

# Decoding IgE Fc Receptors

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## Abstract

Immunoglobulin E (IgE) plays a central role in the pathogenesis of allergic diseases by interacting with two membrane receptors: high-affinity FcεRI and low-affinity FcεRII (CD23). Allergen-induced IgE-occupied FcεRI aggregation on the mast cell or basophil cell surface leads to the activation of intracellular signaling events and eventually the release of pre-formed and *de novo* synthesized inflammatory mediators. The role of FcεRII in allergic diseases has been proposed to include regulation of IgE synthesis, enhanced histamine release from basophils, and a contribution to Ag–IgE complex presentation but the exact function of CD23 remains poorly understood. This review summarizes some new developments in IgE Fc-receptor studies with an emphasis on regulation of FcεRI expression and signal transduction, including monomeric IgE, lipid raft segregation, and some recently identified negative regulators. A better understanding of signaling events following IgE FcR aggregation will shed new light on how allergy patients might be treated more safely and effectively.

## Key Words

Allergy  
Anti-IgE therapy  
CD23  
FcεRI  
FcεRII  
IgE receptors  
Immunoglobulins  
ITAMs  
ITIMs  
Lipid raft segregation  
RabGEF1

## Introduction

Immunoglobulin (Ig) molecules consist of two variable antigen binding (Fab) regions and one relatively constant (Fc) region that interacts with different effector molecules such as complement and Fc receptors (FcRs) on phagocytes and other immune cells. Five

different classes (IgA, IgD, IgE, IgG, and IgM) of Igs (antibodies, Abs) in mammals have distinct structures, distributions, and biologic functions. IgM and IgG have a crucially important role in protection against invading bacteria, viruses, and fungi, whereas

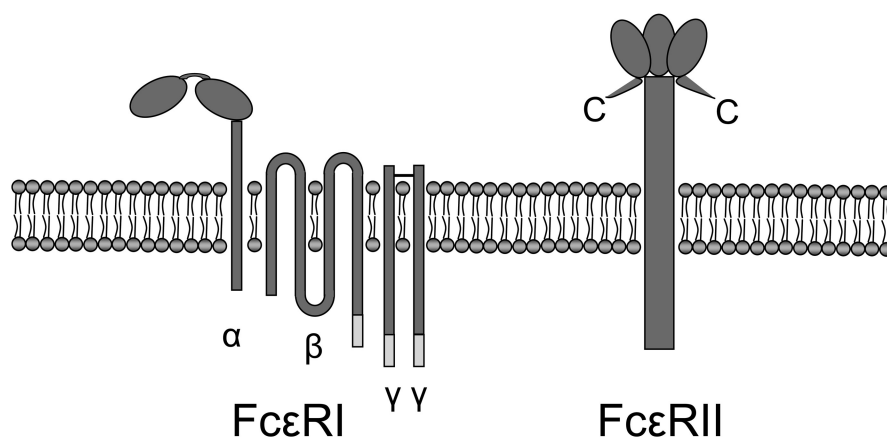


**Fig. 1.** IgE Fc receptors. FcεRI displays a  $\alpha\beta\gamma_2$  tetramer and FcεRII is a C-lectin trimer.

IgA has a pivotal role in mucosal surface defense. While the exact function of IgD remains largely unknown, IgE is known to play a central role in the pathogenesis of allergic inflammatory diseases. To date, two different types of membrane FcRs for IgE, the high-affinity receptor (FcεRI) and the low-affinity receptor (FcεRII), have been identified (1).

FcεRI is constitutively expressed in human and murine mast cells and basophils. It is composed of four subunits ( $\alpha\beta\gamma_2$ ): an IgE-binding  $\alpha$ -chain; a receptor-stabilizing and signal-amplifying  $\beta$ -chain; and a disulfide-bond-linked  $\gamma$ -chain homodimer, which is the major signal transducer (Fig. 1). Aggregation of FcεRI occupied by allergen-induced IgE, leads to phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAM), found within  $\beta$  and  $\gamma$  chains, by activation of Lyn, a Src family protein tyrosine kinase (PTK) which is normally weakly associated with the  $\beta$  chain. Phosphorylated ITAMs then recruit Lyn and Syk (another Src family PTK), respectively. Following Syk activation, the linker for activation of T cells (LAT) plays a key role as a scaffold that acti-

vates several signaling pathways, including the phospholipase C (PLC) and calcium ( $\text{Ca}^{2+}$ ) pathways and the mitogen-activated protein kinase (MAPK) pathway (2). Recently, another Src family PTK, Fyn, was also shown to associate with FcεRI  $\beta$ -chain and play a complementary role in this signal transduction through activation of the phosphatidylinositol 3 kinase (PI3K) pathway (3). These signaling events eventually lead to degranulation, secretion of granule-stored inflammatory mediators including histamine and serotonin, and synthesis and release of eicosanoids (leukotrienes and prostaglandins), cytokines, and chemokines (4) (Fig. 2).

The low-affinity receptor FcεRII (CD23) is different from FcεRI in that it does not belong to the Ig receptor superfamily but is a type II transmembrane protein with a calcium-dependent lectin domain in the C-terminal extracellular end (Fig. 1). The human CD23 gene has been mapped to chromosome 19p13 and exists in two isoforms: CD23a expressed constitutively on B cells and CD23b found on a wide variety of cells including T cells, macrophages, eosinophils, and other hematopoietic cells having been induced by IL-4 (Table 1). CD23

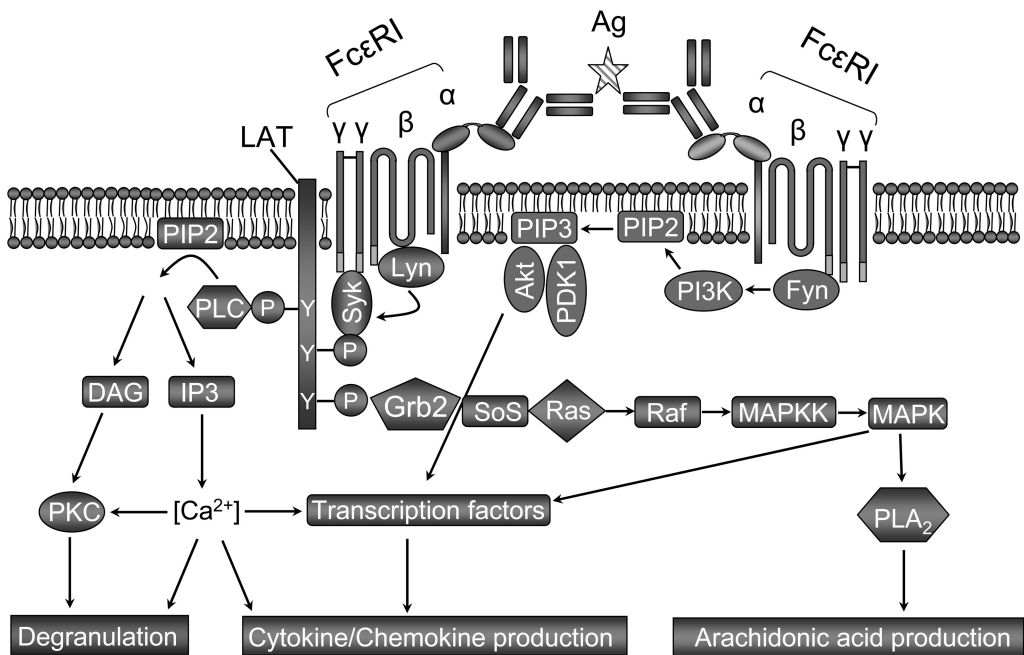


**Fig. 2.** The classical signaling cascade in activated mast cells. Following allergen-induced IgE-occupied FcεRI aggregation, FcεRIβ-associated Lyn and Fyn are activated to initiate the downstream signaling cascade.

**Table 1.** Structures and biological functions of FcεRI and FcεRII

|                         | FcεRI                                                                                                                                                                                                                                                                           | FcεRII (CD23)                                                                                                                                                                                        |
|-------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Affinity                | High                                                                                                                                                                                                                                                                            | Low                                                                                                                                                                                                  |
| Structure               | Ig superfamily, αβγ <sub>2</sub> or αγ <sub>2</sub>                                                                                                                                                                                                                             | Type II TM with C-lectin domain, two isomers: CD23a and CD23b                                                                                                                                        |
| Chromosome localization | 1q23, 11q13, 1q23                                                                                                                                                                                                                                                               | 19p13                                                                                                                                                                                                |
| Cell distribution       | Mast cells, basophils, APC                                                                                                                                                                                                                                                      | CD23a: B cells<br>CD23b: T cells, macrophages, eosinophils, dendritic cells                                                                                                                          |
| ITAM                    | Yes                                                                                                                                                                                                                                                                             | No                                                                                                                                                                                                   |
| Biological functions    | <ul style="list-style-type: none"> <li>• Induce degranulation</li> <li>• Induce synthesis and release of inflammatory mediators (LTs and PGs)</li> <li>• Induce synthesis and release of cytokines and chemokines (IL-4 and MCP)</li> <li>• Help Ag-IgE presentation</li> </ul> | <ul style="list-style-type: none"> <li>• Regulate of IgE synthesis</li> <li>• Enhance histamine release</li> <li>• Help Ag-IgE presentation</li> <li>• Promote B cell growth and survival</li> </ul> |

at the cell surface is labile and a soluble fragment (sCD23) is released from both human and murine cells. Matrix metalloprotease is suggested to be responsible for the initial cleavage

of membrane-bound CD23 and then to yield an array of sCD23s with different molecular weights. Although CD23 has been implicated in a variety of biological activities, probably

due to its extensively distributed ligands including IgE, here we deal only with its role in allergic inflammatory responses (5). We focus on regulation of FcεRI expression and signal transduction.

## High-Affinity IgE Receptor (FcεRI)

### Cell Distribution and Function

FcεRI was initially thought to be expressed exclusively on mast cells and basophils but firm evidence shows that FcεRI has a broader distribution, and is found on Langerhans cells, eosinophils, monocytes, and cells derived from monocytes including dendritic cells (DCs), on the surfaces of which the receptor is found as a non-classical trimer  $\alpha\gamma_2$ , rather than the classical tetramer  $\alpha\beta\gamma_2$  presented on mast cells and basophils. The human FcεRI  $\beta$ -gene has been mapped to chromosome 11q13 whereas both the FcεRI  $\alpha$ - and  $\gamma$ -genes are located in chromosome 1q23, suggesting the possibility of their coordinate regulation (Table 1). Mature mast cells usually stay in the vascularized tissues and have a long life span whereas basophils typically mature in the bone marrow and then circulate in the peripheral blood. Although basophils were often considered to have a minor role in the pathogenesis of allergic inflammation, due to their low circulating population and short life spans, a recent study has demonstrated that basophils play a critical role in the development of IgE-mediated chronic allergic inflammation independently of mast cells (6). Transfer of FcεRI-expressing basophils into FcεRI-deficient mice restored the delayed-onset of allergic inflammation but not the immediate reaction, indicating the important role of mast cells and basophils in the immediate and delayed allergic response, respectively. Even though the detailed mechanism of action of basophils in chronic allergic inflammation remains unclear, it seems unlikely that basophils act as inflammatory

effector cells but rather as initiators or propagators, which, after activation, have been shown to be able to secrete large amounts of Th2 cytokines and chemokines including IL-4 and IL-13, which are key mediators of allergic inflammation (6).

FcεRI expressing on antigen-presenting cells such as Langerhans cells and DCs can deliver IgE-bound antigens into the MHC class II antigen-presentation pathway and induce Th2 response, which is finely tuned in vivo (Fig. 3). For example, human plasmacytoid dendritic cells (pDCs) have a high density of type I IFN, are the hallmark of the Th1 response which can be induced by binding of CpG-oligodeoxynucleotide (ODN) to Toll-like receptor 9 (TLR9). However, pDCs are also found in allergic lesions and in the presence of FcεRI. This dichotomy was thoroughly investigated recently (7). IgE crosslinking was found to reduce TLR9 expression and inhibit the ability of pDCs to secrete IFN- $\alpha$  when stimulated with CpG-ODN, whereas TLR9 exposure to CpG-ODN first caused a marked reduction (around 15-fold) of FcεRI mRNA levels and even loss of the FcεRI protein expression in pDCs. These findings indicate crosstalk between IgE-mediated allergic inflammation and bacteria DNA-initiated specific innate immune response in pDCs, so that the Th1/Th2 balance is broken and the response ensues. FcεRI is also found on the surface of human airway smooth muscle cells (ASMs) in both cultured cell lines and primary cells from asthmatic bronchial biopsies (8). Crosslinking of FcεRI on ASMs by IgE and anti-IgE induces a rapid mobilization of intracellular calcium, one of the crucial signals to trigger smooth muscle contraction. This then causes release of inflammatory cytokines and chemokines, IL-4, IL-5, IL-13, and eotaxin, which can amplify and perpetuate airway inflammation. The addition of anti-FcεRI  $\alpha$ -chain blocking

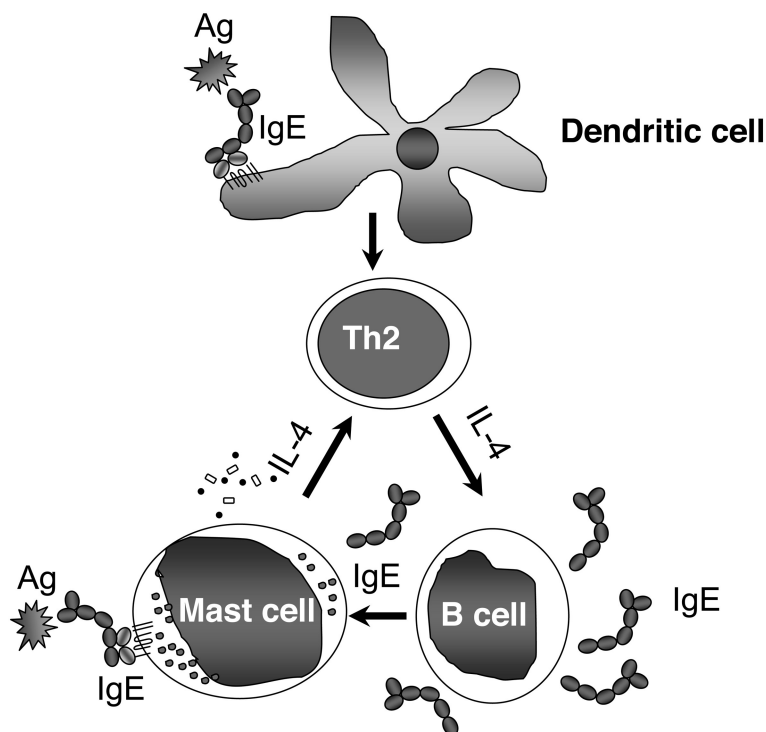


**Fig. 3.** Antigen presentation mediated by FcεRI-bearing dendritic cells (DCs). DCs can present Ag to CD4+ Th cells and induce Th2 responses, leading to a positive feedback loop.

antibodies against IgE binding sites significantly inhibits this release, suggesting a promising therapeutic strategy.

### Regulation of FcεRI Expression

Two types of FcεRI, tetramer  $\alpha\beta\gamma_2$  and trimer  $\alpha\gamma_2$ , are expressed on cell surfaces. This expression is strongly influenced by at least two factors: expression of FcεRIβ-chain and local IgE concentration. They need to have three subunits assembled in order to be expressed on murine cell surfaces, and that  $\alpha\gamma_2$  has 100-fold less density than  $\alpha\beta\gamma_2$  on human cells, highlights the critical role of FcεRIβ in the assembly of FcεRI. FcεRIβ<sub>T</sub>, a splice variant of FcεRIβ, includes the fifth intron of the FcεRIβ gene, which contains a

stop codon and therefore produces a truncated version of FcεRIβ. When FcεRIβ<sub>T</sub> associates with FcεRIα, which contains an endoplasmic reticulum (ER) retention sequence that allows FcεRI to enter the Golgi for glycosylation and migration to the surface of membrane, FcεRIβ<sub>T</sub> masks this sequence and the complex can then be targeted for proteasomal degradation (9). A more recent study shows that post-translational processing in ER is the rate-limiting step in the expression of functional FcεRI at the cell surface. FcεRIβ<sub>T</sub> slows the conversion of α-chain to its proper conformation and induces the accumulation of unglycosylated and misfolded α-chain, which is subjected to proteasomal degradation (10).

Even though the gene sequence of FcεRI has been well established, its *cis* and *trans*

acting factors are still poorly understood. Therefore, polymorphisms of the FcεRI gene, especially the FcεRIβ gene, have been intensively investigated. A single nucleotide polymorphism (SNP) was identified in the coding region of FcεRIβ. This E237G variant was shown to correlate with higher expression of FcεRI on circulating basophils. Two other SNPs tightly linked to the E237A in the FcεRIβ promoter region were also found to increase promoter activity. This study provides a direct linkage between the FcεRIβ SNPs and atopy (11). Another SNP in the promoter region of FcεRIα gene was also shown to increase transcription activity by introducing an additional GATA-1 site to the promoter (12).

It is long known that treatment of cultured mast cells or basophils with IgE leads to upregulation of cell surface FcεRI. While FcεRI is constitutively synthesized and assembled, IgE controls the loss of receptor once it appears on cell surface. Ligation with IgE prevents FcεRI from internalization and degradation that otherwise constantly removes the free FcεRI from cell surfaces (13). Although the protective function of IgE has been widely accepted, the mechanism of loss of receptor remains unknown. The identification of a high-affinity natural autoantibody against the IgE binding site on the FcεRIα from human cord blood may shed light on how the turnover of FcεRI is regulated (13). This natural antibody, CBMα8, is strictly in germline configuration and has high affinity, comparable to that of IgE. Inhibition experiments indicate both CBMα8 and an anti-FcεRIα autoantibody from healthy and chronic urticaria donors have the common epitope on FcεRIα, which coincides with that bound by IgE. Since the mature anti-FcεRIα Ab has much lower affinity than natural Ab, the authors propose that random mutations in this autoantibody during maturation may lower its affinity below a threshold level that

would be needed for removal of the autoreactive clone, thereby permitting escape from tolerance. Under physiological conditions, most autoepitopes on FcεRIα are masked by IgE because of its extremely high affinity. However, the small amount of low affinity anti-FcεRIα autoantibody may mediate the internalization of FcεRI resulting in a dynamic balance.

## Regulation of FcεRI Signaling

### Monomeric IgE and FcεRI Signaling

It has long been documented that the FcεRI aggregation, rather than IgE binding that protects FcεRI from internalization and further degradation, is essential to trigger mast cell responses (1, 14). However, this view has been challenged by a series of reports that monomeric IgE (mIgE) binding to FcεRI does initiate cell signaling events and further leads to several important cellular activities, such as enhancing mast cell survival (15), degranulation and cytokine production (16), cell adhesion (17), and migration (18). It appears that IgE molecules display heterogeneity. Different IgE molecules induce various degrees of activation: highly cytokinergic (HC) IgEs (for example, SPE-7), induce the production and secretion of various cytokines and other activation events including degranulation, whereas poorly cytokinergic (PC) IgEs (including H1-DNP-ε-206), do so inefficiently. The structural difference that accounts for functional divergence is as yet unknown (19). Although this phenomenon that mIgE alone can initiate cell signaling was originally considered a breakthrough at the requirement for the initiation of signaling events, two recent reports provide compelling illustrations of how mIgEs may trigger FcεRI aggregation. James and colleagues (20) have studied the crystal structures and pre-steady-state binding kinetics of mIgE, the SPE-7,

revealing that SPE-7 binding site may exist as at least two distinct isomers, which greatly differ in their specificities and affinities. One has high affinity for the immunizing hapten (DNP), termed hapten-binding Ab; the other has physiological affinity for a DNP-unrelated protein antigen (Trx-Shear 3), termed protein-binding Ab, which possesses a promiscuous, low-affinity binding site for aromatic ligands including DNP. This conformational diversity of mIgE implies that some unknown multivalent ligands may crosslink mIgE and cause FcεRI aggregation. A more recent report suggests that non-physiological experimental conditions were used in all mIgE studies (21). The exceptionally high FcεRI cell surface expression and all receptors are occupied by the same mIgE, which is in contrast to the *in vivo* physiological situation where the low concentration of the whole specified IgEs repertoire of IgEs may contribute to triggering FcεRI aggregation upon binding of mIgE. Therefore, rather than a novel mechanism, the role of some non-physiological agents is the likely explanation for the observation of initiation of signaling events by mIgE ligation alone.

### Lipid Segregation and FcεRI Signaling

A recent advance in understanding the role of submembranous compartments in FcεRI signaling has two general aspects: segregation of transmembrane proteins in lipid raft plays a fundamental role in orchestrating cell signaling events and the membrane-associated cytoskeleton plays an important role in regulating signaling. Cell signaling is thought to take place in specialized compartments. One is lipid rafts, characteristics of which include low density, detergent-resistance, composed of tightly packed glycosphingolipids and cholesterol, and organized in a liquid-ordered ( $L_O$ ) phase. Because signaling molecules are concentrated in lipid rafts, whereas others are

excluded, these rafts have been proposed to function as signaling platforms. Early studies show that ligand-mediated crosslinking of FcεRI on mast cells causes their association with detergent-resistant lipid rafts, which correlates with the initiation of tyrosine phosphorylation of FcεRI. Depletion of cholesterol in cell membrane leads to loss of both crosslinked FcεRI and Lyn from lipid rafts, thereby inhibiting Lyn-mediated phosphorylation of FcεRI ITAM (22). According to a current model (Fig. 4), FcεRI is excluded from lipid rafts in resting mast cells, whereas the downstream signaling molecules, such as Lyn, covalently associate with fatty acids and therefore concentrate in lipid rafts. Upon aggregation, FcεRI translocates into rafts and becomes proximal to raft-localized Lyn. However, it is still poorly understood how the earliest events in FcεRI signaling take place, including how crosslinked receptors associate with lipid rafts, how receptor ITAMs are phosphorylated, and the exact function of lipid rafts in the signaling cascade.

One hypothesis concerning the function of lipid rafts is that the raft environment protects the phosphorylated tyrosine residue of Lyn from dephosphorylation by phosphatases that may be excluded from the rafts. In order to test this hypothesis, Young and colleagues (23) co-transfect FcεRI and Lyn in a Chinese hamster ovary (CHO) cell line, revealing a high basal level of Lyn kinase activity and spontaneous phosphorylation of FcεRI. Co-expression of a lipid-excluded transmembrane tyrosine phosphatase, protein-tyrosine phosphatase  $\alpha$  (PTP $\alpha$ ), but not other tyrosine phosphatases such as SH2 domain-containing protein tyrosine phosphatase 1 (SHP-1), CD45, and a lipid raft-preferring chimeric version of PTP- $\alpha$ , suppresses Lyn kinase activity and markedly reduces the level of spontaneous phosphorylation of FcεRI. However, the structural basis for crosslink-mediated FcεRI asso-

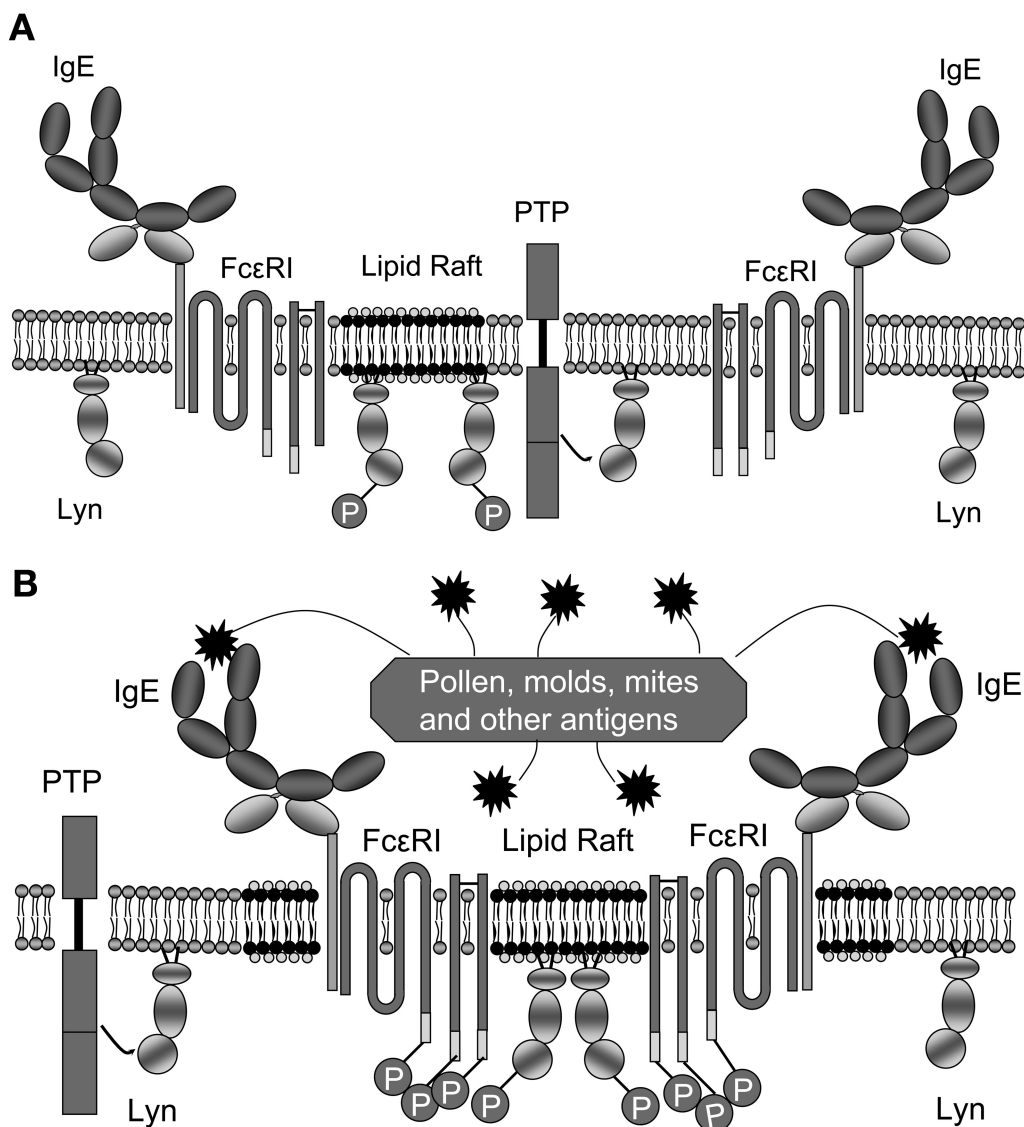


**Fig. 4.** The current model for FcεRI aggregation in lipid rafts. (A) Distribution of membrane components in non-stimulated cells. (B) Distribution of membrane components following FcεRI aggregation. Multiple antigens including pollen, molds, house dust mite, and cockroach have many antigenic epitopes, by which they can crosslink with FcεRI receptors through IgE antibodies on mast cells or basophils. (Adapted from ref. 22.)

ciation with lipid rafts remains incompletely understood. A series of single-chain chimeric IgE receptors containing the extracellular domain from human FcεRIα-chain, the cytoplasmic domain of T cell receptor ζ-chain, and

six different transmembrane (TM) segments of FcεRIα/γ were constructed to investigate the role of TM in FcεRI signaling (24). The results indicate that the magnitude of signaling and the degree of the functional responses medi-

ated by these receptors are strongly dependent on their TM sequences, which either allow or deny success of the crosslinked receptors to the detergent-resistant lipid rafts. Transmembranous disulfide-mediated oligomerization of one chimeric receptor, which mimics the *in vivo*  $\alpha\gamma_2$ , greatly enhances signaling responses.

Another compartment, submembranous F-actin skeleton, consists of an F-actin crosslink connected to the plasma membrane by a series of linkages between F-actin skeleton and transmembrane proteins. Like lipid rafts, the F-actin skeleton is resistant to detergents but its density is relatively high. This compartment also plays a role in cell signaling as well as many other cell activities. Lipid rafts and submembranous F-actin skeleton dynamically interact and thus act in concert to spatially organize cell-signaling events. By using patterned lipid bilayers containing a specific ligand for IgE on a silicon oxide surface, which can define temporally and spatially IgE-receptor clusters at room temperature, Wu et al. (25) investigated the relationship between the F-actin skeleton and lipid rafts in signaling. The results indicate that the lipid raft function is tightly regulated by the actin skeleton, because recruitment of Lyn and other inner leaflet components of lipid rafts to the patterned bilayers is strictly dependent on F-actin polymerization and prevented by cytochalasin D. A more recent study shows that SH2-domain-containing inositol polyphosphate-5-phosphatase 1 (SHIP1), a submembranous F-actin skeleton compartment component, is constitutively associated with filamin-1, an actin-binding protein, and responsible for the actin-dependent downregulation of Fc $\epsilon$ RI signaling upon receptor aggregation (26).

To study how the signaling molecules assemble and interact dynamically in living cells upon Fc $\epsilon$ RI aggregation, Larson and col-

leagues (27) used two-photon confocal cross-correlation fluorescent correlation spectroscopy (CC-FCS) to monitor Lyn and Fc $\epsilon$ RI protein-protein interactions along with protein-membrane interaction (diffusion) simultaneously during IgE signal transduction. Firstly, the lateral mobility of both the crosslinked receptor and Lyn-EGFP are decreased, suggesting direct protein interaction. Then, the association between Fc $\epsilon$ RI and Lyn is temporally changed and continues long after the initial interaction between the receptor and Lyn. Lyn also exhibits a sustained decrease in lateral diffusion, but PM-EGFP, which consists of the membrane-targeting sequence of Lyn and EGFP and serves as an inner leaflet lipid raft marker, does not. These results indicate that the lipid environment is not sufficient to drive interaction between IgE-Fc $\epsilon$ RI and Lyn-EGFP. Rather, the interaction is driven by protein-protein association either directly or through some unknown molecules, which might cause Lyn to continuously decrease its lateral diffusion. The time scale of changes of both interaction and diffusion suggests that these events are secondary to the initial interaction.

### Negative Regulation of Fc $\epsilon$ RI Signaling

Significant progress has been made in understanding the regulation of Fc $\epsilon$ RI signaling in the past a few years. Overall, the negative regulators are dominant in resting cells; after Fc $\epsilon$ RI aggregation, positive regulators are activated and outweigh negative regulators, leading to various biological responses, the degree and duration of which are determined by the balance of two types of counter regulators. A recent study shows that, after being challenged with Ag, IgE-sensitized mast cells display bell-shaped dose-response curves for Ag-triggered degranulation (controlled by the amount of Ag as well as the amount of IgE). The reduced response at

high, supraoptimal Ag concentrations might be a result of quick recruitment and activation of a large amount of negative regulators, such as SHIP1 (28). In another study, the molecular mechanism underlying this regulation was investigated by using different combinations of stimuli: monomeric IgE, IgE + anti-IgE, IgE + low amounts of Ag, and IgE + high amounts of Ag. The results indicate that upon low-intensity stimulation (all stimuli except IgE + high amounts of Ag), Lyn positively regulates degranulation, cytokine production, and cell survival, whereas Lyn works as a negative regulator of high intensity stimulation (IgE + high amounts of Ag) through phosphorylation and activation of SHIP and SHP-1 (29). Therefore, it seems that negative regulators determine both the threshold and the upper limit of the signaling. Because negative regulation always gives the opportunity for new therapeutic approaches, it has attracted considerable attention in recent years. The following are some signaling molecules of particular recent interest.

### *FcεRIβ*

FcεRIβ functions as a signal amplifier, which recruits Src tyrosine kinase and regulates the cell-surface expression of FcεRI. However, it has also been reported to have a previously unrecognized negative regulatory role involving an additional tyrosine residue, located between the two canonical tyrosine residues of the consensus ITAM (YX<sub>3</sub>YX<sub>3</sub>Y). When this tyrosine is mutated to phenylalanine (YFY), the mitogen-activated protein kinase (MAPK) pathway is activated, NF-κB activity is enhanced, and eventually cytokine (IL-6, -13) synthesis and secretion increase. Although the phosphorylation of FcεRI and the association with Lyn is complete, and the phosphorylation of some downstream molecules, including Syk, LAT, PLCγ, and the phosphorylation of SHIP-1 is markedly

reduced, this might cause hyperresponses of cells upon stimulation. Therefore, FcεRIβ may contribute to a negative regulation through recruitment of SHIP-1 and Lyn (30).

### *Lyn*

Lyn, one of the Src-family tyrosine kinases, is weakly associated with FcεRIβ in resting mast cells and therefore was initially thought to be the sole positive regulator for signal initiation. However, it was not until the development of autoimmune disease was found in Lyn<sup>-/-</sup> mice that the role of Lyn as a negative regulator of signal transduction was revealed (31). Lyn<sup>-/-</sup> mice are found to have hyperresponsive B cells after B cell receptor (BCR) ligation. Lyn<sup>-/-</sup> mast cells have enhanced degranulation, suggesting a previously unrecognized crosstalk between Lyn and Fyn. Lyn is the major tyrosine kinase upon stimulation, and is responsible for the phosphorylation of FcεRI ITAM, Syk recruitment, activation of PLC, and increase of intracellular calcium concentrations. On the other hand, Fyn is principally required for the signaling of Gab2, a scaffold protein which recruits PI-3 kinase and controls PIP3 production and is, thus, also critical for degranulation and cytokine production (3). The negative regulatory function of Lyn takes place at two levels. First, Lyn is able to phosphorylate immunoreceptor tyrosine-based inhibitory motifs (ITIMs), which then recruit and activate phosphatases, including SHIP, SHP-1, SHP-2, PTP-α, and others, eventually leading to downregulation of the signaling (32). The second level involves the inhibitory loop of COOH-terminal Src kinase (Csk) and its adaptor, Csk-binding protein (Cbp). Lyn is responsible for phosphorylation of adaptor Cbp, which recruits Csk to the lipid rafts and phosphorylates the C-terminal tyrosine residue of Src-tyrosine kinase family (Lyn and Fyn), resulting in an inactivation of these kinases, which are kept within lipid rafts (33).

## *Syk*

Syk plays an essential role in signal transduction upon FcεRI aggregation, as shown by Syk-deficient mast cells that are defective in receptor-induced degranulation, cytokine synthesis and secretion, and survival. A conserved tyrosine residue in the linker domain of Syk (Tyr<sup>317</sup>) was reported to be a negative regulator (34). Phosphorylation of Tyr<sup>317</sup> creates a binding site for c-Cbl, an E3 ubiquitin ligase, causing the ubiquitination and subsequent degradation of Syk. The Wiskott–Aldrich syndrome protein-interacting protein (WIP) is considered to interfere with c-Cbl ubiquitination of Syk and prevent its degradation (35). The capacity of WIP-deficient mast cells to degranulate and secrete IL-6 after FcεRI aggregation is impaired. Phosphorylation of Syk and downstream PLCγ, calcium mobilization, and JNK kinase activity are also markedly decreased in WIP-deficient mice. These results indicate that WIP regulates the FcεRI-mediated mast cell activation by stabilizing Syk.

## *FcγRIIB*

FcγRIIB, the inhibitory receptor for IgG, has long been reported to inhibit IgE-induced mast cell activation. Although it has also been extensively studied and used in the therapeutic practices, the exact molecular mechanisms are still not completely understood. A recent study (26) shows that SHIP-1, the main inhibitory molecule in vivo, may be involved in the FcγRIIB-dependent negative regulation. The results suggest that a high-molecular-weight isoform of SHIP-1 is constitutively associated with the actin-binding protein, filamin-1, in the F-actin submembranous cytoskeleton. Upon co-aggregation, FcγRIIB and FcεRI rapidly interact with the F-actin skeleton and engage SHIP-1 and filamin-1. Later, filamin-1 and F-actin dissociate from the FcR complex, whereas SHIP-1 still

remains associated with the complex, leading to diminished the signaling eventually.

## *FcαRI*

IgA has long been considered to be an anti-inflammatory Ab. Supportive evidence comes from patients with selective IgA deficiency, which shows increased susceptibility to autoimmune and allergic diseases. In addition, IgA downregulates IgG-mediated phagocytosis, cytokine production, and other inflammatory responses. However, the molecular basis for this inhibitory function remains largely unknown. A recent report illustrates that IgA has an anti-inflammatory effect through the crosslinking of FcαRI and then the recruitment of SHP-2, which inhibits the signaling induced by FcεRI aggregation (36). The prevention of allergic hyperresponses and the accompanying airway inflammation by anti-FcαRI Fab treatment in FcαRI transgenic mice highlights its potential for treating allergic patients.

## *RabGEF1*

RabGEF1, a Ras binding protein, was identified recently as a novel negative regulator for mast cell activation (37). The synthesis and release of lipid mediators and cytokines upon FcεRI aggregation are mediated through Ras signaling pathways. RabGEF1 is able to bind to Ras in vitro and inhibit its activation. All RabGEF1<sup>-/-</sup> mice spontaneously develop severe skin inflammation and RabGEF1<sup>-/-</sup> mast cells show upregulation of Ras signaling pathways as well as enhanced degranulation and release of lipid mediators and cytokines upon stimulation.

## **Therapeutic Strategies**

The current therapeutic strategies for allergic diseases include antihistamines, corticosteroid, and bronchodilators, which mainly relieve allergic symptoms and accompanying

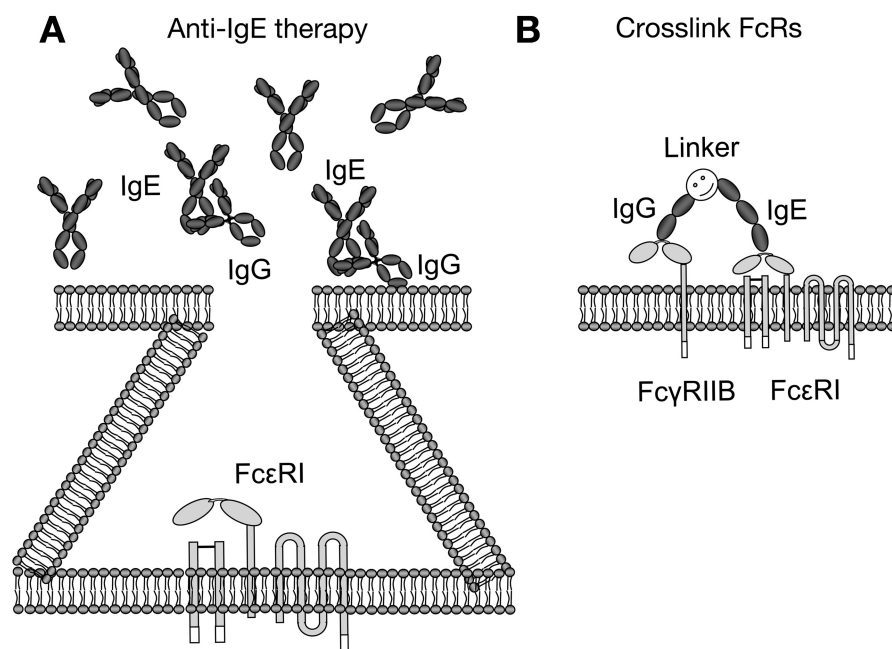


**Fig. 5.** The current therapeutic strategies for allergy. (A) Anti-IgE therapy and (B) crosslinking of FcγR and FcεR receptors. (Adapted from ref. 14.)

inflammatory responses, but are not able to effectively treat the basic cause of these diseases. With increasing insight to the molecular mechanisms underlying FcεRI aggregation-induced allergic inflammatory responses, new mAb-based therapeutic approaches have been proposed and studied in both animal models and allergic patients.

### Anti-IgE Therapy

The rationale behind anti-IgE therapy is simple: the signal transduction and inflammatory responses are reduced by interfering with the binding of IgE molecules to both high- and low-affinity receptors. Omalizumab is a murine anti-human IgE mAb against an epitope on the IgE Cε3 that specifically binds to the FcεRIα. Omalizumab has a high affinity for IgE, thereby inhibiting binding of serum IgE to its receptors. Omalizumab does not bind to cell-bound IgE because the anti-

body-binding site and FcεRIα overlap (Fig. 5A). Clinical studies show that treatment with omalizumab significantly decreases the serum IgE level and allergen-induced bronchoconstriction after exposure to allergens. Other studies also indicate that omalizumab reduces asthma exacerbations, improves asthma symptoms, concomitantly improves upper airway symptoms accompanying allergic rhinitis, improves quality of life, and reduces need for steroid usage as well as rescue medication. It is safe, well tolerated, and conveniently administrated (38,39).

### Anti-FcR Therapy

A recent study shows that anti-FcαRI Fab treatment reduces manifestations of allergic asthma in FcαRI transgenic mice, suggesting that anti-FcαRI treatment is a potential therapy. However, FcαRI has not been found to be expressed on either mast cells or basophils

and this might limit its use in clinical applications (36). Another receptor, Fc $\gamma$ RIIB, has recently been reported to inhibit IgE-induced mast cell activation through recruitment of SHIP-1, one of the major inhibitory molecules *in vivo*. Therefore, anti-Fc $\gamma$ RIIB, which can crosslink and then redistribute Fc $\gamma$ RIIB in cell plasma membrane, may also be an applicable strategy (26).

### Crosslink FcRs

A more elegant, and maybe more practical, strategy is to construct chimeric fusion proteins. For example, a single chimeric human bifunctional protein, with the structure  $\gamma$  hinge-CH $\gamma$ 2-CH $\gamma$ 3-linker-CH $\epsilon$ 2-CH $\epsilon$ 3-CH $\epsilon$ 4, is able to crosslink inhibitory receptor Fc $\gamma$ RIIB and Fc $\epsilon$ RI, leading to a dose- and time-dependent inhibition of antigen-induced IgE-mediated histamine release from IgE-sensitized human mast cells and basophils (Fig. 5B). This approach has also shown to be effective in transgenic mice expressing human Fc $\epsilon$ RI, suggesting a potential role in the treatment of allergic patients (40,41).

### Fc $\epsilon$ RII

The role of Fc $\epsilon$ RII (CD23) in allergic diseases has been studied extensively. It has been proposed to include regulation of IgE synthesis, enhancement of histamine release from basophils, Ag-IgE complex presentation, and involvement in B cell growth and survival. However, the exact function of CD23 in allergic processes remains poorly understood. For example, *in vitro*, CD23 is shown to upregulate IgE synthesis through increased B cell proliferation and antibody synthesis upon binding to CD21 but CD23 deficient mice exhibit abnormally high serum IgE level, whereas mice overexpression of CD23 show a decrease in IgE synthesis and reduced susceptibility to systemic anaphy-

laxis. A recent study sheds light on possible mechanisms underlying this paradox (42). By using surface plasma resonance (SPR) and nuclear magnetic resonance (NMR), Hibbert (42) and colleagues investigate the three-dimensional structure of CD23 and the interaction between CD23 and its two ligands, IgE and CD21. The results reveal that CD23 can bind to IgE and CD21 simultaneously and form a stable trimer, which is resistant to proteolysis. Therefore, soluble CD23 can upregulate IgE synthesis by engaging with membrane-bound IgE and CD21 on B cell surface, leading to the B cell proliferation and ultimately, increased IgE synthesis. However, when the IgE concentration reaches that at which IgE binds to membrane-bound CD23 and form a stable trimer, membrane-bound CD23 is protected from cleavage and IgE synthesis is downregulated.

Another study on chronic idiopathic urticaria (CIU) reveals an autoantibody against CD23-induced histamine release from basophils via eosinophil activation (43). This effect appears to be mediated by the release of major base protein (MBP) from eosinophils upon engagement of CD23. This allergen-independent, MBP-mediated histamine release may contribute to the perpetuation of symptoms in the “late-phase” of an allergic reaction when eosinophils infiltrate the location of inflammation (43).

IgE can enhance the production of antigen-specific Ab more than 100-fold when passively administrated together with its specific antigen. This positive feedback requires CD23. One possible explanation is that B cells can take up IgE-Ag complex via CD23 and efficiently present Ag to CD4<sup>+</sup> T cells. However, an argument against this hypothesis could be that B cells are unable to present Ag to naïve T cells. In order to test if B cells are required in this process, Andrew et al. (44) investigated the antigen specific immune

responses in both CD23<sup>+/+</sup> and CD23<sup>-/-</sup> mice. The results indicate that CD23<sup>+</sup> B cells are responsible for presenting IgE–Ag to Th cells, which display a marked proliferation and activation after presentation (44).

Both in vitro and in vivo studies have shown that crosslinking CD23 leads to downregulation of IgE. Therefore, anti-CD23 mAb (lumiliximab) could be an ideal therapeutic approach. A recent study reveals that lumiliximab treatment not only decreases IgE synthesis, but also plays a role in modulating immune responses to allergens, including the arrest of allergen-induced B and T cell proliferation, decreased synthesis and secretion of inflammatory cytokines, and the downregulation of cell surface expression of co-stimulatory molecule CD86 (45).

## Conclusion and Future Directions

A central feature of the allergic reaction is that Ag crosslinks to FcεRI, and initiates the signaling events. During the past few years, knowledge of FcεRI cell distribution and surface expression, particularly regulation of cell signaling, including monomeric IgE, lipid rafts, and increasing number regulators, has been enriched greatly. However, the situation in vivo is extremely complicated and so often oversimplified. Although significant progress has been

made in understanding the big picture of signaling, many questions still remain unsolved. What *cis* and *trans* factors control the receptor expression? How does the initial phosphorylation happen? What kinase is responsible for this initiation? How do aggregated receptors enter into lipid rafts? Which regulator plays a dominant role in the downregulation responses? With the rapid development of new technology, all these questions will be answered in the future.

Clearly, a better understanding of signaling events upon IgE FcR aggregation is shedding new light on how to treat allergic patients more safely and efficiently. Currently available non-anaphylactogenic monoclonal Abs or bifunctional chimeras have provided a better therapeutic strategy than the conventional approaches. Although these antibody-based approaches have their own disadvantages, they undoubtedly broaden our horizons, and also encourage further investigations focusing on the selective targeting of mIgE on B cells, thus blocking IgE synthesis before sensitization.

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