**Review**

The Emerging Picture of Mast Cell Activation: The Complex Regulatory Network of High-Affinity Receptor for Immunoglobulin E Signaling

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Received June 8, 2017

It is now well known that immunoglobulin E (IgE) and mast cells (MCs) are important participants in allergic diseases. MCs contain electron-dense secretory granules which are filled with inflammatory mediators. The interaction of an allergen (antigen) with an antigen-specific IgE-bound high-affinity receptor for IgE (FcεRI) is an essential step in MC activation as well as subsequent downstream signaling events. What we know is that IgE and FcεRI activate a complex regulatory network (i.e., signaling molecules and messengers) that governs both the type of MC activation and the symptoms of allergic disease. This review focuses on recent discoveries that shed new light on FcεRI signaling networks, holding promise for the development of new therapeutic solutions in the treatment of allergic diseases.

**Key words** allergy; mast cell; immunoglobulin E; high-affinity immunoglobulin E receptor (Fc epsilon RI)

1. INTRODUCTION

Mast cells (MCs) are bone marrow-derived hematopoietic immune cells. MCs are found in tissues subject to contact with outside environmental stimuli, such as the skin, gastrointestinal tract, and bronchus. MCs are phenotypically classified into two distinct subpopulations: mucosal MCs (MMCs) and connective tissue MCs (CTMCs). These populations differ in location, ultrastructure, and function. Both types of MCs express the high-affinity immunoglobulin E (IgE) receptor (FcεRI) on their surfaces. The majority of MCs are found to be in perivascular distribution within tissues in rodents and in humans. It was previously considered that antigen-specific IgE binds passively to FcεRI on MCs. However, Cheng et al. showed novel binding mechanisms in MCs in connective or mucosal tissues: these MCs acquired IgE by extending cell processes into vessels to extract IgE from the blood. After sensitization with IgE, cross-linking of IgE-bound FcεRI by multivalent antigen activates the MCs. Activated MCs secrete preformed and newly synthesized mediators such as histamine, cytokines, chemokines, and lipid mediators, and subsequently recruit immune cells (e.g., eosinophils, neutrophils, natural killer cells, and T cells) at the site of inflammation. This review focuses on recent studies of early signaling events, such as FcεRI aggregation and activation, spatial regulation, and the complex function of second messengers in MCs.

2. MECHANISM OF FcεRI ACTIVATION

FcεRI is expressed by MCs and basophils in both rodents and humans. It consists of a tetrameric protein complex. The α-subunit is a transmembrane protein. The β- and γ-subunits are tetraspan transmembrane protein and a disulfide-linked homodimeric protein, respectively. The α-subunit is necessary for binding IgE. The stoichiometry of the IgE:FcεRI complex is 1:1. The α-subunit is a glycoprotein; the glycosylation site in the α-subunit seems to be important for protein folding in the endoplasmic reticulum (ER) and for transporting it to the cell membrane. The β- and γ-subunits each possess an immunoreceptor tyrosine-based activation motif (ITAM) that plays important roles in MC signal transduction. The ITAM consensus sequence comprises (D/E) xxYxxL-(x5,11)-YxxL (L/I), and the phosphorylation of tyrosine residues in ITAMs is involved in the transduction of downstream signals. The γ-subunit ITAM (Yγ1TGLNTRSQETY225ETL) contains two canonical tyrosine residues (Yγ17, Yγ55), and the β-subunit ITAM (Yβ3EELHVY229SPIY232SEL) has another non-canonical tyrosine residue (Yβ25) between two canonical tyrosine residues (Yβ219 and Yβ230). The N-terminal canonical tyrosine residue (Yγ17) in the β-subunit, but not the C-terminal tyrosine residue (Yγ55), is important for binding with Lyn (a member of the Src family of protein tyrosine kinases). After FcεRI engagement, Lyn phosphorylates tyrosine residues in the β- and γ-subunits. A mutational analysis of these tyrosine residues in β-subunit ITAM demonstrated that the non-canonical tyrosine residue (Yγ25) has an inhibitory role in cytokine release, whereas there is no effect on the degranulation response. The tyrosine residues in the γ-subunit also play different roles in regulating MC functions. It has been recognized that both the tyrosine residues (Yγ17, Yγ55) in γ-subunit ITAM serve as docking sites for spleen tyrosine kinase (Syk). Our finding revealed that the C-terminal canonical tyrosine residue (Yγ55) plays an important role in regulating Syk activation, since tyrosine residue (Yγ55) is a potential target of preferential dephosphorylation by Src homology region 2 domain-containing phosphatase (SHP)-1 and SHP-2, and thus controls the extent of Syk activation. In addition, the phosphorylation at tyrosine residue (Yβ25) is greater in low-affinity ligand-stimulated FcεRI interactions relative to high-affinity ligand-stimulated FcεRI interactions, a topic that will be revisited in the next section. After activation of Syk, Syk phosphorylates a number of its target molecules, including the linker of activated T cells (LAT1) and the linker for the activation of T-cells family member 2 (LAT2). LAT1 phosphorylation activates phospholipase Cγ (PLCγ), and subsequently promotes calcium signals in MCs, inducing their degranulation and the release of inflammatory mediators that could affect other immune cells and tissues.
induce allergic disease.

3. COMPLEXITY OF FceRI ACTIVATION; SPATIAL REGULATION

Early studies from Fewtrell and Metzger in 1980, using chemically oligomerized IgE, demonstrated that MC degranulation could be elicited by an IgE aggregate as small as a dimer, but that monomers of IgE failed to elicit a response. The use of a monovalent hapten (dinitrophenyl (DNP)-Lys) diminished FcεRI aggregation by disrupting interaction between the multivalent antigen (DNP10–40-human serum albumin (HSA)) and the DNP-specific IgE. This inhibition by monovalent hapten abrogates FceRI phosphorylation and prevents effective responses (i.e., degranulation). These findings argue that the aggregation of FceRI has an essential role in the induction of MC degranulation and cytokine/chemokine release.

However, in the past two decades, there has been considerable work demonstrating that monomeric IgE binding to FceRI on nonhuman MCs can induce various responses, such as prolonged MC survival under suboptimal growth factor conditions, cytokine production, and some detectable molecular signals. The formation of FceRI aggregates may be important in MC signal transduction; nevertheless, the effects of “monomeric IgE” on FceRI aggregation and MC activation may be different. In addition, whether monomeric IgE in human MCs shows similar effects remains unknown. The relevance of monomeric IgE to human MC pathophysiology has yet to be elucidated.

Sil et al., using trivalent ligands with rigid DNA spacers of varying lengths, have studied the relationship between spacer length of the ligand and activation of FceRI. The results showed an inverse correlation between spacer length and FceRI phosphorylation. Varying the concentration of antigens also has a marked effect on cellular responses, such as degranulation and cytokine production. Indeed, suboptimal antigen concentration elicits a low rate of the kinds of MC responses observed with a greater antigen concentration. On the other hand, antigen hyperconcentration also seems to diminish these MC responses. These data suggest the need for an optimum antigen concentration to achieve a sufficient degree of FceRI aggregation and to induce subsequent molecular events that are required for MC effector responses. However, differences in the extent of FceRI occupancy with antigen-specific IgE seem to affect different MC responses. Gonzalez-Espinoza et al. have demonstrated that the production of chemokines (e.g., monocyte chemoattractant protein-1 (CCL2), macrophage inflammatory protein-1α (CCL3), and macrophage inflammatory protein-1β (CCL4)) requires very low FceRI occupancy by antigen-specific IgE, thus suggesting that weak clustering of FceRI might suffice for eliciting this kind of response. Of note, low receptor occupancy is accompanied by the induction of p38 mitogen-activated protein kinase (p38 MAPK) phosphorylation, whereas high receptor occupancy induces c-Jun NH2-terminal kinase (JNK1) and extracellular signal-regulated kinase 2 (ERK2) phosphorylation. These data strongly suggest that FceRI might be capable of receiving signal intensity information, which may distinguish intensity-specific signaling pathways resulting in appropriate MC responses.

4. ANTIGEN AFFINITY IN FceRI ACTIVATION

IgE binding to FceRI occurs with very high affinity ($K_a \geq 10^{10} \text{M}^{-1}$), but it is reversible with a half-life of $>6\text{d}$. It is well known that different allergic patients can produce IgE antibodies that recognize the same antigen with different affinities. It has long been questioned how such differences in the affinity of antigens for IgE are interpreted by FceRI, and what cellular outcomes may occur in MCs. To explore these questions, we utilized two previously-described antigens that have different affinities for DNP-specific IgE antibody, (2-nitrophenyl (2NP) and DNP), conjugated to an immunoglobulin Fab fragment as the carrier protein. To assess downstream molecular signaling, we used varying concentrations of high- or low-affinity antigens that caused similar levels and kinetics of FceRI phosphorylation (a 100-fold greater concentration of low-affinity antigen relative to the high-affinity antigen). Under these conditions, the lower concentration of high-affinity antigen induced degranulation and cytokine (e.g., tumor necrosis factor α (TNF-α), interleukin (IL)-6, and IL-13) release. In contrast, the higher concentration of low-affinity antigen led to significantly enhanced chemokine (e.g., CCL2, CCL3, and CCL4) release, while not inducing degranulation or cytokine release (Fig. 1). To investigate the mechanisms underlying these different outcomes, we studied the dynamics of FceRI aggregation by total internal reflection fluorescence microscopy. Small and highly mobile FceRI aggregates were observed under high-affinity antigen stimulation, whereas large and less mobile receptor aggregates were observed under low-affinity antigen stimulation (Fig. 1). We also investigated signal transduction under stimulation with these different affinity antigens. Whereas the association of Lyn with FceRI did not differ, the association of another Src family of protein tyrosine kinases, Fgr, with FceRI was increased under low-affinity antigen stim-

**Biography**

Dr. Ryo Suzuki was born in Miyagi, Japan. He earned his Ph.D. in Pharmaceutical Sciences from Nagoya City University in 2001. Subsequently, he became a Research Resident in the Department of Dementia Research, National Institute of Longevity Sciences (Aichi, Japan). He became Lecturer in the Graduate School of Pharmaceutical Sciences, Nagoya City University, in 2002. He was a Visiting Fellow at the National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health (Maryland, U.S.A.) in 2005, and was promoted to Research Fellow in 2008. He became an Assistant Professor in the Graduate School of Pharmaceutical Sciences, Nagoya City University in 2014. In 2017, he was promoted to full professor in the Faculty of Pharmaceutical Sciences, Institute of Medical, Pharmaceutical, and Health Sciences, Kanazawa University. His major research interest centers on the molecular and cellular mechanisms of allergic diseases.
ulation relative to high-affinity antigen stimulation.\textsuperscript{20} We also observed that low-affinity antigen phosphorylated LAT2 preferentially to LAT1, whereas high-affinity antigen significantly phosphorylated LAT1.\textsuperscript{20} These findings demonstrate that the balance in phosphorylation of the two related adaptor proteins, LAT1 and LAT2, is dependent on antigen affinity-dependent signaling events.

We further investigated the physiological effects of the different responses to these antigens. To address these questions, we utilized a mouse model of passive cutaneous anaphylaxis. This mouse model is based on the elicitation of MC responses locally at the site of inflammation, and allows us to measure physiological differences in response to high- or low-affinity antigens \textit{in vivo}. Both high- and low-affinity antigens led to similar levels of ear swelling, dermal thickening, and numbers of immune cells infiltrating the site of inflammation. However, the types of infiltrating cells differed depending on whether the antigen used was high- or low-affinity. Whereas neutrophils were the dominant cell type infiltrating under a high-affinity antigen challenge, monocyte/macrophages were more abundant with the low-affinity antigen challenge.\textsuperscript{20} Our findings argue that differences in the affinity of an antigen for an IgE antibody might be interpreted by FcεRI, resulting in changes in the immune response such as mediator release and immune cell recruitment. The physiological relevance of the differences in immune cell recruitment is still unclear. One speculation is that this may cause different symptoms in allergic disease.

5. SECOND MESSENGERS

5.1. Calcium Signals Ca\textsuperscript{2+} signals are essential to diverse cellular functions including differentiation, cell proliferation, cell death, and gene transcription. In MC, Ca\textsuperscript{2+} signals are essential for degranulation and cytokine release. After FcεRI engagement, signals are initiated by the PLC\textgamma mediated production of inositol 1,4,5-trisphosphate (IP\textgamma), resulting in the release of Ca\textsuperscript{2+} from intracellular stores (e.g., the ER). Depletion of Ca\textsuperscript{2+} from the ER activates Ca\textsuperscript{2+} channels on the cell membrane and induces an influx of Ca\textsuperscript{2+} from the extracellular space. This is referred to as store-operated calcium entry (SOCE). The key component of SOCE, the Orai-1 channel, was identified by single-nucleotide polymorphism array and RNA interference (RNAi) screening in 2006 by different groups.\textsuperscript{21,22} Other Orai isoforms (i.e., Orai-2 and Orai-3) have been identified based on their homology with the Orai gene.\textsuperscript{23} Further, the stromal interaction molecule (STIM)-1 and STIM-2, Ca\textsuperscript{2+}-sensors located in the ER, which interact with the Orai channel in the plasma membrane, have also been identified.\textsuperscript{23,24} As ER Ca\textsuperscript{2+} stores empty, STIM-1 and STIM-2 activate Orai channels, resulting in the opening of Ca\textsuperscript{2+} channels and an influx of Ca\textsuperscript{2+} into MC. The Orai-STIM interaction is enabled to ensure a sustained level of cytosolic Ca\textsuperscript{2+}. Many factors are involved in regulating Orai channel functions. The glycosylation of Orai modulates SOCE in a cell-type-specific manner. In human MCs, the inhibition of sulfotransferase alters the glycosylation of Orai-1 and increases SOCE.\textsuperscript{25} Cholesterol also regulates Orai channel activity. Derler \textit{et al.} demonstrated that cholesterol depletion induces increased Ca\textsuperscript{2+} entry through endogenous Orai-1 channels, thus leading to increased degranulation in MCs.\textsuperscript{26} As mentioned above, there are three isoforms of Orai with some differences in electrophysiological and pharmacological properties.\textsuperscript{27} We demonstrated that Orai-2 is located on the secretory granules in MC, and regulates Ca\textsuperscript{2+} release from intracellular Ca\textsuperscript{2+} stores. Knockdown of Orai-2 from MC resulted in a defective degranulation response.\textsuperscript{28} In addition, we have demonstrated that another cation channel, transient receptor potential cation channel 1 (TRPC1), also participates in FcεRI-mediated Ca\textsuperscript{2+} influx and degranulation in MCs.\textsuperscript{29} Medic \textit{et al.} explored the role of TRPC1 in an anaphylactic mouse model. Contrary to the role of TRPC1 in MC \textit{in vitro}, they found that TRPC1 deficiency caused a delayed recovery of antigen-induced anaphylactic reactions.\textsuperscript{30} Thus, some TRPCs forming complexes with Orai-1 appear to participate in Ca\textsuperscript{2+} influx in MCs.\textsuperscript{31,32}

5.2. Other Second Messengers The zinc ion (Zn\textsuperscript{2+}), similar to Ca\textsuperscript{2+}, is also an essential factor for homeostasis.\textsuperscript{33} Yamasaki \textit{et al.} showed that Zn\textsuperscript{2+} acts as an intracellular signaling molecule and is involved in MC activation. They also showed that Zn\textsuperscript{2+} concentration is increased in cytosol, which is named the “Zn wave,” following FcεRI engagement.\textsuperscript{34} The Zn\textsuperscript{2+} wave occurs within several minutes after FcεRI engagement and originates from the ER. Yamasaki \textit{et al.} also
found that the L-type calcium channel (LTCC) located in the ER is responsible for Zn\(^{2+}\) release, and that it positively regulates inflammatory cytokine production by controlling nuclear factor-kappa B (NF-xB) activity.\(^{30}\) Nishida et al. have shown that the Zn\(^{2+}\) transporter Znt5/Slc30a5 is required for an MC-mediated delayed-type allergic response (e.g., contact hypersensitivity), but not for immediate reaction (e.g., passive cutaneous anaphylaxis).\(^{30}\) A previous study demonstrated that secretory granules in MC are rich in Zn\(^{2+}\).\(^{37}\) In human MCs, Zn\(^{2+}\) was rapidly released after FcεRI engagement. Interestingly, the kinetics for Zn\(^{2+}\) release were different from those for degranulation.\(^{38}\) Ho et al. have found that Zn\(^{2+}\) also works as a potent inhibitor of caspases, which play an important role in apoptosis. Depletion of Zn\(^{2+}\) by chelation with N,N,N',N'-tetakis(2-pyridylmethyl)ethylenediamine (TPEN) results in greatly increased susceptibility of MCs to toxin-induced caspase activation.\(^{39}\) Thus, Zn\(^{2+}\) is involved in multiple steps of FcεRI-induced MC activation and in allergic responses. We now seek a better understanding of the roles of Zn\(^{2+}\) in MCs in the context of both normal and pathological conditions. Clarification of the functions of Ca\(^{2+}\) and/or Zn\(^{2+}\) should begin to address questions about MC biology.

Sphingosine-1-phosphate (S1P) is a lipid mediator involved in diverse biological processes, including the immune system. S1P affects MC function by acting through its receptors on the cell membrane. Many of its functions are regulated by G-protein-coupled S1P receptors (S1P\(_{1-5}\)). S1P is formed inside cells by the phosphorylation of sphingosine (SPH) mediated by two conserved sphingosine kinases (SphK1 and SphK2). In MCs, Src kinases (Lyn and Fyn) activate SphK1 and SphK2 after FcεRI engagement.\(^{40,41}\) SphK1 is a key regulator of human MCs, and SphK2 predominates in mouse MCs.\(^{42}\) This difference of predominant isoform seems to vary not only by species but also by the levels of MC maturation. In addition, S1P functions as a signaling molecule, independent of cell surface receptors. S1P specifically binds to the histone deacetylases HDAC1 and HDAC2, which are its first described intracellular targets.\(^{43}\) These interactions regulate the histone acetylation and transcriptional activity of target genes. The complex role of S1P is that S1P is secreted from activated MCs through the transporter multidrug resistance protein 1 (ABCC1, a member of the ATP-binding cassette transporter family).\(^{44}\) It has been reported that MCs express S1P\(_{1}\) and S1P\(_{2}\) preferentially among reported that MCs express S1P\(_{1}\) and S1P\(_{2}\) preferentially among the five receptors for S1P. S1P\(_{1}\) is involved in chemotactic functions of MCs toward antigens. S1P\(_{2}\) is involved in degranulation,\(^{41}\) though its function varies depending on cell type and experimental conditions.\(^{45,46}\) The mechanism by which S1P\(_{1}\) might regulate MC degranulation remains unclear.

I have pointed out new aspects of several important messengers in MC signaling. The diverse functions of these messengers in response to FcεRI stimulation remain to be elucidated.

6. CONCLUSION

In this review, I have highlighted FcεRI signals that control MC activation, especially focusing on the complexity of FcεRI activation and of the signaling network. A broad range of experimental findings shows that numerous proteins and factors are involved in MC activation and allergic responses. Our knowledge of FcεRI signaling is still limited. For example, it remains largely unclear how the complex regulatory network of FcεRI activation responds to antigens of different affinities. Innovative experimental methods may help to uncover these mechanisms, and may facilitate the development of new drugs for the treatment of allergic diseases.

Acknowledgments The author wishes to acknowledge the many co-authors and contributors to the work summarized herein. The author particularly acknowledges Prof. Naohide Hirashima at Nagoya City University. The author is supported by Grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan. The author is also supported by Grants from the Takeda Science Foundation and The Naito Foundation. The author acknowledges support by the intramural research program of the National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, U.S.A.

Conflict of Interest The author declares no conflicts of interest.

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