positively microtubule growth [27]. SG movement towards the plus-end involved the kinesin-1 motor recruited in a PI3K-dependent manner to Ca^{2+} -binding C2 domain-containing Synaptotagmin-like protein3 (Slp3) bound to SG-localized Rab27b GTPase [28] (**Fig. 2**). The fusion accessory mammalian uncoordinated 18 (Munc18) family member Munc18-2 (STXBP2), besides its role in fusion (see below), also plays a role in microtubule coupling. Localized to SG via the Syntaxin3 (STX3) fusion protein it relocates after stimulation along microtubules into lamellipodia remaining associated with large (fused) SG [29]. Munc18-2 knockdown affected SG collapse into the plasma membrane with SGs appearing stationary docked along microtubules. In parallel, the interaction of Munc18-2 with β -tubulin decreased suggesting that Munc18-2 dynamically docks SG during microtubular transport [29].

Fusion machinery

Bilayer mixing in MCs implicates both fusion between SG and with the plasma membrane. It involves the evolutionary conserved N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) family of proteins (**Fig. 2**). Composed of target t-SNARES and vesicular v-SNARES they form via their ~60 aa SNARE-helical motifs a fusion-catalyzing tetrameric trans-SNARE complex. The first identified t-SNARE in MCs was SNAP-23. Introduction of blocking Abs into permeabilized MC blocked secretion [30]. During stimulation SNAP-23 relocates from the plasma membrane into forming cytoplasmic degranulation channels, a feature compatible with compound exocytosis. A second t-SNARE identified was STX3, which reversely relocates from SG to the plasma membrane [28,29]. Inhibition of fusion in STX3-

deficient MCs was partial, whereas no effect was seen in STX4-deficient MC [31]. Some studies found a role of plasma membrane-localized STX4 in degranulation indicating possible compensatory mechanisms [29,32,33]. Concerning v-SNAREs an ~50% inhibition of degranulation was found in vesicular associated membrane protein8 (VAMP8) knockdown or knockout MCs [34-36]. After stimulation VAMP8 relocates from SGs to the plasma membrane forming enhanced complexes with STX4 and SNAP-23 [35]. VAMP7 and VAMP2 v-SNAREs may additionally contribute [32,37], albeit for the latter no effect was observed in knockout MCs [36]. As VAMP2 formed enhanced complexes with t-SNAREs after stimulation, but only in VAMP8-deficient MC, this may suggest a certain plasticity in SNARE usage [35].

SNARE-complex formation is regulated by phosphorylation. SNAP-23 gets phosphorylated on Ser95/120 enhancing SNARE assembly [38]. This involved I κ B kinase 2/ β (IKK β 2) [39] albeit this was challenged later [40]. By contrast, phosphorylation of VAMP8 by PKC β at the interface for SNARE zippering negatively regulated fusion as supported by phosphomimetic mutants [41]. Regulatory phosphorylation was also demonstrated for STX3 whose constitutive phosphorylation on Thr14 restrains fusion [42], much like STX4 phosphorylation by a Rab3D associated kinase [43]. The STX-binding protein tomosyn-1 (STXBP5) negatively regulates fusion. As knockdown enhances degranulation, it likely blocks productive SNARE complex formation when bound to STX [44]. During stimulation tomosyn-1 becomes phosphorylated on Thr by PKC δ , inducing its stimulation-dependent dissociation from STX4 and association with STX3. The latter may represent a negative feedback regulatory mechanism.

Several SNARE accessory proteins, some receptive to changes in intracellular Ca²⁺ levels via their C2-domains, regulate the final docking, priming and fusion steps. According to the neuronal “*release of inhibition* model” fusion is triggered within milliseconds from a primed state involving a multimeric assembly of Ca²⁺-sensors [45]. Two neuronal Synaptotagmin1 (Syt1) molecules interact with a preassembled SNARE prefusion complex stabilized by

Complexin1 (CPLX1). Ca^{2+} increases induce a conformational change in Syt1 and CPLX1, which enables SNARE zippering, pulling together opposing membranes further assisted by parallel phospholipid binding of Syt1 that favors membrane curvature. As MC exocytosis does not occur in milliseconds it is likely that the neuronal prefusion complex represents a short-lived intermediate state in MCs. Clearly, MCs use CPLX and Syt isoforms. Absence of the SG-localized Ca^{2+} -sensor Syt2 and knockdown of CPLX2 markedly inhibited MC secretion [46,47].

Prior to fusion, MC SGs get docked and primed (**Fig. 2**). Docking involves Munc13-4 a multifunctional effector that interacts via a noncanonical binding motif with Rab27a/b on SGs [48]. TIRF experiments indicated that Rab27a/b-Munc13-4 complexes are necessary to dock motile SG beneath the plasma membrane; docking is lost with interaction-interrupting mutants [48]. Rab27a may additionally function in the short-range actin motility as in knockout MCs degranulation is slightly enhanced [24]. While the phenotype of Rab27a or b-deficient cells was mild, the phenotype of Munc13-4-deficient MCs led to a marked inhibition of degranulation suggesting additional essential functions [24,49]. Evidence from homologous proteins in neuronal cells [45] suggests that Munc13-4 together with Munc18-2 cooperatively promote proper SNARE assembly in the right parallel configuration [49,50] (**Fig.2**). MC express Doc2 α , another C2 domain-containing protein with MC degranulation being decreased in KO MCs [51]. Doc2 α interacts with SG-localized Munc13-4 [51] and may represent an additional Ca^{2+} -sensor assisting Munc13-4 to fulfill its docking/priming function.

4. Regulation of signaling-dependent cytokine and chemokine gene transcription in response to antigenic stimulation

IgE receptor stimulation also promotes *de novo* gene transcription. Within a few hours, activated MCs secrete a large number of newly synthesized proteins [52,53]. Whole genome

transcriptome analysis revealed that human skin MCs upregulated 260 and downregulated 84 genes after stimulation through FcεRI for two hours [54]. Mouse MC upregulated 1089 genes, and downregulated 280 genes [55]. The upregulated genes are significantly enriched in gene sets that encode signaling molecules, transcription factors, proteases and other enzymes, cell surface molecules, and cytokines/chemokines. Whereas the down regulated genes are significantly enriched in sets involved in cell proliferation, signal transduction, apoptosis or enriched in genes encoding receptors [55].

How genes detect the proximal signals triggered by antigenic stimulation is not completely understood. A number of transcription factors (TFs) are activated through phosphorylation modification after antigenic stimulation. These TFs are called signal-dependent TFs (SDTFs). SDTFs STAT5, NFAT, AP1, NFκB and EGR2 are critical in FcεRI-upregulated gene expression [56]. Our knowledge of enhancer locations to which SDTFs bind in response to antigenic stimulation is still limited. Enhancers are segments of DNA located in the non-coding regions of genes. They activate gene transcription by delivering important accessory factors to the promoter [57-59]. It is generally believed that binding of the SDTFs to enhancers activates them [60]. Activated enhancers then interact with other enhancers to form loops with promoters by bringing transcription factors and coactivators together with basic transcriptional complexes. Binding of SDTFs, other TFs and co-factors to enhancers also creates accessible regions within enhancers and recruit histone modifying enzymes to add acetyl or methyl groups to histones. Histone modifications can be used to identify potential enhancers [60]. For example, the poised enhancers can be identified by monomethylation of lysine residue 4 on histone 3 (H3K4me1) and active enhancers can be marked by acetylation of lysine residue 27 on histone 3 (H3K27ac) modification [61-64]. The accessible regions can be located by ATAC-seq [65].

A recent study used the next generation sequencing method to examine enhancers that can respond to antigenic or IL-33 stimulation and found that Ca²⁺-dependent open chromatin

(COC) domains within enhancers in human blood-derived MCs in response to FcεRI or IL-33 stimulation [66]. TF binding motif analysis revealed enrichment of ATF3, BATF and AP1-binding sites in COC domains (**Fig. 3**). Whether ATF3, BATF and AP-1 indeed play a critical role in promoting gene transcription in response to FcεRI-crosslinking awaits further experiments that knockout or knockdown ATF3, BATF and AP-1 expression in MCs. Recent data show that GATA2 binds to super-enhancers in resting mouse MCs prior to FcεRI-stimulation. Deletion of the *Gata2* gene in mouse MCs abolished the upregulation of gene transcription in response to IgE receptor crosslinking, suggesting that GATA2 is essential in recruiting SDTFs to FcεRI-induced enhancers [55]. TF binding motif analysis revealed that GATA2, EGR2 and AP1-binding sites are enriched in the FcεRI crosslinking-induced enhancers (**Fig 3**). Further experiments are underway to examine the role of EGR2 and AP1 in promoting FcεRI-induced gene transcription. We also found that FcεRI-induced enhancers can cooperate with constitutively active enhancers to generate maximal gene transcription (**Fig 3**) [67].

Figures legends

Figure 1. FcεRI signaling pathways that lead to degranulation and de novo synthesis of eicosanoids, cytokines and chemokines. Aggregation of IgE-occupied FcεRI induces activation of the SFK Lyn, which phosphorylates tyrosine residues of ITAM motifs (indicated yellow rectangles) of β and γ chains. Syk is recruited to the phosphorylated γ chains. Activation of Syk leads to phosphorylation of LAT1 and LAT2. These proteins serve as scaffolds for multimolecular signaling complexes for the binding of adaptor molecules such as Gads, Grb2, SLP-76 and Shc; guanosine triphosphate exchangers such as Sos and Vav; and the enzymes, PLC-γ2 and PLC-γ1. PLC-γ catalyzes the hydrolysis of PIP₂ to DAG and IP₃, which results in the activation of PKCs and the release of intracellular Ca²⁺, respectively. These signals lead to degranulation. Phosphatidylinositol 3-kinase (PI3K) is activated when its adaptor subunit p85 is bound by Gab2 phosphorylated by Fyn or/and Syk. The generation of phosphatidylinositol 3,4,5-trisphosphate (PIP₃) by PI3K p110δ catalytic subunit leads to the recruitment of PH domain-containing molecules such as Btk, PDK1, and Akt. Sos- and Vav-regulated GDP-GTP exchange of Ras, Rac and Rho leads to the activation of MAPK cascades. Erk signaling regulates phospholipase A₂ activation, leading to the generation of eicosanoids. Increased Ca²⁺ concentration activates Ca²⁺/calmodulin-dependent serine phosphatase calcineurin. Calcineurin dephosphorylates NFAT and allow it to translocate into the nucleus. FcεRI stimulation leads to

degradation of I κ B and allows nuclear localization of NF- κ B. These transcription factors regulate the transcription of cytokines and other proinflammatory factors.

Figure 2. The SG transport and fusion machinery in mast cells. For reason of simplicity the docking and fusion steps are detailed only at the PM. Note however, that in MC fusion between SG is part of the compound exocytosis process during which relevant actors redistribute to the plasma membrane and inversely to the SGs. Long-range transport towards the plus-end of microtubules involves the kinesin-1 motor recruited in a PI3K-dependent manner to Synaptotagmin-like protein 3 (Slp3) (containing Ca²⁺-binding C2 domains) that binds via its Slp homology domain (SHD) domain to the SG-localized Rab27b GTPase. The fusion promoting protein Munc18-2 also participates in the interaction with β -tubulin via a so far unknown effector. When reaching the plasma membrane actin disassembly (not shown) allows docking of SG at the plasma membrane involving several fusion accessory proteins such as Munc13-4 bound to Rab27a/b assisted by Doc2 some of which contain Ca²⁺/phospholipid binding C2 domains. This allows fusion priming and loose SNARE-zippering, which in MC likely represents a short-lived intermediate state (not depicted) followed by the assembly of the stable trans-SNARE complex that drives membrane bilayer mixing and is triggered at high Ca²⁺ concentration by conformational rearrangements of C2-domain containing Syt2 and CPLX2 and the coordinated action of Munc18-2 and Munc13-4 that coordinate the correct assembly of the SNARE complex.

Fig 3. The induced enhancers detect signals and cooperate with constitutively active enhancers to promote gene transcription. Signal-dependent transcription factors are activated through phosphorylation by kinases that are activated by aggregation of Fc ϵ RI bound with IgE and their cognate antigens. These TFs, such as pAP-1, pEGR2, pATF3 and pBATF bind to the consensus DNA binding motifs in the antigenic stimulation-induced enhancers to activate the induced enhancers, which then interact with constitutively active enhancers, forming a higher order of transcriptional complex with promoters to promote gene transcription in response to antigenic stimulation. TF, transcription factor; MLL3/4, histone-lysine N-methyltransferase, H3 lysine-4 specific; P300, histone acetyltransferase p300; Pol II, RNA polymerase II; p, phosphorylation. Green dot, monomethylation of lysine residue 4 on histone 3 (H3K4me1); red dot, acetylation of lysine residue 27 on histone 3 (H3K27ac) modification.

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Conflict of interest statement:

All authors declare that they do not have any conflict of interest.

References and recommended readings

1. Kinet JP: **The high-affinity IgE receptor (Fc epsilon RI): from physiology to pathology.** *Annu Rev Immunol* 1999, **17**:931-972.
2. Pribluda VS, Pribluda C, Metzger H: **Transphosphorylation as the mechanism by which the high-affinity receptor for IgE is phosphorylated upon aggregation.** *Proc Natl Acad Sci U S A* 1994, **91**:11246-11250.
3. Paolini R, Jouvin MH, Kinet JP: **Phosphorylation and dephosphorylation of the high-affinity receptor for immunoglobulin E immediately after receptor engagement and disengagement.** *Nature* 1991, **353**:855-858.
4. Siraganian RP, de Castro RO, Barbu EA, Zhang J: **Mast cell signaling: the role of protein tyrosine kinase Syk, its activation and screening methods for new pathway participants.** *FEBS Lett* 2010, **584**:4933-4940.
5. Costello PS, Turner M, Walters AE, Cunningham CN, Bauer PH, Downward J, Tybulewicz VL: **Critical role for the tyrosine kinase Syk in signalling through the high affinity IgE receptor of mast cells.** *Oncogene* 1996, **13**:2595-2605.
6. Zhang J, Berenstein EH, Evans RL, Siraganian RP: **Transfection of Syk protein tyrosine kinase reconstitutes high affinity IgE receptor-mediated degranulation in a Syk-negative variant of rat basophilic leukemia RBL-2H3 cells.** *J Exp Med* 1996, **184**:71-79.
7. Ma HT, Beaven MA: **Regulation of Ca²⁺ signaling with particular focus on mast cells.** *Crit Rev Immunol* 2009, **29**:155-186.
8. Yu M, Lowell CA, Neel BG, Gu H: **Scaffolding adapter Grb2-associated binder 2 requires Syk to transmit signals from FcepsilonRI.** *J Immunol* 2006, **176**:2421-2429.
9. Ali K, Bilancio A, Thomas M, Pearce W, Gilfillan AM, Tkaczyk C, Kuehn N, Gray A, Giddings J, Peskett E, et al.: **Essential role for the p110delta phosphoinositide 3-kinase in the allergic response.** *Nature* 2004, **431**:1007-1011.
10. Parravicini V, Gadina M, Kovarova M, Odom S, Gonzalez-Espinosa C, Furumoto Y, Saitoh S, Samelson LE, O'Shea JJ, Rivera J: **Fyn kinase initiates complementary signals required for IgE-dependent mast cell degranulation.** *Nat Immunol* 2002, **3**:741-748.
11. Kambayashi T, Koretzky GA: **Proximal signaling events in Fc epsilon RI-mediated mast cell activation.** *J Allergy Clin Immunol* 2007, **119**:544-552; quiz 553-544.
12. Metcalfe DD, Peavy RD, Gilfillan AM: **Mechanisms of mast cell signaling in anaphylaxis.** *J Allergy Clin Immunol* 2009, **124**:639-646; quiz 647-638.
13. Suzuki R: **The Emerging Picture of Mast Cell Activation: The Complex Regulatory Network of High-Affinity Receptor for Immunoglobulin E Signaling.** *Biol Pharm Bull* 2017, **40**:1828-1832.
14. Xiao W, Nishimoto H, Hong H, Kitauro J, Nunomura S, Maeda-Yamamoto M, Kawakami Y, Lowell CA, Ra C, Kawakami T: **Positive and negative regulation of mast cell activation by Lyn via the FcepsilonRI.** *J Immunol* 2005, **175**:6885-6892.
15. Gonzalez-Espinosa C, Odom S, Olivera A, Hobson JP, Martinez ME, Oliveira-Dos-Santos A, Barra L, Spiegel S, Penninger JM, Rivera J: **Preferential signaling and induction of allergy-promoting lymphokines upon weak stimulation of the high affinity IgE receptor on mast cells.** *J Exp Med* 2003, **197**:1453-1465.

16. Andrews NL, Pfeiffer JR, Martinez AM, Haaland DM, Davis RW, Kawakami T, Oliver JM, Wilson BS, Lidke DS: **Small, mobile FcεRI receptor aggregates are signaling competent.** *Immunity* 2009, **31**:469-479.
17. Suzuki R, Leach S, Liu W, Ralston E, Scheffel J, Zhang W, Lowell CA, Rivera J: **Molecular editing of cellular responses by the high-affinity receptor for IgE.** *Science* 2014, **343**:1021-1025.
- .. Study showing that affinity of antigen to IgE is translated to different in vitro and in vivo outcomes of FcεRI signaling.
18. Nishida K, Yamasaki S, Ito Y, Kabu K, Hattori K, Tezuka T, Nishizumi H, Kitamura D, Goitsuka R, Geha RS, et al.: **Fc{epsilon}RI-mediated mast cell degranulation requires calcium-independent microtubule-dependent translocation of granules to the plasma membrane.** *J Cell Biol* 2005, **170**:115-126.
19. Draber P, Sulimenko V, Draberova E: **Cytoskeleton in mast cell signaling.** *Front Immunol* 2012, **3**:130.
20. Pivniouk VI, Snapper SB, Kettner A, Alenius H, Laouini D, Falet H, Hartwig J, Alt FW, Geha RS: **Impaired signaling via the high-affinity IgE receptor in Wiskott-Aldrich syndrome protein-deficient mast cells.** *Int Immunol* 2003, **15**:1431-1440.
21. Kettner A, Kumar L, Anton IM, Sasahara Y, de la Fuente M, Pivniouk VI, Falet H, Hartwig JH, Geha RS: **WIP regulates signaling via the high affinity receptor for immunoglobulin E in mast cells.** *J Exp Med* 2004, **199**:357-368.
22. Law M, Lee Y, Morales JL, Ning G, Huang W, Pabon J, Kannan AK, Jeong AR, Wood A, Carter C, et al.: **Cutting Edge: Drebrin-Regulated Actin Dynamics Regulate IgE-Dependent Mast Cell Activation and Allergic Responses.** *J Immunol* 2015, **195**:426-430.
23. Foger N, Jenckel A, Orinska Z, Lee KH, Chan AC, Bulfone-Paus S: **Differential regulation of mast cell degranulation versus cytokine secretion by the actin regulatory proteins Coronin1a and Coronin1b.** *J Exp Med* 2011, **208**:1777-1787.
24. Singh RK, Mizuno K, Wasmeier C, Wavre-Shapton ST, Recchi C, Catz SD, Futter C, Tolmachova T, Hume AN, Seabra MC: **Distinct and opposing roles for Rab27a/Mlph/MyoVa and Rab27b/Munc13-4 in mast cell secretion.** *FEBS J* 2013, **280**:892-903.
25. Wollman R, Meyer T: **Coordinated oscillations in cortical actin and Ca²⁺ correlate with cycles of vesicle secretion.** *Nat Cell Biol* 2012, **14**:1261-1269.
- .. Study showing that cytoskeletal reorganization necessary for degranulation is tightly coupled to Ca²⁺ oscillations. SGs are captured at high cortical F-actin and low Ca²⁺ followed by vesicle passage through the cortex with increasing Ca²⁺ levels and SG fusion occurring at peak levels
26. Colin-York H, Li D, Korobchevskaya K, Chang VT, Betzig E, Eggeling C, Fritzsche M: **Cytoskeletal actin patterns shape mast cell activation.** *Commun Biol* 2019, **2**:93.
27. Sulimenko V, Hajkova Z, Cernohorska M, Sulimenko T, Sladkova V, Draberova L, Vinopal S, Draberova E, Draber P: **Microtubule nucleation in mouse bone marrow-derived mast cells is regulated by the concerted action of GIT1/betaPIX proteins and calcium.** *J Immunol* 2015, **194**:4099-4111.
28. Munoz I, Danelli L, Claver J, Goudin N, Kurowska M, Madera-Salcedo IK, Huang JD, Fischer A, Gonzalez-Espinosa C, de Saint Basile G, et al.: **Kinesin-1 controls mast cell degranulation and anaphylaxis through PI3K-dependent recruitment to the granular Slp3/Rab27b complex.** *J Cell Biol* 2016, **215**:203-216.

- .. Study indicating that long-range SG transport involves kinesin-1 that gets coupled to SG via a SLP3/Rab27b complex in a PI3Kinase-dependent manner.
- 29. Brochetta C, Suzuki R, Vita F, Soranzo MR, Claver J, Madjene LC, Attout T, Vitte J, Varin-Blank N, Zabucchi G, et al.: **Munc18-2 and syntaxin 3 control distinct essential steps in mast cell degranulation.** *J Immunol* 2014, **192**:41-51.
- 30. Guo Z, Turner C, Castle D: **Relocation of the t-SNARE SNAP-23 from lamellipodia-like cell surface projections regulates compound exocytosis in mast cells.** *Cell* 1998, **94**:537-548.
- 31. Sanchez E, Gonzalez EA, Moreno DS, Cardenas RA, Ramos MA, Davalos AJ, Manllo J, Rodarte AI, Petrova Y, Moreira DC, et al.: **Syntaxin 3, but not syntaxin 4, is required for mast cell-regulated exocytosis, where it plays a primary role mediating compound exocytosis.** *J Biol Chem* 2019, **294**:3012-3023.
- 32. Woska JR, Jr., Gillespie ME: **Small-interfering RNA-mediated identification and regulation of the ternary SNARE complex mediating RBL-2H3 mast cell degranulation.** *Scand J Immunol* 2011, **73**:8-17.
- 33. Yang Y, Kong B, Jung Y, Park JB, Oh JM, Hwang J, Cho JY, Kweon DH: **Soluble N-Ethylmaleimide-Sensitive Factor Attachment Protein Receptor-Derived Peptides for Regulation of Mast Cell Degranulation.** *Front Immunol* 2018, **9**:725.
- 34. Lippert U, Ferrari DM, Jahn R: **Endobrevin/VAMP8 mediates exocytotic release of hexosaminidase from rat basophilic leukaemia cells.** *FEBS Lett* 2007, **581**:3479-3484.
- 35. Tiwari N, Wang CC, Brochetta C, Ke G, Vita F, Qi Z, Rivera J, Soranzo MR, Zabucchi G, Hong W, et al.: **VAMP-8 segregates mast cell-preformed mediator exocytosis from cytokine trafficking pathways.** *Blood* 2008, **111**:3665-3674.
- 36. Puri N, Roche PA: **Mast cells possess distinct secretory granule subsets whose exocytosis is regulated by different SNARE isoforms.** *Proc Natl Acad Sci U S A* 2008, **105**:2580-2585.
- 37. Sander LE, Frank SP, Bolat S, Blank U, Galli T, Bigalke H, Bischoff SC, Lorentz A: **Vesicle associated membrane protein (VAMP)-7 and VAMP-8, but not VAMP-2 or VAMP-3, are required for activation-induced degranulation of mature human mast cells.** *Eur J Immunol* 2008, **38**:855-863.
- 38. Hepp R, Puri N, Hohenstein AC, Crawford GL, Whiteheart SW, Roche PA: **Phosphorylation of SNAP-23 regulates exocytosis from mast cells.** *J Biol Chem* 2005, **280**:6610-6620.
- 39. Suzuki K, Verma IM: **Phosphorylation of SNAP-23 by I κ B kinase 2 regulates mast cell degranulation.** *Cell* 2008, **134**:485-495.
- 40. Peschke K, Weitzmann A, Heger K, Behrendt R, Schubert N, Scholten J, Voehringer D, Hartmann K, Dudeck A, Schmidt-Supprian M, et al.: **I κ B kinase 2 is essential for IgE-induced mast cell de novo cytokine production but not for degranulation.** *Cell Rep* 2014, **8**:1300-1307.
- 41. Malmersjo S, Di Palma S, Diao J, Lai Y, Pfuetzner RA, Wang AL, McMahon MA, Hayer A, Porteus M, Bodenmiller B, et al.: **Phosphorylation of residues inside the SNARE complex suppresses secretory vesicle fusion.** *EMBO J* 2016, **35**:1810-1821.
- 42. Tadokoro S, Shibata T, Inoh Y, Amano T, Nakanishi M, Hirashima N, Utsunomiya-Tate N: **Phosphorylation of syntaxin-3 at Thr 14 negatively regulates exocytosis in RBL-2H3 mast cells.** *Cell Biol Int* 2016, **40**:589-596.

43. Pombo I, Martin-Verdeaux S, Iannascoli B, Le Mao J, Deriano L, Rivera J, Blank U: **IgE receptor type I-dependent regulation of a Rab3D-associated kinase. A possible link in the calcium-dependent assembly of SNARE complexes.** *J Biol Chem* 2001, **12**:12.
44. Madera-Salcedo IK, Danelli L, Tiwari N, Dema B, Pacreau E, Vibhushan S, Birnbaum J, Agabriel C, Liabeuf V, Klingebiel C, et al.: **Tomosyn functions as a PKCdelta-regulated fusion clamp in mast cell degranulation.** *Sci Signal* 2018, **11**.
- .. Study showing that the STX-binding protein tomosyn-1 (STXBP5) regulates the availability of fusion-competent STX proteins. STX-binding of tomosyn-1 is regulated by Thr phosphorylation by PKCδ.
45. Brunger AT, Choi UB, Lai Y, Leitz J, White KI, Zhou Q: **The pre-synaptic fusion machinery.** *Curr Opin Struct Biol* 2019, **54**:179-188.
46. Tadokoro S, Nakanishi M, Hirashima N: **Complexin II facilitates exocytotic release in mast cells by enhancing Ca²⁺ sensitivity of the fusion process.** *J Cell Sci* 2005, **118**:2239-2246.
47. Melicoff E, Sansores-Garcia L, Gomez A, Moreira DC, Datta P, Thakur P, Petrova Y, Siddiqi T, Murthy JN, Dickey BF, et al.: **Synaptotagmin-2 controls regulated exocytosis but not other secretory responses of mast cells.** *J Biol Chem* 2009, **284**:19445-19451.
48. Elstak ED, Neeft M, Nehme NT, Voortman J, Cheung M, Goodarzifard M, Gerritsen HC, van Bergen En Henegouwen PM, Callebaut I, de Saint Basile G, et al.: **The munc13-4-rab27 complex is specifically required for tethering secretory lysosomes at the plasma membrane.** *Blood* 2011, **118**:1570-1578.
49. Rodarte EM, Ramos MA, Davalos AJ, Moreira DC, Moreno DS, Cardenas EI, Rodarte AI, Petrova Y, Molina S, Rendon LE, et al.: **Munc13 proteins control regulated exocytosis in mast cells.** *J Biol Chem* 2018, **293**:345-358.
50. Gutierrez BA, Chavez MA, Rodarte AI, Ramos MA, Dominguez A, Petrova Y, Davalos AJ, Costa RM, Elizondo R, Tuvim MJ, et al.: **Munc18-2, but not Munc18-1 or Munc18-3, controls compound and single-vesicle-regulated exocytosis in mast cells.** *J Biol Chem* 2018, **293**:7148-7159.
- . Complete analysis of all Munc18 isoforms in knockout models show that it is the Munc18-2 isoform, which is the relevant SNARE accessory protein in mast cells
51. Higashio H, Nishimura N, Ishizaki H, Miyoshi J, Orita S, Sakane A, Sasaki T: **Doc2 alpha and Munc13-4 regulate Ca(2+) -dependent secretory lysosome exocytosis in mast cells.** *J Immunol* 2008, **180**:4774-4784.
52. Mukai K, Tsai M, Saito H, Galli SJ: **Mast cells as sources of cytokines, chemokines, and growth factors.** *Immunol Rev* 2018, **282**:121-150.
53. Draber P, Halova I, Polakovicova I, Kawakami T: **Signal transduction and chemotaxis in mast cells.** *Eur J Pharmacol* 2016, **778**:11-23.
54. Motakis E, Guhl S, Ishizu Y, Itoh M, Kawaji H, de Hoon M, Lassmann T, Carninci P, Hayashizaki Y, Zuberbier T, et al.: **Redefinition of the human mast cell transcriptome by deep-CAGE sequencing.** *Blood* 2014, **123**:e58-67.
55. Li Y, Gao J, Kamran M, Harmacek L, Danhorn T, Leach SM, O'Connor BP, Hagman JR, Huang H: **GATA2 regulates mast cell identity and responsiveness to antigenic stimulation by promoting chromatin remodeling at super-enhancers.** *Nat Commun* 2021, **12**:494.
- .. This paper demonstrates that GATA2 promotes chromatin accessibility at the super-enhancers of MC mast cell identity genes and primes both typical and super-enhancers at genes that respond to antigenic stimulation in mouse mast cells.

56. Cildir G, Pant H, Lopez AF, Tergaonkar V: **The transcriptional program, functional heterogeneity, and clinical targeting of mast cells.** *J Exp Med* 2017, **214**:2491-2506.
57. Schaffner W: **Enhancers, enhancers - from their discovery to today's universe of transcription enhancers.** *Biol Chem* 2015, **396**:311-327.
58. Schoenfelder S, Fraser P: **Long-range enhancer-promoter contacts in gene expression control.** *Nat Rev Genet* 2019, **20**:437-455.
59. Spitz F, Furlong EE: **Transcription factors: from enhancer binding to developmental control.** *Nat Rev Genet* 2012, **13**:613-626.
60. Rosenfeld MG, Lunyak VV, Glass CK: **Sensors and signals: a coactivator/corepressor/epigenetic code for integrating signal-dependent programs of transcriptional response.** *Genes Dev* 2006, **20**:1405-1428.
61. Calo E, Wysocka J: **Modification of enhancer chromatin: what, how, and why?** *Mol Cell* 2013, **49**:825-837.
62. Creighton MP, Cheng AW, Welstead GG, Kooistra T, Carey BW, Steine EJ, Hanna J, Lodato MA, Frampton GM, Sharp PA, et al.: **Histone H3K27ac separates active from poised enhancers and predicts developmental state.** *Proc Natl Acad Sci U S A* 2010, **107**:21931-21936.
63. Heintzman ND, Stuart RK, Hon G, Fu Y, Ching CW, Hawkins RD, Barrera LO, Van Calcar S, Qu C, Ching KA, et al.: **Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome.** *Nat Genet* 2007, **39**:311-318.
64. Rada-Iglesias A, Bajpai R, Swigut T, Brugmann SA, Flynn RA, Wysocka J: **A unique chromatin signature uncovers early developmental enhancers in humans.** *Nature* 2011, **470**:279-283.
65. Corces MR, Trevino AE, Hamilton EG, Greenside PG, Sinnott-Armstrong NA, Vesuna S, Satpathy AT, Rubin AJ, Montine KS, Wu B, et al.: **An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues.** *Nat Methods* 2017, **14**:959-962.
66. Cildir G, Toubia J, Yip KH, Zhou M, Pant H, Hissaria P, Zhang J, Hong W, Robinson N, Grimbaldston MA, et al.: **Genome-wide Analyses of Chromatin State in Human Mast Cells Reveal Molecular Drivers and Mediators of Allergic and Inflammatory Diseases.** *Immunity* 2019, **51**:949-965 e946.
- .. this paper describes that antigenic stimulation induces formation of new enhancers that depend on Ca²⁺ signaling in human mast cells.
67. Kamran M, Liang J, Liu B, Li Y, Gao J, Keating A, Mohamed F, Dai S, Reinhardt R, Jiong Y, et al.: **The Clusters of Transcription Factors NFATC2, STAT5, GATA2, AP1, RUNX1 and EGR2 Binding Sites at the Induced IL13 Enhancers Mediate IL13 Gene Transcription in Response to Antigenic Stimulation.** *J Immunol* 2020, **205**:3311-3318.

Conflict of interest statement:

All authors declare that the do not have any conflict of interest.

Figure 1

Figure 2