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IgE receptor and signal transduction in mast cells and basophils

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There are many aspects of mast cell and basophil biology that are being explored today. Notably, there is a wide variety of studies of the roles these two cell types play in the development of a multitude of diseases and the role they play in the general immune response. But the original reasons for studying these two cell types – because they are considered crucial to immediate hypersensitivity reactions – remain a driving force for research. These two cell types express the complete high affinity IgE receptor and aggregation of this receptor results in the secretion of multiple potent mediators that cause many of the signs and symptoms of an allergic reaction. Understanding more about the biology of the receptor and the signaling that it initiates remains important to the development of new therapeutic approaches to inhibit this reaction.

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FcεRI biology

The high affinity IgE receptor is a protein that confers on a cell the ability to bind antigen-specific IgE antibody. It is composed of three subunits, alpha, beta, and a disulfide-linked pair of gamma subunits. There is a great deal of interesting structure to the biology of this receptor, much of which is still being uncovered. In human cells, unlike murine cells, the fact that the receptor can be expressed on the cell surface without the beta subunit has led to observations of FcεRI expression on many unexpected cell types. All granulocytes have been reported to express FcεRI although there remains some controversy over levels of expression and functionality in eosinophils and neutrophils [1]. Monocytes, dendritic cells, and langerhans cells also express modest levels of FcεRI and functions have been described. Beyond the hematopoietic cells, there are some reports of smooth muscle [2] and nerve cells expressing FcεRI [3,4]. Many of these cell types do not express the beta subunit. But it is also

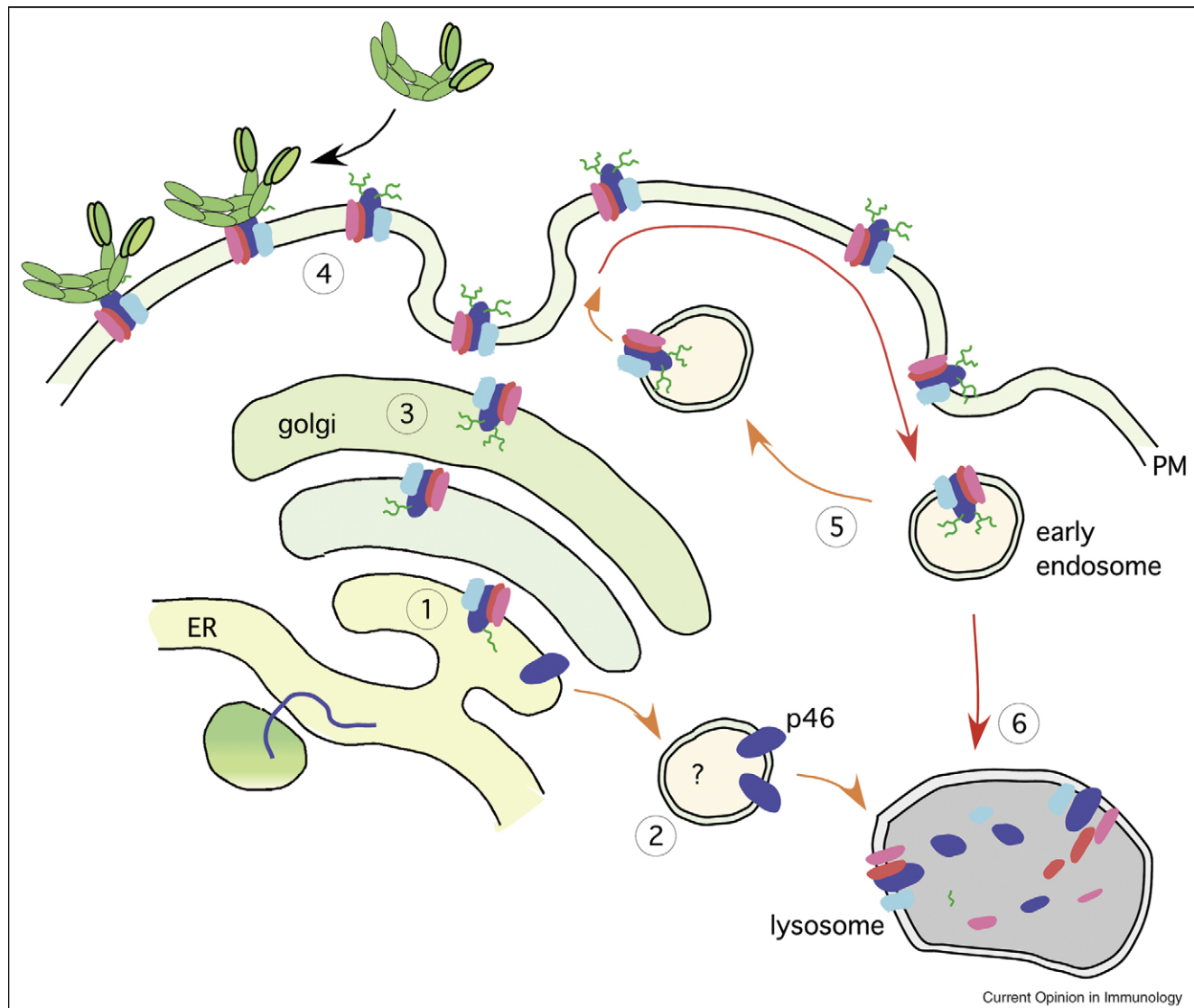
apparent that basophils express variable ratios of FcεRIβ:FcεRIα, so even in a cell that can express the beta subunit, there are variable levels of FcεRIβ expression [5]. It is not known whether this has an influence on function but since FcεRIβ is a better chaperone than FcεRIγ for protecting FcεRIα from degradation before golgi processing, there is, at minimum, the likelihood that more receptor could be expressed when FcεRIβ is present. Recent studies have refined our understanding of this chaperone effect by either the beta or gamma subunit. In contrast with mouse FcεRIα, it is the extracellular domain of alpha that allows human FcεRIα to be expressed on the cell surface without FcεRIβ [6]. An interesting implication of the difference between mice and men is that many cells might express the alpha subunit but it is the expression of the beta subunit that is tied to being a basophil or mast cell. Once the expression of the alpha subunit is freed from the requirement for the beta subunit, as it is in humans but not mice, FcεRIα expression begins appearing in many other cell types, whether or not it is expressed on the cell surface.

One hallmark of FcεRI biology is the dependence of cell surface expression on occupancy of the receptor by IgE. Empty receptors reside on the cell surface for only hours ($T_{1/2}$ of 24 h) while receptor with bound IgE appears permanently expressed [7]. Recent studies have shown that empty receptor is internalized and degraded unless IL-3 is present [7]. The presence of IL-3 slows degradation of internalized receptor and some receptors appear to re-cycle to the cell surface. This dynamics between IgE and receptor expression accounts for the relationship between plasma IgE and basophil and mast cell receptor expression levels. It leads to a log(base e)–linear relationship between receptor expression and IgE level, respectively. The log–linear nature of the relationship means that it is possible to swamp receptors with non-specific IgE, that is, receptor expression cannot precisely keep pace with the amount of IgE in its environment and this has clinical consequences.

Figure 1 shows the current understanding of human FcεRI expression modulation. It is important to note that it is a steady state of cell surface expression, that is, even in the absence of IgE there will be a low density of receptor expression (in circulating basophils, this density appears to be approximately 8000 per cell). As the figure indicates, the role of IgE is to allow retention of surface-expressed IgE but it has no impact on synthesis of receptor. However, synthesis of FcεRI does appear to be under the control of cytokines (it is not known if loss from the cell surface is regulated by cytokines but as noted

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Figure 1



Cartoon of the FcεRI life-cycle. Crucial points in processing and handling are noted with an encircled number. Further processing of the receptor is dependent on the chaperone actions of either FcεRIβ or FcεRIγ (1) during translation in the endoplasmic reticulum (ER). In human cells, there appears to be a large pool of immature receptor that migrates on SDS electrophoresis gels at 45–50 kDa apparent molecular weight. This immature receptor accumulates *in vivo* but is rapidly degraded in culture; it is not further processed for surface expression (2). After removal of carbohydrates associated with immature proteins and replacement with a complex array of carbohydrates by the golgi (3) receptor is placed on the cell surface (PM = plasma membrane). The cell surface FcεRIα subunit has an apparent molecular weight that is broad, ranging from 58 to 66 kDa. It stays on the surface permanently if IgE binds to FcεRIα (4). If unoccupied, it is lost by an endocytotic process but in the presence of certain cytokines (5), a limited fraction may undergo recycling to the cell surface. Otherwise the unoccupied receptor is degraded in a Bafilomycin-sensitive lysosome (6).

above, IL-3 can influence re-cycling of the receptor). Recent studies in mice have shown that IL-10 can regulate FcεRI expression [8] in both mouse and human mast cells. The mechanism of suppression has not been examined. T regulatory cells, in a reaction that is independent of IL-10 but dependent on cell–cell contact, can also regulate FcεRI expression [9], at least in mouse mast cells. The transcription factors involved in expression of FcεRIα are slowly being delineated. Most recently, Elf-1

was found to downregulate FcεRIα promoter activity by competing with the PU.1 transcription factor that aids FcεRIα gene transcription [10].

Monomeric IgE and signaling

It is evident that monomeric IgE induces some kind of signal when binding to FcεRIα because binding prevents endocytosis. But the nature of this signaling remains unknown. In murine cells, a p28 protein has been identified

that associates and dissociates from $\text{Fc}\epsilon\text{RI}\alpha \pm \text{IgE}$ [11]. Whether this is a protein involved in sensing occupancy of $\text{Fc}\epsilon\text{RI}\alpha$ is not yet known. However, it is also clear that the absence of src-family kinase activation or syk activity does not inhibit the ability of monomeric IgE to stabilize surface receptor [7*,12]. By contrast, the kind of monomeric IgE-induced signaling that leads to some mast cell functions (cytokine release, survival, adhesion) in the absence of crosslinking does require canonical early signals such as lyn and syk activity [12]. The ability of monomeric IgE to induce mast cell functions remains a story with unknown biological significance. It is well demonstrated in mice but the ability of human IgE to induce human mast cell or basophil functions is less clear. There are reports of weak levels of cytokine release in cultured human mast cells [13] but the list and magnitude of functions is short and modest. There are continued refinements to the story for murine mast cells, notably, that IgE accelerates the development of murine mast cells and modulates mast cell phenotype [14]. Murine IgE has been classified as either highly or poorly cytokinergic, a definition that relates to the ability of a monomeric IgE species to induce mast cell functions (notably, cytokine release). In humans, IgE has been classified as IgE+ or IgE– on the basis of whether passive transfer of the IgE confers on basophils the ability to respond to Histamine-Releasing Factor (HRF) [15]. This would seem to have a possible relationship to the designation of murine IgEs being poorly or highly cytokinergic. However, recent studies indicate that IgE+ does not have the properties of highly cytokinergic IgE unless the human cells have been treated/primed with high concentrations of cytokines [16].

It is worth noting that in studies that examine monomeric IgE for its ability to induce cytokine release (or enhance survival), experiments are often performed with cells that show high levels of $\text{Fc}\epsilon\text{RI}$ expression but have not been previously exposed to IgE (they may express high levels of receptor because the high levels of cytokines used in these cultures shift the steady state of receptor expression toward high levels, probably due to increased synthesis). In this context, it is useful to explore evidence that spontaneous aggregation of receptor may generate early signals. Several lines of research are pertinent. First, studies have shown that receptors need only to be within 120–200 Å of each other to initiate canonical signaling [17]. Second, weak signaling is limited by kinetic proofreading [18] which otherwise serves to limit propagation of an early signal. But cytokine release is not subject to the kinetic proofreading mechanism so that weak signals leak first into the cytokine generating pathways [19]. Third, high densities of receptor lead to many spontaneous aggregates [20*]. Simulations suggest that with 150 000 receptors per cell, 10% are in close enough proximity (>120, <200 Å) to initiate a signal. Studies of human basophils and RBL cells show that as few as 50 stable aggregates can produce mediator release [21,22].

Spontaneous aggregates are very transient, persisting for less than 50 ms unless there is a weak association (unpublished calculations). Perhaps IgE provides this association; a change in Brownian motion diffusion rate of only 2-fold would increase many spontaneous aggregate persistence times to greater than 200 ms. Studies with simple bivalent haptens suggest that weak signaling is possible with aggregate persistence of 200 ms [23]. Taking all these independent studies together leads to the suggestion that high densities of receptors in highly primed cells might result in spontaneous aggregates that induce signaling strong enough to induce cytokine release but not degranulation, a hallmark of the monomeric IgE effect. It remains to be determined how receptor density and cell priming influence the ability of monomeric IgE to induce cytokine release and most importantly whether receptor occupancy by IgE alters the diffusion-related dissociation time between non-crosslinked receptors.

Aggregated receptor signaling

Src-family kinases

The canonical explanation for how an aggregated receptor induces signaling usually states that the first step requires the weak association of lyn kinase (a src-family kinase member) with the beta subunit of $\text{Fc}\epsilon\text{RI}$, the trans-phosphorylation of an adjacent beta subunit (as part of the 'aggregate'), and the tight association of lyn through its SH2 domain to this phosphorylated beta ITAM (immunotyrosine-activation-motif) results in the enhanced activity of lyn. The enhanced enzymatic activity of lyn then phosphorylates the ITAM of the gamma subunits resulting in the recruitment and activation of syk (spleen tyrosine kinase, a ZAP-70 family member). Much of the resulting signaling results from the activities of syk. However, in recent years, this model has become too simple of a description to explain experimental observations. Several years ago, a second src-family kinase member was implicated in the early signaling; in murine mast cells, fyn was found to initiate signaling cascades distinct from lyn and syk [24]. More recently, a third family kinase member, hck, has been implicated [25*]. The picture that is emerging is for a subtle balance of events whereby lyn may have a role in initiating signaling that is both positive and negative. Fyn generates signaling that is predominantly activating in character, and hck negatively regulates lyn that negatively regulates fyn. An additional complication is that the beta ITAM is not a canonical ITAM but contains a third tyrosine that has a negative influence on the ability of the other two tyrosines to act in a standard way [26,27]. To date, little is known about the additional src-family kinases in human cells although previous studies have shown that fyn plays a role in signal transduction in culture-derived human mast cells [24]. There has been one attempt to examine fyn phosphorylation changes in human basophils but no changes were observed [28]. However, an absence of changes in phosphorylation state is not necessarily an

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indication that fyn does not have a role in signaling in these cells.

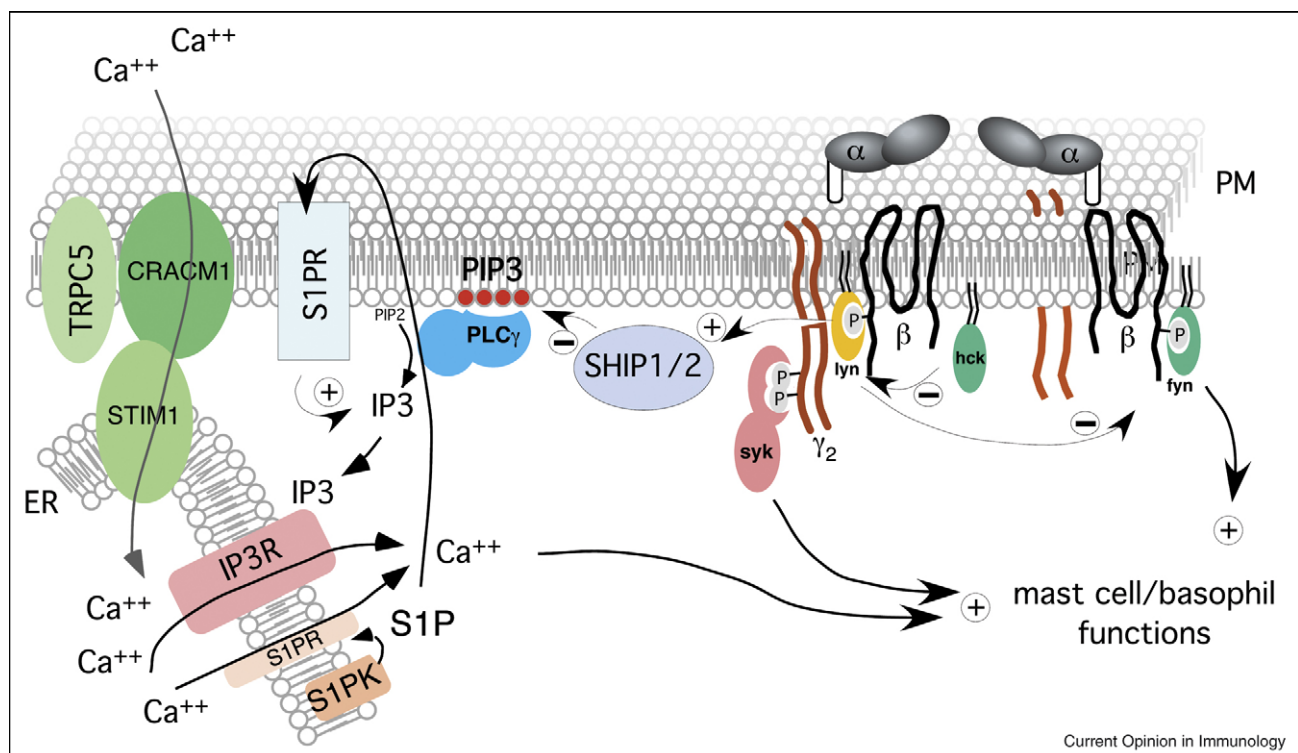
In rat basophilic leukemia cells, lyn kinase was determined to be a rate-limiting molecular species. In other words, there is not enough lyn present (or more precisely, not enough lyn available to FcεRI) to service all the IgE receptors [29]. By contrast, syk expression was robust and not rate limiting. This situation is reversed in human basophils. Although syk is crucial to the function of these cells, relative to other leukocytes, they express very little syk [30] and syk expression is the most variably expressed signaling element examined to date, with expression levels well-correlated to IgE-mediated histamine release [31••].

Several years ago, studies in mice suggested that SHIP is a gatekeeper of IgE-mediated function. SHIP knockout mice had mast cells that were leaky and were easily induced to secrete. It is not entirely clear that this is the case in humans, at least for basophils (the mast cell

story has yet to be explored). SHIP expression in basophils is tightly regulated and variance in its expression contributes little to the variance of maximum histamine release found in the general population [31••]. However, there are phenotypes of basophils where SHIP is poorly expressed and the cells appear more sensitive to stimulation [32], usually with stimuli that do not normally induce secretion. There are also indications that SHIP levels are altered in patients with chronic urticaria [33]. While there is, as yet, no causal demonstration for a role for SHIP in IgE-mediated secretion in human mast cells or basophils, there are other indications that it does play some role [28,34]. Most of the work to date has explored the role of SHIP1 in murine mast cells, but SHIP2 is also expressed in hematopoietic cells and a recent study in mice suggests that SHIP2 also influences IgE-mediated signaling but in ways that are distinct from the effects of SHIP1 [35•].

Increases in cytosolic calcium are required for normal IgE-mediated secretion. Years ago, this was determined

Figure 2



Cartoon of some early signaling steps that are discussed in the text. On the far right, in the plasma membrane, is depicted an aggregate of the heterotrimeric version of FcεRI. The associated src-family kinases, lyn, fyn, and hck are shown with their postulated interactions. Syk activation is central to downstream signaling and the generation of signals that lead to both the production of PIP3 (phosphatidylinositol 3,4,5 trisphosphate), the activation of phospholipase C and therefore the ultimate elevation of cytosolic Ca²⁺ from intracellular stores. Depletion of the intracellular stores leads to activation of the CRACM1 store-operated plasma membrane channel with assistance by a TRP channel, TRPC5 in rat basophilic leukemia cells. Early signaling also activates a sphingosine kinase to generate sphingosine-1-phosphate that may act internally and externally. The external action would activate a S1P receptor and a GTP-binding protein cascade of signaling that also includes generation of inositol 3,4,5 trisphosphate (IP3).

to be a process that required a calcium uptake mechanism that was sensitive to the depletion of internal stores of calcium, the so-called store-operated calcium channel. Other cells use a similar calcium channel but until recently, the channel eluded discovery. In 2006, several groups identified Orai1/CRACM1 as a part of this store-operated channel. The ER protein, STIM1, was found to be the sensor of empty stores of internal calcium. STIM1 signals CRACM1 to let calcium into the cell. This discovery is of general importance but because mast cell and basophil function is crucially dependent on calcium influx, it is of particular importance to these cells. CRACM1 is now identified as necessary for calcium influx in mast cells [36^{••},37^{••}]. However, the precise details of Orai1/CRACM1 involvement are, perhaps, more subtle. The TRP calcium channels (28 members within 6 subfamilies) are important for some types of calcium entry and a recent study in RBL cells has drawn the conclusion that in RBL cells, TRPC5, working in concert with CRACM1, serves to modulate IgE-mediated calcium entry [38[•]]. This result has a similarity to recent observations that different TRP channel proteins/subunits can mix and match to form ion channels with different properties. For example, different heterodimers can produce TRP channels with a wide range of temperature sensitivity [39]. The finding that TRP channels cooperate with CRACM1 raises the possibility that there may be subtle differences in calcium entry mechanisms in mast cells or basophils of different phenotypes. In this context, there has been a long-standing recognition that optimal IgE-mediated release in human skin mast cells occurs at 32 °C rather than 37 °C [40]. Unpublished studies by our group have shown that the calcium response is blunted at 37 °C and as the stimulated cell is cooled to 32 °C, the calcium response increases. It is possible that some fine tuning of the CRACM1 channel is involved. CRACM1 is also only one of a family of three related proteins (CRACM1-3), so there may be additional complications. Studies in RBL cells suggested that Zn²⁺ can utilize the calcium entry pathway of this cell and inhibit calcium entry [41] but Zn²⁺ does not have this effect in human basophils [42[•]], again suggesting a subtle difference in the nature of the store-operated channel.

A full-fledged secretory response in mast cells may include several positive feedback mechanisms, both internal and external. In recent years, there have been several studies that suggest that substances secreted in the early stages of the mast cell response feedback onto activating receptors of the 7-transmembrane variety. Thus, the full-fledged secretory response has biochemical characteristics one associates with immunoreceptors and characteristics one associates with GTP-binding protein-linked receptors. For example, the lipid metabolite, sphingosine-1-phosphate (S1P) is generated during the initial IgE-mediated reaction [43^{••}]. This

small lipid has been suggested to regulate internal calcium store release [44] but when secreted, it interacts with Edg receptors on the same cell to induce a canonical GTP-binding protein-mediated increase in cytosolic calcium and other activating Edg receptor responses [45,46[•]]. This type of feedback opens the door to therapeutic agents that specifically modulate either the generation of S1P or its feedback to an Edg receptor. Recent studies in mice have demonstrated that inhibition of S1P generation does indeed reduce the IgE-mediated reaction and *in vivo*, blunts the inflammatory reaction that characterizes a mast cell response [43^{••}] (Figure 2).

Summary

It is clear that there remain some important problems in understanding the full breadth of the biology of the high affinity IgE receptor, especially as it is expressed in humans. The loss of receptor due to an absence of its ligand (IgE) is an interesting problem whose understanding might provide new avenues of therapy. The ongoing questions and controversy about the ability of monomeric IgE to cause other responses in mast cells and basophils are very important to resolve because if monomeric IgE is a natural regulator of mast cell and basophil function in humans, there may be a lot of allergic disease biology that can be better understood.

With respect to signaling, the ever-increasing complexity of the reaction begs for some guiding principles regarding the role of aggregation states in driving the various processes. The relative roles of the various pathways initiated by aggregation and how nature has chosen to regulate the relative strengths of the various pathways before they are even activated is also an interesting set of questions whose answers might help us to understand the variety of hypersensitivity reactions *in vivo* and the source of biological variation that makes some patients more ill than others.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Alphonse MP, Saffar AS, Shan L, HayGlass KT, Simons FE, Gounni AS: **Regulation of the high affinity IgE receptor (Fc epsilonRI) in human neutrophils: role of seasonal allergen exposure and Th-2 cytokines.** *PLoS ONE* 2008, 3:e1921.
2. Gounni AS, Wellemans V, Yang J, Bellesort F, Kassiri K, Gangloff S, Guenounou M, Halayko AJ, Hamid Q, Lamkhieoued B: **Human airway smooth muscle cells express the high affinity receptor for IgE (Fc epsilonRI): a critical role of Fc epsilonRI in human airway smooth muscle cell function.** *J Immunol* 2005, 175:2613-2621.
3. van der Kleij H, Charles N, Rivera J, Bienenstock J: **Expression of functionally active Fc epsilon and Fc gamma receptors on mouse superior cervical ganglion neuritis.** *Collegium Internationale Allergologicum; Curacao: 2008.*

6 Allergy and Hypersensitivity

4. Ganguly S, Grodzki C, Sugden D, Moller M, Odom S, Gaildrat P, Gery I, Siraganian RP, Rivera J, Klein DC: **Neural adrenergic/cyclic AMP regulation of the immunoglobulin E receptor alpha-subunit expression in the mammalian pinealocyte: a neuroendocrine/immune response link?** *J Biol Chem* 2007, **282**:32758-32764.
- A surprising observation that FcεRI can be found in neural tissue cells.
5. Saini S, Richardson JJ, Wofsy C, Lavens-Phillips, Bochner B, MacGlashan DW Jr: **Expression and modulation of FcεRIα and FcεRIβ in human blood basophils.** *J All Clin Immunol* 2001, **107**:832-841.
6. Hartman ML, Lin SY, Jouvin MH, Kinet JP: **Role of the extracellular domain of Fc epsilon RI alpha in intracellular processing and surface expression of the high affinity receptor for IgE Fc epsilon RI.** *Mol Immunol* 2008, **45**:2307-2311.
7. MacGlashan DW Jr: **Endocytosis, re-cycling and degradation of unoccupied FcεRI in human basophils.** *J Leukoc Biol* 2007, **82**:1003-1010.
- Study showing the life-cycle behavior of human FcεRI in peripheral blood basophils. This study demonstrates that the unoccupied receptor is lost by endocytosis and that IL-3 controls recycling of receptor.
8. Kennedy Norton S, Barnstein B, Brenzovich J, Bailey DP, Kashyap M, Speiran K, Ford J, Conrad D, Watowich S, Moralle MR et al.: **IL-10 suppresses mast cell IgE receptor expression and signaling in vitro and in vivo.** *J Immunol* 2008, **180**:2848-2854.
9. Kashyap M, Thornton AM, Norton SK, Barnstein B, Macey M, Brenzovich J, Shevach E, Leonard WJ, Ryan JJ: **Cutting edge: CD4 T cell-mast cell interactions alter IgE receptor expression and signaling.** *J Immunol* 2008, **180**:2039-2043.
- A demonstration that expression of FcεRI in murine mast cells can be regulated by cell-cell contact with a murine T cell.
10. Wang QH, Nishiyama C, Nakano N, Shimokawa N, Hara M, Kanada S, Ogawa H, Okumura K: **Suppressive effect of Elf-1 on FcεpsilonRI alpha-chain expression in primary mast cells.** *Immunogenetics* 2008, **60**:557-563.
11. Charles N, Monteiro RC, Benhamou M: **p28, a novel IgE receptor-associated protein, is a sensor of receptor occupation by its ligand in mast cells.** *J Biol Chem* 2004, **279**:12312-12318.
12. Kitaura J, Eto K, Kinoshita T, Kawakami Y, Leitges M, Lowell CA, Kawakami T: **Regulation of highly cytokinergic IgE-induced mast cell adhesion by Src, Syk, Tec, and protein kinase C family kinases.** *J Immunol* 2005, **174**:4495-4504.
13. Matsuda K, Piliponsky AM, Iikura M, Nakae S, Wang EW, Dutta SM, Kawakami T, Tsai M, Galli SJ: **Monomeric IgE enhances human mast cell chemokine production: IL-4 augments and dexamethasone suppresses the response.** *J Allergy Clin Immunol* 2005, **116**:1357-1363.
14. Kashiwakura JI, Xiao W, Kitaura J, Kawakami Y, Maeda-Yamamoto M, Pfeiffer JR, Wilson BS, Blank U, Kawakami T: **Pivotal Advance: IgE accelerates in vitro development of mast cells and modifies their phenotype.** *J Leukoc Biol* 2008, **84**:357-367.
15. MacDonald SM, Lichtenstein LM, Proud D, Plaut M, Naclerio RM, MacGlashan DW Jr, Kagey SA: **Studies of IgE-dependent histamine releasing factors: heterogeneity of IgE.** *J Immunol* 1987, **139**:506-512.
16. Xie L, Schroeder JT, Langdon JM, Sora-Scott RS, Kawakami T, MacDonald SM: **Human IgE+ and IgE- are not equivalent to mouse highly cytokinergic IgE.** *J Allergy Clin Immunol* 2008, **121**:1027-1033.
17. Paar JM, Harris NT, Holowka D, Baird B: **Bivalent ligands with rigid double-stranded DNA spacers reveal structural constraints on signaling by Fc epsilon RI.** *J Immunol* 2002, **169**:856-864.
18. McKeithan TW: **Kinetic proofreading in T-cell receptor signal transduction.** *Proc Natl Acad Sci U S A* 1995, **92**:5042-5046.
19. Liu ZJ, Haleem-Smith H, Chen H, Metzger H: **Unexpected signals in a system subject to kinetic proofreading.** *Proc Natl Acad Sci U S A* 2001, **98**:7289-7294.
20. Schweitzer-Stenner R, Pecht I: **Death of a dogma or enforcing the artificial: monomeric IgE binding may initiate mast cell response by inducing its receptor aggregation.** *J Immunol* 2005, **174**:4461-4464.
- A very useful set of calculations regarding the presence of spontaneous aggregates at high densities of FcεRI that may have application to the studies of monomeric IgE stimulating mast cells. It is notable, however, that this paper does not address the issue of aggregate lifetime.
21. Fewtrell C, Metzger H: **Larger oligomers of IgE are more effective than dimers in stimulating rat basophilic leukemia cells.** *J Immunol* 1980, **125**:701-710.
22. MacGlashan DW Jr, Peters SP, Warner J, Lichtenstein LM: **Characteristics of human basophil sulfidopeptide leukotriene release: releasability defined as the ability of the basophil to respond to dimeric cross-links.** *J Immunol* 1986, **136**:2231-2239.
23. Dembo M, Goldstein B, Sobotka AK, Lichtenstein LM: **Histamine release due to bivalent penicilloyl haptens: the relation of activation and desensitization of basophils to dynamic aspects of ligand binding to cell surface antibody.** *J Immunol* 1979, **122**:518-528.
24. 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.
25. Hong H, Kitaura J, Xiao W, Horejsi V, Ra C, Lowell CA, Kawakami Y, Kawakami T: **The Src family kinase Hck regulates mast cell activation by suppressing an inhibitory Src family kinase Lyn.** *Blood* 2007, **110**:2511-2519.
- Following on the heels of studies implicating a second src-family kinase in FcεRI signaling, this study adds another layer of complexity into the very earliest steps of FcεRI signaling by proposing that a third src-family member is involved.
26. Furumoto Y, Nunomura S, Terada T, Rivera J, Ra C: **The FcεpsilonRIβ immunoreceptor tyrosine-based activation motif exerts inhibitory control on MAPK and IkappaB kinase phosphorylation and mast cell cytokine production.** *J Biol Chem* 2004, **279**:49177-49187.
27. Nunomura S, Gon Y, Yoshimaru T, Suzuki Y, Nishimoto H, Kawakami T, Ra C: **Role of the FcεpsilonRI beta-chain ITAM as a signal regulator for mast cell activation with monomeric IgE.** *Int Immunol* 2005, **17**:685-694.
28. MacGlashan DW Jr, Vilarino N: **Nonspecific desensitization, functional memory and the characteristics of SHIP phosphorylation following IgE-mediated stimulation of human basophils.** *J Immunol* 2006, **177**:1040-1051.
29. Torigoe C, Inman JK, Metzger H: **An unusual mechanism for ligand antagonism.** *Science* 1998, **281**:568-572.
30. Ishmael S, MacGlashan DW Jr: **Kinetics of Syk expression in human basophils differentiating from CD34+ progenitors.** *FASEB J* 2008, **22**:1071-1075.
31. MacGlashan DW Jr: **Relationship between Syk and SHIP expression and secretion from human basophils in the general population.** *J Allergy Clin Immunol* 2007, **119**:626-633.
- This study provides important background on the expression levels of 6 critical signaling elements in peripheral blood basophils obtained from a general population sample. Notably, variance in the expression of syk accounts for a major portion of the variance in IgE-mediated histamine release in general population.
32. Vonakis BM, Gibbons S Jr, Sora R, Langdon JM, MacDonald SM: **Src homology 2 domain-containing inositol 5' phosphatase is negatively associated with histamine release to human recombinant histamine-releasing factor in human basophils.** *J Allergy Clin Immunol* 2001, **108**:822-831.
33. Vonakis BM, Vasagar K, Gibbons SP Jr, Gober L, Sterba PM, Chang H, Saini SS: **Basophil FcεpsilonRI histamine release parallels expression of Src-homology 2-containing inositol phosphatases in chronic idiopathic urticaria.** *J Allergy Clin Immunol* 2007, **119**:441-448.
34. Gibbs BF, Rathling A, Zillikens D, Huber M, Haas H: **Initial Fc epsilon RI-mediated signal strength plays a key role in regulating basophil signaling and deactivation.** *J Allergy Clin Immunol* 2006, **118**:1060-1067.

35. Leung WH, Bolland S: **The inositol 5'-phosphatase SHIP-2 negatively regulates IgE-induced mast cell degranulation and cytokine production.** *J Immunol* 2007, **179**:95-102.
A great deal of attention has previously been focused on expression and activity of SHIP1 in mast cell function. This study explores the role of SHIP2, which is often present with SHIP1 in basophils or mast cells.
36. Vig M, DeHaven WI, Bird GS, Billingsley JM, Wang H, Rao PE, Hutchings AB, Jouvin MH, Putney JW, Kinet JP: **Defective mast cell effector functions in mice lacking the CRACM1 pore subunit of store-operated calcium release-activated calcium channels.** *Nat Immunol* 2008, **9**:89-96.
An important demonstration of the criticality of the CRACM1 protein in the cytosolic calcium response in mast cells and therefore the ability of mast cells to mediate allergic inflammation.
37. Baba Y, Nishida K, Fujii Y, Hirano T, Hikida M, Kurosaki T: **Essential function for the calcium sensor STIM1 in mast cell activation and anaphylactic responses.** *Nat Immunol* 2008, **9**:81-88.
An important demonstration of the criticality of both CRACM1 and STIM1 in mediating the cytosolic calcium response in mast cells.
38. Ma HT, Peng Z, Hiragun T, Iwaki S, Gilfillan AM, Beaven MA: **Canonical transient receptor potential 5 channel in conjunction with Orai1 and STIM1 allows Sr^{2+} entry, optimal influx of Ca^{2+} , and degranulation in a rat mast cell line.** *J Immunol* 2008, **180**:2233-2239.
A study of the CRACM1 channel with fascinating implications for control of this critical calcium channel by another calcium channel family, the TRP channels.
39. Cheng W, Yang F, Takanishi CL, Zheng J: **Thermosensitive TRPV channel subunits coassemble into heteromeric channels with intermediate conductance and gating properties.** *J Gen Physiol* 2007, **129**:191-207.
40. Lawrence ID, Warner JA, Cohan VL, Hubbard WC, Kagey SA, Lichtenstein LM: **Purification and characterization of human skin mast cells. Evidence for human mast cell heterogeneity.** *J Immunol* 1987, **139**:3062-3069.
41. Beaven MA, Rogers J, Moore JP, Hesketh TR, Smith GA, Metcalfe JC: **The mechanism of the calcium signal and correlation with histamine release in 2H3 cells.** *J Biol Chem* 1984, **259**:7129-7136.
42. Musset B, Morgan D, Cherny VV, MacGlashan DW Jr, Thomas LL, Rios E, DeCoursey TE: **A pH-stabilizing role of voltage-gated proton channels in IgE-mediated activation of human basophils.** *Proc Natl Acad Sci U S A* 2008, **105**:11020-11025.
A first look at the pH channel in human basophils and how it may regulate function of these cells.
43. Olivera A, Mizugishi K, Tikhonova A, Ciaccia L, Odom S, Proia RL, Rivera J: **The sphingosine kinase-sphingosine-1-phosphate axis is a determinant of mast cell function and anaphylaxis.** *Immunity* 2007, **26**:287-297.
A demonstration of the importance of the sphingosine-1-phosphate receptor and autocrine actions of mast cell generated sphingosine-1-phosphate on the behavior of mast cells *in vivo*.
44. Choi OH, Kim JH, Kinet JP: **Calcium mobilization via sphingosine kinase in signaling by the Fc ϵ RI antigen receptor.** *Nature* 1996, **380**:634-636.
45. Jolly PS, Bektas M, Olivera A, Gonzalez-Espinosa C, Proia RL, Rivera J, Milstien S, Spiegel S: **Transactivation of sphingosine-1-phosphate receptors by Fc ϵ RI triggering is required for normal mast cell degranulation and chemotaxis.** *J Exp Med* 2004, **199**:959-970.
46. Oskeritzian CA, Alvarez SE, Hait NC, Price MM, Milstien S, Spiegel S: **Distinct roles of sphingosine kinases 1 and 2 in human mast-cell functions.** *Blood* 2008, **111**:4193-4200.
Further refinement of the role of the sphingosine axis of signaling in mast cells.