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# IgE-dependent signaling as a therapeutic target for allergies

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## Abstract

Atopic diseases are complex, with many immunological participants, but the central element in their expression is IgE antibody. In an atopic individual, the immune system pathologically reacts to environmental substances by producing IgE, and these allergen-specific IgE antibodies confer to IgE receptor-bearing cells responsiveness to the environmental substances. Mast cells and basophils are central to the immediate hypersensitivity reaction that is mediated by IgE. In humans, there are various other immune cells, notably dendritic cells and B cells, which can also bind IgE. For mast cells, basophils and dendritic cells, the receptor that binds IgE is the high affinity receptor, FcεRI. For B cells and a few other cell types, the low affinity receptor, FcεRII, provides the cell a means to sense the presence of IgE. This overview will focus on events following activation of the high-affinity receptor because FcεRI generates the classical immediate hypersensitivity reaction.

## Introduction: Starting Points

A reasonable starting point for targeting IgE-mediated reactions is IgE itself, but this topic will be considered last because today this strategy usually involves monoclonal antibodies rather than cell permeant small-molecules. In addition, one theme of this review will be how to approach acute vs. chronic treatments for allergic diseases and monoclonal antibody vs. small-molecule strategies may be differentiated by their value for chronic and acute treatments, respectively. The starting point for considering small-molecule approaches will be the earliest steps in the signal transduction reaction that is initiated in mast cells and basophils following their exposure to allergens. This review will also focus on approaches in which the target is well-defined rather than explore the very wide range of compounds, both synthetic (so-called mast cell stabilizers) and natural (e.g., plant-derived compounds), that have been demonstrated to inhibit mast cell secretion. Several decades of study have provided biochemists with several interesting targets. As with any therapeutic approach, the question that remains unknown until actual clinical testing is whether any chosen therapeutic target is effectively localized to mast cells/basophils. The last decade or so of study has not been encouraging if one expects to find an FcεRI-specific target. All of the FcεRI-associated signaling elements that have been identified have also been identified as central participants in other immune cells crucial for a proper immune response.

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## Early steps in FcεRI-dependent signaling

Working inward from FcεRI, the first step in signaling involves src-family kinases. There remains some confusion about the precise number of src-family members that are crucial for signaling, and the final answer may be subtle, with variations among mast cell subtypes or basophils. However, there is general agreement that lyn kinase is necessary for initiating the reaction [1–4]. Currently, lyn kinase would not be a therapeutic target because this enzyme also participates in the termination of the activation cascade. Studies in both mice and humans have shown that less than maximal inhibition of src-family kinases, and lyn in particular, results in enhanced IgE-mediated functions [5, 6]. The src-family kinases fyn and hck have also been implicated in the IgE-mediated reaction [2, 7], and their roles appear to be restricted to reaction cascades that promote secretion from mast cells. However, the role of fyn and hck in IgE-mediated secretion from human mast cells or basophils has not been explored.

Considerable effort has been made to target the next step in the signaling cascade, the activation of syk, a ZAP-70 family member. Although lyn initiates phosphorylation of FcεRI, which allows syk to be recruited, a very broad range of activation signaling cascades start with the activity of syk [8]. This characteristic motivated the development of syk inhibitors for both atopic and other inflammatory diseases, such as rheumatoid arthritis (RA). Effective inhibition of syk, either through genetic means in mice or pharmacological means in humans completely ablates FcεRI-mediated secretion and essentially any other pro-inflammatory function that has been studied in mast cells/basophils [9, 10]. Recent syk inhibitors show both high potency and selectivity. Surprisingly, limited testing of syk inhibitors *in vivo* has not resulted in remarkable suppression of allergic symptoms [11]. It is speculated that local metabolism blunts the effectiveness of the drugs, although the precise answer is not known. However, even if syk inhibitors are found to be highly selective and efficacious, syk is often involved in signaling in immunoreceptor-bearing cells and all leukocytes (including T cells at early stages of development); all these cells use syk in the earliest steps of signal transduction for some of their functions. Syk knockout mice show perinatal mortality. Some cancer cells show heightened proliferation when syk is down-regulated [12]. So development of these inhibitors for atopic diseases has been put on hold except when it is possible to use them topically. Nevertheless, because these inhibitors are being explored for other chronic inflammatory conditions such as RA (e.g., R-788 has completed phase II trials, [13, 14]) some experience with their use in patients with coincident atopy may happen.

Downstream of syk activation are two other important signaling enzymes whose function might be considered crucial for mast cell or basophil secretion. The phosphotidylinositol 3′ phosphate kinase (PI3K) family of enzymes and Bruton's tyrosine kinase (of the Tec family) are activated either directly or indirectly through the action of syk. However, species differences and subtle differences in the requirements for the isozymes of PI3K in different mast cell types have made it less clear how generally useful inhibitors of these enzymes will be.

The PI3K family consists of a large number of members, although the class IA family is composed of 3 members, α, β, and δ. These enzymes phosphorylate membrane inositol phospholipids, and these phosphorylated phospholipids act as recruitment islands on the inner leaf of the plasma membranes for subsequent steps in the activation cascade (Figure 1). It is generally accepted that mast cells use α, β and δ, with a probable dominance of the δ member in regulating IgE-mediated secretion (the δ form is also considered to be restricted to hematopoietic cells) [15, 16]. The γ form, a class 1B member, is felt to be involved in G-protein linked receptor (GPCR) activation [16]. Studies in murine mast cells

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have advanced the idea that even during IgE-mediated secretion, which might begin as a PI3K δ-mediated process, there are secreted autocrine substances that feedback to amplify the secretory response through GPCRs (sphingosine-1-phosphate being one example) [17, 18]. Under the simple view that PI3Kγ is involved in GPCR activation, an IgE-mediated event could still be dependent on PI3Kγ [17]. In basophils, only PI3Kδ is present for the IgE-dependent activation cascade and PI3Kγ appears to be linked to non-immunoreceptor functions. Although studies in mice suggest that both PI3Kδ and γ contribute to mast cell secretion, there is no solid data regarding the efficacy of selective PI3K isozyme inhibitors in human mast cell studies. In human basophils, a specific PI3Kδ-only inhibitor completely ablates secretion, which is consistent with similar results using pan-specific PI3K inhibitors (in a cell that expresses only PI3Kδ of the class 1A group) [19]. However, PI3K is used for various signaling pathways in nearly all cell types, and this again raises questions about use of selective inhibitors for treatment of atopy. Like syk, knocking out PI3Kα or β in mice is embryonic lethal [20, 21].

The involvement of Bbk in human mast cell and basophil secretion is also somewhat unclear, although studies in murine mast cells suggest it plays some role [22]. Btk may also play a role in mast cell development [23]. Highly selective inhibitors of btk are only recently available, and studies in humans have had to wait for their development because the genetic manipulation that is common for murine studies is not possible. A recent study of a selective non-competitive btk inhibitor, PCI-32765, demonstrated complete inhibition of IgE-mediated functions in human basophils [24]. Based on studies in murine mast cells, this high efficacy was somewhat surprising. Similar results for human mast cells are not yet available. Btk inhibitors have been developed to treat B cell lymphomas, and in a manner similar to the development of syk inhibitors, it may be possible to learn how they operate in patients with coincident atopy.

## Acute vs. chronic treatment

The attractive aspect of inhibitors of these three classes of enzymes (syk, PI3K, and btk) is that inhibition of secretion is essentially 100% at pharmacologically relevant concentrations of the drugs. It is anticipated that 100% efficacy may be necessary to be clinically useful (more on this below). The unattractive aspect is that the targets are found in so many cell types. Even topical administration may cause problems because the locations for topical use are also at the interface between the environment and the mucosal immune response. One approach that was recently advanced takes advantage of two characteristics of the mast cell and basophil response [25]. All activation cascades are accompanied by the initiation of termination cascades so that the reactions do not become runaway. Indeed, a strong case can be made for viewing the termination steps (other terms being down-regulation, tachyphylaxis, or desensitization) as just as important in the expression of the magnitude of the response as the activation cascades (see below). In mast cells and basophils, it appears that all the identified termination pathways are dependent on signaling steps that precede activation of syk [9, 26]. If a mast cell/basophil is exposed to an inhibitor of syk, PI3K or btk (and presumably any crucial syk-dependent process), while also being exposed to IgE-mediated stimulation, then the cell does not secrete (befitting the high efficacy of these inhibitors), but it does desensitize. The drug can be removed and the mast cell/basophil remains desensitized (i.e., it can’t be stimulated to secrete). Presumably, continued exposure to the IgE-mediated stimulus will maintain the desensitized state and the drug need only have been used once. This approach would allow these very powerful drugs to be used once and leverages the nature of the signal transduction reaction to turn these cells off without the threat of secretion during the initiation-phase of the therapy. There are a number of ways that this approach could be manipulated to achieve the desired effect of selectively blunting the mast cell/basophil response.

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A second area of potential use that does not involve chronic administration is in the treatment of an incipient or ongoing anaphylactic reaction. Current treatment of this potentially life-threatening reaction while it is occurring is limited to suppression of the physiological effects of strong mast cell/basophil secretion using epinephrine (high dose steroids are also used but no one understands what these do or even if they are necessary). Use of a highly efficacious and potent inhibitor of mast cell secretion would seem to be a logical approach. However, in animal (*in vivo*) and human (*ex vivo*) studies, it is clear that very little mast cell secretion drives a potent end-organ response. For example, smooth muscle cells in the human airway show maximum contraction with 2–5% histamine release from airway mast cells ([27], where human airways are similar to guinea pig). Therefore, suppression of an ongoing reaction would likely require the level of efficacy that is seen in the test tube for inhibitors of syk, PI3K and btk. It is still debated whether mast cells/basophils are secreting and contributing to the escalating clinical picture 15 minutes after an anaphylactic reaction has started, (i.e., the secretory component of the reaction may be complete within 15 minutes). If the mast cell/basophil contribution is complete within minutes, then this approach would not work in the emergency department setting; the answer will not be known until highly efficacious inhibitors are tried in a clinical setting. Furthermore, patients that know they are at risk for anaphylactic reactions (peanut and bee venom allergies are good examples) carry epi-pens (single dose of epinephrine); it is not a stretch to have them also carry a therapeutic dose of an appropriate and efficacious mast cell inhibitor.

A less direct approach -- that might someday capitalize on the importance of key signaling molecules like syk -- is to control their expression selectively in mast cells/basophils. In humans, expression of syk in mast cells/basophils is uniquely poor relative to most other leukocytes [28]. For example, a peripheral blood basophil expresses 10-fold less syk than its close cousin leukocyte, the eosinophil (that is also highly involved in allergic inflammation). It is estimated that in human basophils, there are 5–8 FcεRI molecules for each molecule of syk (25,000 syk molecules per basophils vs. 150,000 molecules of FcεRI) [29]. Mast cells express about twice as much syk as basophils but still less than most leukocytes [10]. It appears that syk expression is tuned to be just sufficient to drive the IgE-mediated reaction. However, natural variation in the normal population around this average low level of expression results in cells that show a broad range of responsiveness, ranging from no IgE-mediated secretion to very high levels of secretion [29]. Thus far, this highly variable but blunted expression of a signaling molecule is limited to syk [29]. Other signaling molecules do not show the same heterogeneity of expression [30]. It is the fact that syk expression is uniquely tuned in these cells that offers a potential therapeutic approach if the mechanism for this unique tuning is found to be selective for mast cells/basophils. As will be noted below for SHP-1 (Src-homology region 2 (SH2) containing phosphatase-1), regulation of expression of an early signaling molecule does occur with some drugs. Likewise, there are efforts to regulate syk expression using interfering RNA (RNAi) [31]. But without targeting the specific expression control mechanisms, these approaches would run into problems similar to small molecule syk inhibitors.

## Leveraging the termination cascades

As noted above, the activation cascades are always accompanied by termination of signaling cascades (Figure 2). A dramatic demonstration of the relevance of these pathways to controlling the extent of the signaling reaction is found in the SHIP-pathway [32]. SHIP (SH2-containing inositol 5′-phosphatase) is a member of a family of enzymes that regulate the relative presence of phosphorylated inositol-containing phospholipids. For many immunoreceptors, a crucial early step involves the use of PI3K (see above). This complex biochemistry is regulated by activation-sensitive kinases (e.g., PI3K), and by both

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constitutively active and activation-dependent phosphatases. The SH2 domain of SHIP1 provides a means for this enzyme to be recruited into activation complexes to modulate the amplification of the early steps. A SHIP1-knockout mouse expresses a variety of immune system diseases and specifically shows extremely heightened activation of mast cells [33, 34]. In fact, even the binding of monomeric IgE, which does not usually induce an IgE-mediated activation cascade because receptor aggregation is normally required for initiation of these cascades, will activate the cells [35]. There is correlative evidence in humans that enhanced activity of SHIP1 suppresses basophil activation [36, 37]. A new class of drugs that activate SHIP1 (e.g., AQX-1125) are being tested for their usefulness in controlling the IgE-mediated diseases. *In vitro* data appear to support this potential therapeutic approach [38, 39]. The nexus of events related to phosphorylating phospholipids may also represent a therapeutic target if specific aspects of these complex reactions can be found to be selectively active in mast cells/basophils.

Exploiting termination cascades such as SHIP1 (and its associated elements, e.g., dok adaptors, rasGAP-like molecules [40]) [38], is a relatively new tactic, and one of the primary problems is that down-regulatory molecules need to be made active, rather than inhibited, in order to blunt an IgE-medated reaction. An equally powerful approach would be the activation of SHP-1. The SHP-1 family of enzymes are activation-sensitive tyrosine phosphatases that can de-phosphorylate the earliest signaling elements whose activities are dependent on phosphorylation of tyrosines. It is apparent that for the IgE-dependent reaction, strong activation of enzymes like SHP-1 can suppress secretion [41–43]. An excellent example of this phenomenon can be found in the reaction in which an allergen binds to both IgE on the cell surface and to solution-phase IgG. The allergen-bound IgG can then bind to IgG receptors on mast cells/basophils, specifically CD32b, and when this receptor is recruited into the evolving reaction complex, the recruitment of either SHIP1 or SHP-1 into the reaction effectively suppresses secretion [44]. This particular process is exploited in a therapeutic approach discussed below, but it demonstrates the potential power of targeting enzymes involved in terminating the ongoing reaction. Recent studies of the drug AKBA (acetyl-11-keto-β-boswellic acid), an anti-inflammatory in clinical use, suggest that it acts to suppress STAT3 phosphorylation by up-regulating the expression of SHP-1 [45]. Similar approaches might be applicable in mast cells/basophils.

## Downstream pathways

A necessary component of the secretory response of mast cells/basophils is the elevation of intracellular calcium. The mechanisms for producing an elevation are only partially known and potentially specific to these cells or even subsets of mast cells. Considerable excitement resulted from the identification of ICRAC channels as mediators of the store-operated calcium (SOC) influx mechanism [46] and a recent study has identified mRNA for ICRACM1-3 in human lung mast cells but protein only for ICRACM1 and M2 [47]. As with other shared signaling elements, the relative ubiquity of these proteins in mediating calcium transport raises questions about their usefulness as targets. However, there are indications that another mechanism for calcium mobilization may be important. The enzyme family of sphingosine kinases has been associated with the maintenance of the cytosolic calcium response in mast cells [48]. These observations appear to apply to both rodent and human mast cells [18, 49, 50]. Efforts to regulate sphingosine kinase for treatment of cancer have resulted in the development of several inhibitors [51–54]. As noted previously, in murine studies, the release of sphingosine-1-phosphate (S1P, a product of sphingosine kinase) may provide a positive autocrine mechanism of sustaining the mast cell response. It is possible that inhibition of sphingosine kinase(s) may act as this level of the response.

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Parenthetically, serendipity has played a role in identification of a pathway specific inhibitor of mast cell secretion. FTY720 was developed as an inhibitor of the S1P receptor and sphingosine kinase inhibitor, but it has also been found to inhibit cPLA2 [55]. Cytosolic PLA2 is necessary for the cleavage of arachidonic acid from phospholipids, and arachidonic acid is a precursor to the prostaglandins and leukotrienes that are an important mediator released from mast cells/basophils. Consistent with its inhibition of cPLA2, FTY720 was found inhibit prostaglandin D2 generation from a human mast cell line, LAD2, but to not inhibit degranulation.

Although ICRAC may be responsible for the primary influx of calcium, it is clear that fine-tuning of the cytosolic calcium response falls to other transport processes. For example, studies in rodent mast cells indicate that $Ca_V1.2$ (an L-type calcium channel sensitive to nifedipine) modulates the cytosolic calcium response [56]. Studies in rat basophilic leukemia cells identified an association between ICRAC and one of the TRP family of calcium channels [57]. In human skin mast cells, the cytosolic calcium response is regulated by a temperature sensing channel, although this channel has not yet been identified [58]. It is well recognized that potassium channels strongly modulate the functional response of T cells and an excellent example of using this information to alter disease states can be found in studies of a specific Kv1.3 channel blocker [59] and its modulation of T cells involved in autoimmune diseases. In human lung mast cells, the KCa3.1 channel (KCNN4, gene ID 3783) appears to modulate the cytosolic calcium response [60]. Therefore, if ICRAC is not a target, perhaps some of the collateral mechanisms for mediating the calcium response could be pharmacological targets.

## The root of the problem

Although there are numerous opportunities to inhibit the IgE-mediated reaction in mast cells/basophils, the most direct approach would be to simply eliminate the presence of IgE. Proof of this concept is now in hand; omalizumab is an antibody that is being used to treat moderate to severe asthma. This antibody works by binding to a region of IgE that is involved in IgE binding to FcεRI [61]. When administered to patients, free IgE levels (free, in this case, refers to IgE antibody that can still bind to FcεRI) are markedly reduced, whereas total circulating IgE levels actually rise because omalizumab bound to IgE increases its serum half-life by 20-fold. Reduction in free IgE results in marked down-regulation of FcεRI expression on mast cells/basophils due to the fact that this receptor only stays on the cell surface if bound to IgE (there may be therapeutic opportunity here as well) [62–64]. The combined reductions in free IgE and FcεRI expression can blunt future IgE-dependent activation events.

However, the quantitative aspects of this approach are important because the mast cell/basophil is exquisitely sensitive to IgE-mediated stimulation. A typical basophil from an atopic patient expresses 250,000 FcεRI per cell yet it needs only 200–500 for meaningful secretion [29]. Although only a fraction of the total cell-surface IgE is specific for a particular allergen, the sensitivity of these cells is an issue for this approach. A long-standing example of this difficulty comes from attempts to develop small organic molecules to compete with IgE binding to FcεRI.

Small molecule inhibitors of the binding reaction work well in slowing the forward binding reaction, but because the affinity of IgE for FcεRI is so high (dissociation half-life of 5–10 days), most small molecule inhibitors don’t ultimately reduce cell-bound IgE sufficiently to suppress allergen-mediated secretion (although refinements of this approach are still underway, [65, 66]). The studies with omalizumab define the requirements quite well; cell-surface suppression must be on the order of 99% [67]. Although omalizumab is a good

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proof-of-concept test case, this antibody's affinity for IgE is sufficiently weak that huge quantities of omalizumab are needed to effectively treat patients. There is considerable room for improvement even for this specific approach. Newer anti-IgE antibodies have much higher affinities, and these will more than likely improve the cost-benefit ratio of this therapeutic approach. Omalizumab does not appear to modulate the production of IgE, although early studies in mice suggested that it might. Consequently, there is no diminishment of IgE synthesis, only blockage of its binding.

Two newer approaches might achieve blockade of synthesis (Figure 3). One recent strategy uses a two-pronged approach. The antibody under development binds to IgE in a manner similar to omalizumab, which establishes a quality similar to omalizumab. But the Fc-tail of the antibody also binds with high affinity to CD32b [68]. As noted above, this immunoreceptor negatively regulates some cell types. In particular, CD32b engagement down-regulates B cells, and it is expected that this particular anti-IgE antibody will also down-regulate the development of plasma cells that secrete IgE. Preliminary studies support this view [68]. A second therapeutic strategy relies on the observation that membrane-associated IgE (as distinct from IgE bound to a receptor), has a unique peptide not seen on solution phase IgE [69]. An antibody that binds to this unique peptide sequence (CemX) down-regulates B cell development and should suppress plasma cell development, resulting in suppression of IgE synthesis [70].

In addition to these "passive" approaches, there is considerable effort to actively induce the production of highly selective anti-IgE antibodies by immunization with carefully chosen peptides from IgE [71–73]. This approach involves breaking immunological tolerance. There is, however, an advantage to needing to break tolerance. Studies in animals indicate that breaking tolerance is highly transient. Not sustaining the immunization generally results in a rapid decrease in antibody titers to the tolerized peptides. This characteristic serves to quell concerns that it may not be a good idea to permanently suppress free IgE levels. The risk of this approach is the uncontrolled generation of potentially anaphylactic anti-IgE antibodies.

## Concluding remarks

It seems likely that among the several new approaches to reducing the presence of IgE on mast cells/basophils, there will be a successful cost-effective strategy to suppressing this pathological reaction. The biological approaches to reducing free IgE levels may make it possible to strongly suppress any IgE-mediated reaction. But these approaches do not act quickly and cannot provide a therapeutic approach to acute reactions. To accomplish acute management, development of drugs that suppress the mast cell/basophil response is feasible. Studies *in vitro* have demonstrated the remarkable efficacy of new generations of early signaling inhibitors. There are several critical targets that have yet to be adequately tested in humans. The generally non-life-threatening nature of atopic disease, with the exception of anaphylaxis, has blunted enthusiasm for developing these potent inhibitors as chronic therapies for allergies. But there remain some clinical situations where strong inhibitors would be welcome. Perhaps through a coupling of the direct suppression of IgE with inhibitors of mast cell/basophil secretion, there will highly enhanced treatment modalities for these potentially dangerous diseases.

## Literature Cited

1. Sanderson MP, Wex E, Kono T, et al. Syk and Lyn mediate distinct Syk phosphorylation events in FcvarepsilonRI-signal transduction: implications for regulation of IgE-mediated degranulation. Mol Immunol. 2010; 48:171–8. [PubMed: 20828828]

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2. Hong H, Kitaura J, Xiao W, et al. The Src family kinase Hck regulates mast cell activation by suppressing an inhibitory Src family kinase Lyn. Blood. 2007; 110:2511–9. [PubMed: 17513616]
3. Vonakis BM, Haleem-Smith H, Benjamin P, et al. Interaction between the unphosphorylated receptor with high affinity for IgE and Lyn kinase. J Biol Chem. 2001; 276:1041–50. [PubMed: 11010962]
4. Wofsy C, Vonakis BM, Metzger H, et al. One lyn molecule is sufficient to initiate phosphorylation of aggregated high-affinity IgE receptors. Proc Natl Acad Sci U S A. 1999; 96:8615–20. [PubMed: 10411924]
5. Poderycki M, Tomimori Y, Ando T, et al. A minor catalytic activity of Src family kinases is sufficient for maximal activation of mast cells via the high-affinity IgE receptor. J Immunol. 2010; 184:84–93. [PubMed: 19949072]
6. Lavens-Phillips SE, Miura K, MacGlashan DW Jr. Pharmacology of IgE-mediated desensitization of human basophils: Effects of protein kinase C and Src-family kinase inhibitors. Biochem Pharmacol. 2000; 60:1717–27. [PubMed: 11077055]
7. Gomez G, Gonzalez-Espinosa C, Odom S, et al. Impaired FcepsilonRI-dependent gene expression and defective eicosanoid and cytokine production as a consequence of Fyn deficiency in mast cells. J Immunol. 2005; 175:7602–10. [PubMed: 16301670]
8. Siraganian RP, de Castro RO, Barbu EA, et al. Mast cell signaling: the role of protein tyrosine kinase Syk, its activation and screening methods for new pathway participants. FEBS Lett. 2010; 584:4933–40. [PubMed: 20696166]
9. MacGlashan DW Jr. Self-Termination/Anergic Mechanisms in Human Basophils and Mast Cells. Int Arch Allergy Immunol. 2009; 150:109–21. [PubMed: 19439977]
10. Havard S, Scola AM, Kay LJ, et al. Characterization of syk expression in human lung mast cells: relationship with function. Clin Exp Allergy. 2011; 41:378–88. [PubMed: 21255137]
11. Meltzer EO, Berkowitz RB, Grossbard EB. An intranasal Syk-kinase inhibitor (R112) improves the symptoms of seasonal allergic rhinitis in a park environment. J Allergy Clin Immunol. 2005; 115:791–6. [PubMed: 15806000]
12. Yuan Y, Liu H, Sahin A, et al. Reactivation of SYK expression by inhibition of DNA methylation suppresses breast cancer cell invasiveness. Int J Cancer. 2005; 113:654–9. [PubMed: 15455373]
13. Weinblatt ME, Kavanaugh A, Genovese MC, et al. An oral spleen tyrosine kinase (Syk) inhibitor for rheumatoid arthritis. N Engl J Med. 2010; 363:1303–12. [PubMed: 20879879]
14. Friedberg JW, Sharman J, Sweetenham J, et al. Inhibition of Syk with fostamatinib disodium has significant clinical activity in non-Hodgkin lymphoma and chronic lymphocytic leukemia. Blood. 2010; 115:2578–85. [PubMed: 19965662]
15. Smith AJ, Surviladze Z, Gaudet EA, et al. p110beta and p110delta phosphatidylinositol 3-kinases up-regulate Fc(epsilon)RI-activated Ca2+ influx by enhancing inositol 1,4,5-trisphosphate production. J Biol Chem. 2001; 276:17213–20. [PubMed: 11279065]
16. Windmiller DA, Backer JM. Distinct phosphoinositide 3-kinases mediate mast cell degranulation in response to G-protein-coupled versus FcepsilonRI receptors. J Biol Chem. 2003; 278:11874–8. [PubMed: 12529321]
17. Laffargue M, Calvez R, Finan P, et al. Phosphoinositide 3-kinase gamma is an essential amplifier of mast cell function. Immunity. 2002; 16:441–51. [PubMed: 11911828]
18. Jolly PS, Bektas M, Watterson KR, et al. Expression of SphK1 impairs degranulation and motility of RBL-2H3 mast cells by desensitizing S1P receptors. Blood. 2005; 105:4736–42. [PubMed: 15741218]
19. Miura K, Lavens-Phillips S, MacGlashan DW Jr. Localizing a control region in the pathway to LTC4 secretion following stimulation of human basophils with anti-IgE antibody. J Immunol. 2001; 167:7027–37. [PubMed: 11739523]
20. Bi L, Okabe I, Bernard DJ, et al. Proliferative defect and embryonic lethality in mice homozygous for a deletion in the p110alpha subunit of phosphoinositide 3-kinase. J Biol Chem. 1999; 274:10963–8. [PubMed: 10196176]
21. Bi L, Okabe I, Bernard DJ, et al. Early embryonic lethality in mice deficient in the p110beta catalytic subunit of PI 3-kinase. Mamm Genome. 2002; 13:169–72. [PubMed: 11919689]

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22. Kawakami Y, Kitaura J, Hartman SE, et al. Regulation of protein kinase CbetaI by two protein-tyrosine kinases, Btk and Syk. Proc Natl Acad Sci U S A. 2000; 97:7423–8. [PubMed: 10852954]
23. Iwaki S, Tkaczyk C, Satterthwaite AB, et al. Btk plays a crucial role in the amplification of Fc epsilonRI-mediated mast cell activation by kit. J Biol Chem. 2005; 280:40261–70. [PubMed: 16176929]
24. Macglashan D Jr, Honigberg LA, Smith A, et al. Inhibition of IgE-mediated secretion from human basophils with a highly selective Bruton's tyrosine kinase, Btk, inhibitor. Int Immunopharmacol. 2011; 11:475–9. [PubMed: 21238622]
25. MacGlashan DW Jr, Undem BJ. Inducing an Anergic State in Mast Cells and Basophils without Secretion. Journal of Allergy and Clinical Immunology. 2008; 121:1500–6. [PubMed: 18539198]
26. 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; 177:1040–51. [PubMed: 16818760]
27. Undem BJ, Pickett WC, Adams GKd. Antigen-induced sulfidopeptide leukotriene release from the guinea pig superfused trachea. Eur J Pharmacol. 1987; 142:31–7. [PubMed: 2446885]
28. Ishmael SS, MacGlashan DW Jr. Syk expression in peripheral blood leukocytes, CD34+ progenitors, and CD34-derived basophils. J Leukoc Biol. 2010; 87:291–300. [PubMed: 19889729]
29. MacGlashan DW Jr. Relationship Between Syk and SHIP Expression and Secretion from Human Basophils in the General Population. Journal of Allergy and Clinical Immunology. 2007; 119:626–33. [PubMed: 17336612]
30. Ishmael S, MacGlashan D Jr. Early signal protein expression profiles in basophils: a population study. J Leukoc Biol. 2009; 86:313–25. [PubMed: 19436043]
31. Yanni, JM.; Chatterton, JE.; Gamache, DA., et al., inventors. RNAi mediated inhibition of spleen tyrosine kinase-related inflammatory conditions. patent application no. US20090324507.
32. Conde C, Gloire G, Piette J. Enzymatic and non-enzymatic activities of SHIP-1 in signal transduction and cancer. Biochem Pharmacol. 2011; 82:1320–34. [PubMed: 21672530]
33. Huber M, Helgason CD, Damen JE, et al. The src homology 2-containing inositol phosphatase (SHIP) is the gatekeeper of mast cell degranulation. Proc Natl Acad Sci U S A. 1998; 95:11330–5. [PubMed: 9736736]
34. Sly LM, Rauh MJ, Kalesnikoff J, et al. SHIP, SHIP2, and PTEN activities are regulated in vivo by modulation of their protein levels: SHIP is up-regulated in macrophages and mast cells by lipopolysaccharide. Exp Hematol. 2003; 31:1170–81. [PubMed: 14662322]
35. Kalesnikoff J, Huber M, Lam V, et al. Monomeric IgE stimulates signaling pathways in mast cells that lead to cytokine production and cell survival. Immunity. 2001; 14:801–11. [PubMed: 11420049]
36. Eckman JA, Hamilton RG, Gober LM, et al. Basophil Phenotypes in Chronic Idiopathic Urticaria in Relation to Disease Activity and Autoantibodies. J Invest Dermatol. 2008; 128:1956–63. [PubMed: 18356810]
37. Vonakis BM, Saini SS. Syk-deficient basophils from donors with chronic idiopathic urticaria exhibit a spectrum of releasability. J Allergy Clin Immunol. 2008; 121:262–4. [PubMed: 18206515]
38. Kennah M, Yau TY, Nodwell M, et al. Activation of SHIP via a small molecule agonist kills multiple myeloma cells. Exp Hematol. 2009; 37:1274–83. [PubMed: 19703514]
39. Ong CJ, Ming-Lum A, Nodwell M, et al. Small-molecule agonists of SHIP1 inhibit the phosphoinositide 3-kinase pathway in hematopoietic cells. Blood. 2007; 110:1942–9. [PubMed: 17502453]
40. Tamir I, Stolpa JC, Helgason CD, et al. The RasGAP-binding protein p62dok is a mediator of inhibitory FcgammaRIIB signals in B cells. Immunity. 2000; 12:347–58. [PubMed: 10755621]
41. Ozawa T, Nakata K, Mizuno K, et al. Negative autoregulation of Src homology region 2-domain-containing phosphatase-1 in rat basophilic leukemia-2H3 cells. Int Immunol. 2007; 19:1049–61. [PubMed: 17675340]
42. Nakata K, Suzuki Y, Inoue T, et al. Deficiency of SHP1 leads to sustained and increased ERK activation in mast cells, thereby inhibiting IL-3-dependent proliferation and cell death. Mol Immunol. 2011; 48:472–80. [PubMed: 21044800]

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43. Nakata K, Yoshimaru T, Suzuki Y, et al. Positive and negative regulation of high affinity IgE receptor signaling by Src homology region 2 domain-containing phosphatase 1. J Immunol. 2008; 181:5414–24. [PubMed: 18832698]
44. Malbec O, Fong DC, Turner M, et al. Fc epsilon receptor I-associated lyn-dependent phosphorylation of Fc gamma receptor IIB during negative regulation of mast cell activation. J Immunol. 1998; 160:1647–58. [PubMed: 9469421]
45. Phromnoi K, Prasad S, Gupta SC, et al. Dihydroxypentamethoxyflavone down-regulates constitutive and inducible signal transducers and activators of transcription-3 through the induction of tyrosine phosphatase SHP-1. Mol Pharmacol. 2011; 80:889–99. [PubMed: 21816954]
46. Soboloff J, Spassova MA, Tang XD, et al. Orai1 and STIM reconstitute store-operated calcium channel function. J Biol Chem. 2006; 281:20661–5. [PubMed: 16766533]
47. Ashmole I, Duffy SM, Leyland ML, et al. CRACM/Orai ion channel expression and function in human lung mast cells. J Allergy Clin Immunol. 2012
48. Choi OH, Kim JH, Kinet JP. Calcium mobilization via sphingosine kinase in signaling by the FceRI antigen receptor. Nature. 1996; 380:634–6. [PubMed: 8602265]
49. Jolly PS, Bektas M, Olivera A, et al. Transactivation of sphingosine-1-phosphate receptors by FcepsilonRI triggering is required for normal mast cell degranulation and chemotaxis. Journal of Experimental Medicine. 2004; 199:959–70. [PubMed: 15067032]
50. Oskeritzian CA, Alvarez SE, Hait NC, et al. Distinct roles of sphingosine kinases 1 and 2 in human mast-cell functions. Blood. 2008; 111:4193–200. [PubMed: 18178871]
51. Kapitonov D, Allegood JC, Mitchell C, et al. Targeting sphingosine kinase 1 inhibits Akt signaling, induces apoptosis, and suppresses growth of human glioblastoma cells and xenografts. Cancer Res. 2009; 69:6915–23. [PubMed: 19723667]
52. French KJ, Zhuang Y, Maines LW, et al. Pharmacology and antitumor activity of ABC294640, a selective inhibitor of sphingosine kinase-2. J Pharmacol Exp Ther. 2010; 333:129–39. [PubMed: 20061445]
53. Edmonds Y, Milstien S, Spiegel S. Development of small-molecule inhibitors of sphingosine-1-phosphate signaling. Pharmacol Ther. 2011; 132:352–60. [PubMed: 21906625]
54. Schnute ME, McReynolds MD, Kasten T, et al. Modulation of cellular S1P levels with a novel, potent and specific inhibitor of sphingosine kinase-1. Biochem J. 2012
55. Payne SG, Oskeritzian CA, Griffiths R, et al. The immunosuppressant drug FTY720 inhibits cytosolic phospholipase A2 independently of sphingosine-1-phosphate receptors. Blood. 2007; 109:1077–85. [PubMed: 17008548]
56. Yoshimaru T, Suzuki Y, Inoue T, et al. L-type Ca2+ channels in mast cells: activation by membrane depolarization and distinct roles in regulating mediator release from store-operated Ca2+ channels. Mol Immunol. 2009; 46:1267–77. [PubMed: 19128833]
57. Ma HT, Peng Z, Hiragun T, et al. Canonical transient receptor potential 5 channel in conjunction with Orai1 and STIM1 allows Sr2+ entry, optimal influx of Ca2+, and degranulation in a rat mast cell line. J Immunol. 2008; 180:2233–9. [PubMed: 18250430]
58. Lawrence ID, Warner JA, Cohan VL, et al. Purification and characterization of human skin mast cells. Evidence for human mast cell heterogeneity. J Immunol. 1987; 139:3062–9. [PubMed: 2444649]
59. Beeton C, Wulff H, Standifer NE, et al. Kv1.3 channels are a therapeutic target for T cell-mediated autoimmune diseases. Proc Natl Acad Sci U S A. 2006; 103:17414–9. [PubMed: 17088564]
60. Duffy SM, Berger P, Cruse G, et al. The K+ channel iKCA1 potentiates Ca2+ influx and degranulation in human lung mast cells. J Allergy Clin Immunol. 2004; 114:66–72. [PubMed: 15241346]
61. Chang TW, Davis FM, Sun N-C, et al. Monoclonal antibodies specific for human IgE-producing B cells: A potential therapeutic for IgE-mediated allergic diseases. Bio/Technology. 1990; 8:122–6. [PubMed: 1369991]
62. MacGlashan JDW, Bochner BS, Adelman DC, et al. Down-regulation of FceRI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. J Immunol. 1997; 158:1438–45. [PubMed: 9013989]

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63. MacGlashan DW Jr, Xia HZ, Schwartz LB, et al. IgE-regulated expression of FceRI in human basophils: Control by regulated loss rather than regulated synthesis. J Leuk Biol. 2001; 70:207–18.

64. Borkowski TA, Jouvin MH, Lin SY, et al. Minimal requirements for IgE-mediated regulation of surface Fc epsilon RI. J Immunol. 2001; 167:1290–6. [PubMed: 11466345]

65. Hunt J, Bracher MG, Shi J, et al. Attenuation of IgE affinity for FcepsilonRI radically reduces the allergic response in vitro and in vivo. J Biol Chem. 2008; 283:29882–7. [PubMed: 18703499]

66. Harwood NE, McDonnell JM. The intrinsic flexibility of IgE and its role in binding FcepsilonRI. Biomed Pharmacother. 2007; 61:61–7. [PubMed: 17188833]

67. Saini SS, MacGlashan DW Jr, Sterbinsky SA, et al. Down-regulation of human basophil IgE and FceRIa surface densities and mediator release by anti-IgE infusions is reversible in vitro and in vivo. J Immunol. 1999; 162:5623–30.

68. Chu SY, Horton HM, Pong E, et al. Reduction of total IgE by targeted coengagement of IgE B-cell receptor and FcgammaRIIb with Fc-engineered antibody. J Allergy Clin Immunol. 2012; 129:1102–15. [PubMed: 22257644]

69. Peng C, Davis FM, Sun LK, et al. A new isoform of human membrane-bound IgE. J Immunol. 1992; 148:129–36. [PubMed: 1727861]

70. Chen HY, Liu FT, Hou CM, et al. Monoclonal antibodies against the C(epsilon)mX domain of human membrane-bound IgE and their potential use for targeting IgE-expressing B cells. Int Arch Allergy Immunol. 2002; 128:315–24. [PubMed: 12218370]

71. Wang CY, Walfield AM, Fang X, et al. Synthetic IgE peptide vaccine for immunotherapy of allergy. Vaccine. 2003; 21:1580–90. [PubMed: 12639479]

72. Kricek F, Ruf C, Rudolf MP, et al. IgE-related peptide mimotopes. Basic structures for anti-allergy vaccine development. Int Arch Allergy Immunol. 1999; 118:222–3. [PubMed: 10224388]

73. Peng Z, Liu Q, Wang Q, et al. Novel IgE peptide-based vaccine prevents the increase of IgE and down-regulates elevated IgE in rodents. Clin Exp Allergy. 2007; 37:1040–8. [PubMed: 17581197]

74. Weis RM, Balakrishnan K, Smith BA, et al. Stimulation of fluorescence in a small contact region between rat basophil leukemia cells and planar lipid membrane targets by coherent evanescent radiation. J Biol Chem. 1982; 257:6440–5. [PubMed: 6804467]

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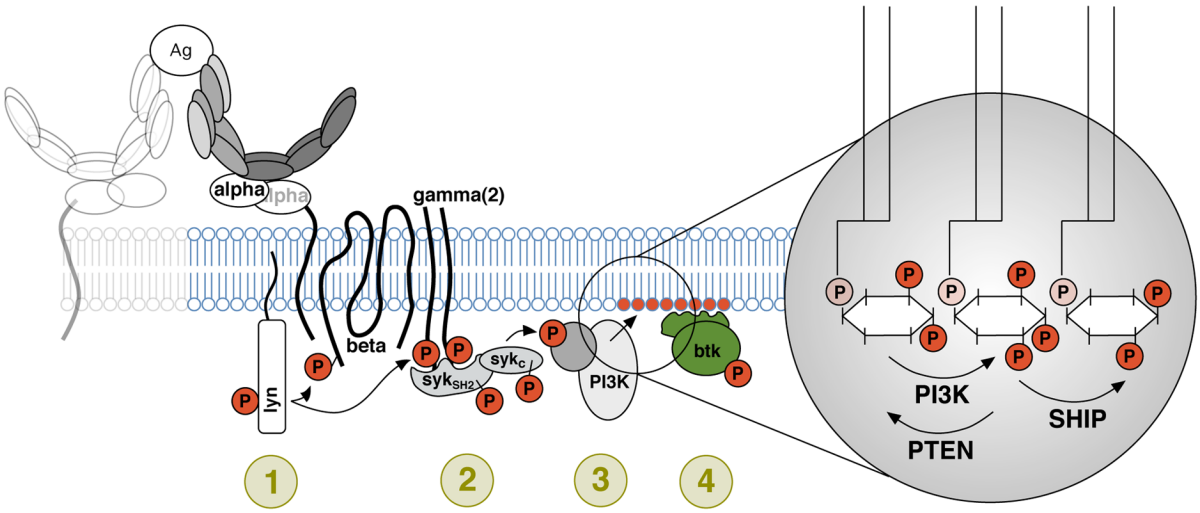


**Figure 1.**

Simplified cartoon of the earliest steps in IgE/FcεRI-dependent signaling. IgE-mediated stimulation of mast cells/basophils begins with aggregation of FcεRI when multivalent substances bind to the cell-surface IgE, which is bound to FcεRI. Some form of aggregation is a requisite for activation, although there is considerable variability in how the aggregate can be formed. For example, in addition to the standard model of antigen:antibody aggregation, simply concentrating FceRI into a localized region of the cell membrane can induce activation [74]. Aggregation appears to be necessary to position the beta subunits of FcεRI so that they are trans-phosphorylated by a closely associated src-family kinase, lyn. This is step **1**. The phosphorylated beta subunit of FcεRI binds lyn more tightly, allowing lyn to phosphorylate the gamma subunit of FcεRI. The phosphorylated gamma subunit recruits syk. This is step **2**. Syk auto-phosphorylates and becomes more active, initiating many subsequent steps. One substrate of syk is the regulatory subunit of PI3K (phosphotidylinositol 3′ kinase) which becomes active and begins phosphorylating inositol phospholipids. This is step **3**. The expanded portion of the cartoon shows just a couple of the possible forms of phosphorylated inositol phospholipids. These particular forms are regulated by multiple enzymes including PI3K, SHIP (SH2-containing inositol-5′-phosphatase) and PTEN (phosphatase and tensin homolog deleted in chromosome 10) the latter of which simply reverses the effects of PI3K. Activity of PTEN appears constitutive but controls the set-point of these reactions. The phosphotidyl-3,4,5-trisphosphate (PIP3) in the plasma membrane acts as a recruitment site for other enzymes including btk (which binds through a PH domain). This is step **4**. Btk is activated through phosphorylation, probably by a src-family kinase (not shown). Each of these enzymes in steps **1–4** mediate multiple downstream signaling steps that are critical for initiation and maintenance of the secretory response in mast cells/basophils. Inhibition of each step can completely ablate IgE-mediated functions, although inhibition of lyn needs to be nearly complete to see suppression due to its role in initiating termination steps.

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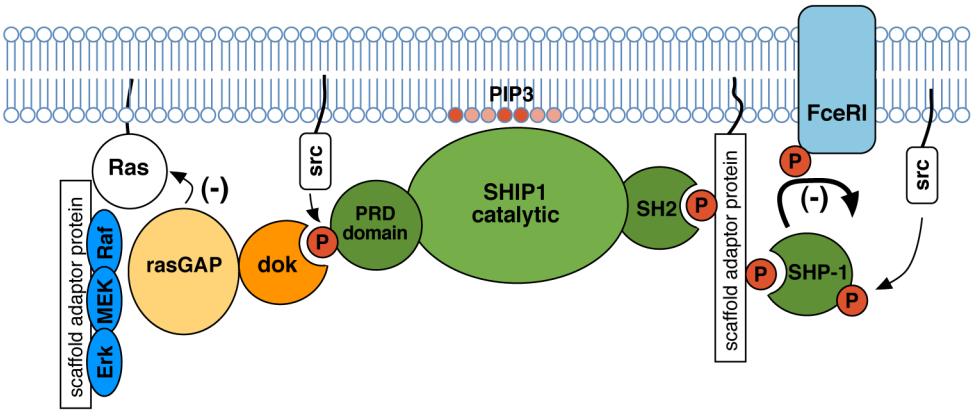


**Figure 2.**

Termination steps during IgE-mediated activation. The cartoon expands on the behavior of SHIP presented in figure 1. This multi-domain enzyme is capable of regulating the presence of PIP3 which recruits various activation enzymes to the plasma membrane. But at the C-terminal end of SHIP, near the proline rich domain (PRD), a tyrosine phosphorylated by a src-family kinase, can recruit the adaptor protein dok (downstream of kinase) for which there are several forms. Dok recruits a rasGAP protein which down-regulates the activity of p21 ras, another small G-protein that is crucial in the initiation of the MAPK (mitogen-activated protein kinases) pathway and other pathways to secretion. The MAPK kinase pathway (raf->MEK->Erk) specifically regulates cPLA2 and the arachidonic acid metabolism pathway but p21Ras controls other pathways as well. On the right side of the cartoon is a representation of the potential role of SHP-1 (SH2-containing tyrosine phosphatase). SHP-1 represents a family of tyrosine phosphatases that are actively recruited into activation cascades, but there are also constitutively acting tyrosine phosphatases that control set-points for activation. The cartoon represents only a small subset of the negative feedback loops that operate during activation, but these two phosphatases act as negative feedback on early steps of the cascade.

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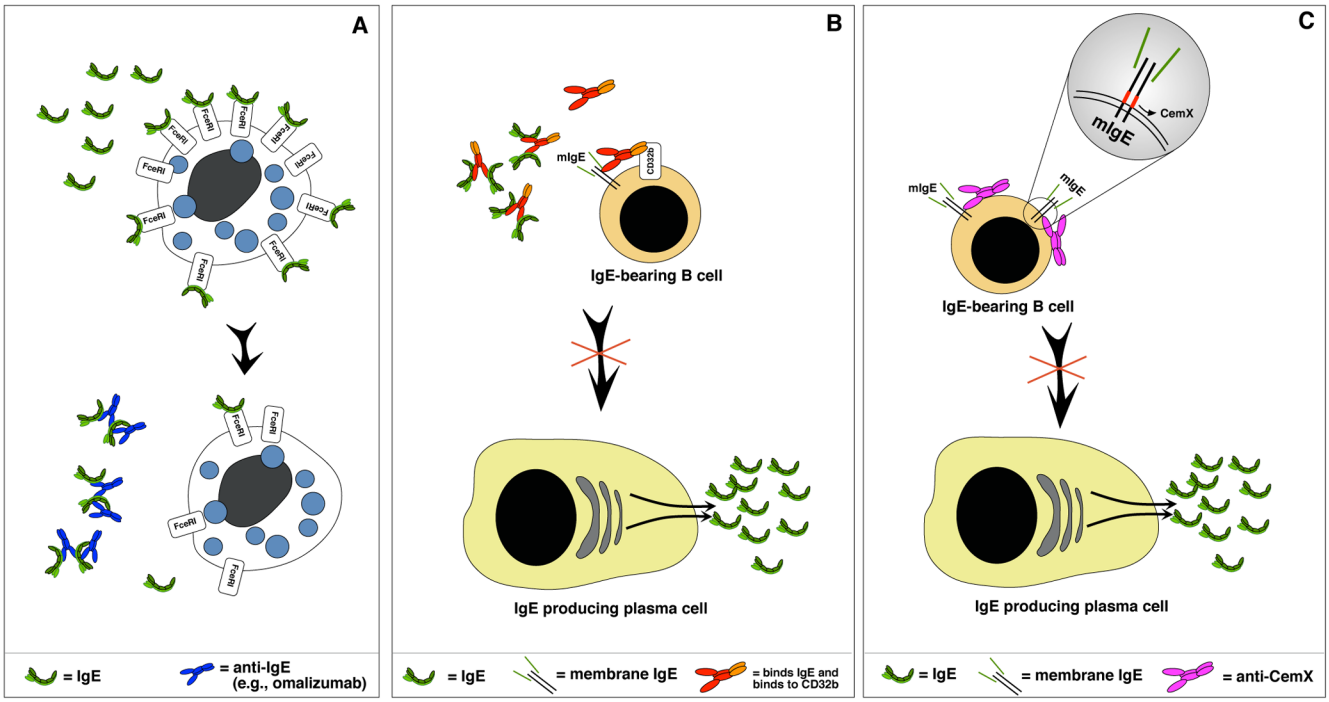


**Figure 3.**

Therapeutic approaches to down-regulating the presence of IgE. Panel A shows the schema for the sole current IgE-reducing approach. Omalizumab is an anti-IgE antibody that binds to an epitope on IgE that is necessary for interaction with FcεRI prevents IgE from binding to FcεRI. Because the specific epitope is sterically inaccessible when IgE is bound to the receptor, this approach should not result in direct activation of mast cells/basophils. However, the approach results in large amounts of circulating total IgE because it does not influence the synthesis of IgE. The current therapeutic, omalizumab, has an affinity that is not equivalent to the natural affinity of IgE for FcεRI. Consequently, high quantities of omalizumab are needed for treatment to effectively reduce free IgE levels. But this process is assisted by the natural biology of FcεRI expression control. An unoccupied cell surface FcεRI is not stable and leaves the cell surface with a half-life (*in vitro*) of 1 day. Consequently, reductions in free IgE also result in reductions in the density of FcεRI on mast cells/basophils. This effect is a double-edged sword because slight increases in free IgE result in more dramatic increases in cell surface IgE than would expected on the basis of equilibrium binding because FcεRI expression increases. However, higher affinity anti-IgE antibodies working by the same mechanism may solve many of the problems associated with omalizumab. Panels B and C illustrate two methods to block the transition of B cells to IgE-secreting plasma cells. Panel B; the illustration shows a hybrid approach in which an anti-IgE antibody is administered that accomplishes the goals of omalizumab but also suppresses IgE synthesis. It does this by engineering the Fc portion of the therapeutic antibody to bind with high affinity to CD32b on B cells. Co-crosslinking the CD32b with the plasma membrane IgE that is exclusively on B cells that have class switched, (i.e. it does not affect B cells destined for synthesis of other antibody classes) suppresses the progression of the B cells towards plasma cells. Panel C; the cartoon depicts a third approach. Fortuitously, membrane-associated IgE includes a short peptide sequence near the transmembrane segment (labeled CemX) that is not found on solution phase IgE. Antibodies to this short sequence target B cells destined to make plasma cells that secrete IgE and crosslinking the membrane IgE induces suppression of the B cell-to-plasma cell transition.