

THE HIGH-AFFINITY IGE RECEPTOR (FcεRI): From Physiology to Pathology

Jean-Pierre Kinet

Harvard Medical School and Beth Israel Deaconess Medical Center, Boston,
Massachusetts 02215; e-mail: jkinet@bidmc.harvard.edu

KEY WORDS: receptor, signaling, atopy

ABSTRACT

The high affinity receptor for immunoglobulin E (designated FcεRI) is the member of the antigen (Ag) receptor superfamily responsible for linking pathogen- or allergen-specific IgEs with cellular immunologic effector functions. This review provides background information on FcεRI function combined with more detailed summaries of recent progress in understanding specific aspects of FcεRI biology and biochemistry. Topics covered include the coordination and function of the large multiprotein signaling complexes that are assembled when FcεRI and other Ag receptors are engaged, new information on human receptor structures and tissue distribution, and the role of the FcRβ chain in signaling and its potential contribution to atopic phenotypes.

INTRODUCTION

The formation of antigen-antibody complexes plays a fundamental role in our immune defense system. Interaction of these complexes with many cells of the immune system results in a variety of critical functions, including phagocytosis, antibody-dependent cytotoxicity, modulation of antibody secretion and cell secretion. These interactions are mediated through the binding of immunoglobulin Fc domains to their corresponding Fc receptors (FcRs). One of these FcRs is FcεRI, a multimeric cell surface receptor that binds immunoglobulin E (IgE) with high affinity. The physiological function of FcεRI has remained elusive, but its role in allergic reactions is well established. The last review entirely

devoted to Fc ϵ RI in Annual Reviews was written in 1986 (1), at a time preceding the molecular cloning of the Fc ϵ RI α , β , and γ subunits. In 1991, my colleague Jeffrey Ravetch and I wrote a review on FcRs, based on the large amount of new information that had been generated from the molecular cloning of the immunoglobulin-binding FcRs (2). This work led to the recognition that FcRs are homologous and belong to the Ig superfamily. Another fundamental piece of information was the unexpected finding that the Fc ϵ RI γ chains (now denominated FcR γ chains) that had been found initially as a subunit of the Fc ϵ RI receptor were also subunits of at least one other Fc receptor, the low affinity IgG receptor (Fc γ RIII) (3–5) and of the T cell antigen receptor (TCR) (6). It was becoming clear that in addition to Fc ϵ RI, other Fc receptors could also be multimeric complexes and that they could share identical subunits. The 1991 review was written more to emphasize the family and modular nature of the FcRs than to elaborate on the fragmented knowledge of their individual characteristics and biological relevance. In that review, Fc ϵ RI was described as a tetrameric complex ($\alpha\beta\gamma_2$) expressed exclusively on the surface of mast cells and basophils whose function is to mediate cellular degranulation and the release of various mediators such as histamine, leukotrienes, and a number of cytokines and chemokines, the hallmark of allergic reactions.

Over the last seven years, a number of important findings have dramatically improved our understanding of Fc ϵ RI biological functions. One unexpected finding has been the recognition of substantial differences between human and rodent Fc ϵ RI. While rodent Fc ϵ RI has an obligatory $\alpha\beta\gamma_2$ tetrameric structure, human Fc ϵ RI can be expressed as both trimeric ($\alpha\gamma_2$) and tetrameric ($\alpha\beta\gamma_2$) structures. The cellular distribution of human and rodent Fc ϵ RI is also different. Rodent Fc ϵ RI is only expressed on mast cells and basophils (and non-B, non-T cells), whereas expression of human Fc ϵ RI extends to monocytes, eosinophils, platelets, Langerhans cells, and dendritic cells. Other important findings include the following: the participation of Fc ϵ RI in the process of antigen presentation; the genetic demonstration that Fc ϵ RI can alter the disease evolution of some parasitic diseases; the molecular and genetic demonstration that the Fc ϵ RI β chain serves as an amplifier of Fc ϵ RI biological function; the possible association between various human Fc ϵ RI β chain polymorphisms and atopic phenotypes; the control of receptor expression by its own ligand IgE; and the possibility that the Fc ϵ RI α chain may be the target of autoantibodies in chronic urticaria. In addition, remarkable progress has been made in understanding the intracellular signaling mechanisms by which immunoreceptors, including Fc ϵ RI, are activating cells. The present review focuses on all of the advances listed above and discusses their biological implications.

DIFFERENCES BETWEEN RODENT AND HUMAN Fc ϵ RI SYSTEMS

Rodent Fc ϵ RI: a Tetrameric Structure

In rats and mice, from which it was first isolated, Fc ϵ RI is exclusively expressed in mast cells, basophils, and non-B non-T cells, and is a tetramer made of one α chain, one β chain, and two identical, disulfide-linked γ chains (7, 8). The α , β , and dimeric γ chains are maintained as a complex in the plasma membrane through a combination of hydrophobic and electrostatic noncovalent interactions involving both covalently and noncovalently bound lipids (9, 10).

The α chain, a member of the immunoglobulin superfamily, comprises two extracellular immunoglobulin-related domains, one transmembrane domain containing an aspartic residue, and a short cytoplasmic tail (8, 11–13). The α chain contains seven N-linked glycosylation sites, all of which are utilized. These N-linked oligosaccharides are not intrinsically necessary for proper folding of the α chain but are required in the endoplasmic reticulum (ER) to mediate the proper interaction between the α chain and the ER folding machinery (14). Thus, the α chain is heavily glycosylated and appears as a broad, very heterogeneous band centered around the 45-kDa marker when resolved by standard SDS-PAGE; however, the weight of the α chain protein core is 27 kDa.

The β subunit (FcR β) has four transmembrane domains separating amino and carboxy terminal cytoplasmic tails (8, 15). In mast cells, FcR β is a subunit not only of Fc ϵ RI but also of the low affinity IgG receptor (Fc γ RIII) (16). The γ subunit (FcR γ) is a member of the $\gamma/\zeta/\eta$ family of antigen receptor subunits, which are notable for their structure, consisting essentially of a transmembrane region and cytoplasmic tail, and for their utilization among many families of antigen receptors. The γ , ζ and η chains can be found in homo- or heterodimeric forms with Fc γ RIII, Fc γ RI, Fc ϵ RI, Fc α R, and the TCR depending on the cellular context (3–6, 17–24). FcR γ homodimer has also been shown recently to associate with glycoprotein VI on human platelets to form a collagen receptor (25–27).

Assembly of Fc ϵ RI

When the cDNA encoding the α chain was first isolated, transfection of this cDNA failed to induce expression of a functional IgE-binding α chain on the surface of the transfected cells. An explanation for this failure awaited the subsequent cloning of the β and γ chains. The cloning of β and γ cDNAs permitted us to co-transfect the three cDNAs, resulting in the cell surface expression of a large number of IgE-binding Fc ϵ RI complexes (7, 8). Indeed, the three subunits α , β , and γ were required for efficient cell surface expression of Fc ϵ RI. A further analysis of this co-transfection requirement determined

that the α chain contains endoplasmic reticulum (ER) retention signals, among which is a di-lysine motif located in its cytoplasmic domain (28). In the absence of the other chains and because of the presence of these ER retention signals, the transfected α chain remains and is degraded in the ER. However, assembly of the α chain with the co-transfected β and γ chains masks the ER retention signals, allowing the $\alpha\beta\gamma_2$ complex to be exported to the Golgi apparatus and from there, to the plasma membrane.

However, when the same experiment was attempted with human α , β , and γ chains, it became clear that requirements for surface expression of the Fc ϵ RI complex were different in the human system. Indeed, $\alpha\gamma_2$ complexes could be as easily exported to the cell surface as $\alpha\beta\gamma_2$ complexes, demonstrating that human γ was sufficient by itself to counterbalance the human α chain ER retention signals (29, 30). Although the precise molecular explanation for this species difference is still unclear, there is a suggestion that an additional ER retention signal may be located in the rodent α extracellular domain (see below Fc ϵ RI: a modular unit).

These transfection data turned out to be more important than initially recognized. They demonstrated a species difference between rodent and human Fc ϵ RI and showed that human β , unlike rodent β , is not necessary for cell surface expression of Fc ϵ RI. Moreover they were also the first suggestion that two Fc ϵ RI isoforms ($\alpha\beta\gamma_2$ and $\alpha\gamma_2$) may in fact exist in humans.

Human Fc ϵ RI: Different Cell Distribution

As mentioned above, the high affinity IgE receptor Fc ϵ RI was initially thought to be expressed exclusively on mast cells and basophils. However, G. Stingl's group in collaboration with us reported that Fc ϵ RI is also expressed on cutaneous Langerhans cells (31). This finding, reported simultaneously by another group (32), led ourselves and our collaborators to question the selectivity of expression of Fc ϵ RI on human cells. In fact, Fc ϵ RI was detected on eosinophils of some hypereosinophilic patients (33), on monocytes of patients with a number of atopic disorders (34) and, later, on circulating dendritic cells (35) and platelets (36). While some of these findings were initially controversial, in particular Fc ϵ RI expression on subsets of eosinophils, most of them have now been reproduced by other groups (37–41). In addition, unambiguous genetic evidence now confirms the extended pattern of human Fc ϵ RI expression (see next section).

It is useful to comment here on why it took so long to recognize the expression of Fc ϵ RI on cells as common as human monocytes while so many clusters of differentiation (CDs) have been detected and characterized. Two points need to be made. First, there was no available reagent until the early 1990s, when anti-human Fc ϵ RI α monoclonal antibodies (mAb) became available (34, 42). Partly for this reason, the binding of IgE, which had been detected on many of these

cells, had been interpreted as binding to CD23 (the low affinity IgE receptor, Fc ϵ RII). Second, the level of expression of Fc ϵ RI on monocytes, eosinophils, Langerhans cells, and dendritic cells is low when compared to the level of expression seen on mast cells and basophils (as judged by flow cytometry analysis) and is therefore more difficult to detect. For example, normal human monocytes express 10 to 100 times less receptors than circulating basophils. Even the upregulated levels of Fc ϵ RI on monocytes of patients with various allergic diseases (34) are still well below the levels expressed by mast cells and basophils. At present, the basis for the difference of expression levels between the cell types and the mechanisms of the regulation of these levels is not known and it is quite possible that additional cell types will be shown in the future to express Fc ϵ RI under specific circumstances.

Genetic Control of the Cell Specific Expression of Human Fc ϵ RI

The difference of Fc ϵ RI cell distribution between humans and mice has now been confirmed genetically. First, a 2.9-kb fragment 5' of the initiation codon from the human Fc ϵ RI α gene is active in a reporter gene assay in mouse monocytic cell line, and as expected in mast cells and basophils but not in fibroblasts or B and T cells (A Brini, J-P Kinet, unpublished). Moreover, we generated a transgenic mouse using a 9.5-kb Fc ϵ RI α gene, whose 5-exon structure has been described (43–45), under the control of the 2.9-kb promoter and showed that the transgene induces expression of human Fc ϵ RI α not only on mast cells and basophils (46) but also on monocytes/macrophages, Langerhans cells, and eosinophils but not on B and T cells nor on neutrophils (47). Therefore, the 2.9-kb fragment contains the information necessary to achieve cell-specific expression of human Fc ϵ RI. Another group reported the generation of a transgenic mouse line, using as promoter sequence, a smaller 1.3-kb fragment upstream of the start codon (48). These authors claimed that this promoter is specific for mast cells. However, possible expression of Fc ϵ RI on monocytes, eosinophils, and Langerhans cells was not analyzed and may therefore have been missed. Alternatively, the regulatory elements governing Fc ϵ RI cellular specificity may lie in the 2.9 kb fragment in a region upstream of the 1.3 kb fragment.

Analysis of the Fc ϵ RI receptor structure in the "humanized" transgenics shows that the 9.5-kb transgene recapitulates not only the appropriate cell specificity of expression of human Fc ϵ RI but also the appropriate cell type/structure observed in human cells (47), as discussed in the next section.

Human Fc ϵ RI: Two Isoforms

The observation that human Fc ϵ RI cell is expressed on a variety of cells other than mast cells and basophils led to another surprising finding. In contrast to

the easily detectable α and γ transcripts, those for the β chain were not detected in monocytes even using the sensitive polymerase chain reaction (PCR) (34 and D Dombrowicz, J-P Kinet, unpublished observations). Our inability to detect FcR β transcripts in monocytes, Langerhans cells, and dendritic cells suggested that the structure of Fc ϵ RI on these cells is indeed different from that on mast cells and basophils. Thus, in these cells, either Fc ϵ RI lacks the FcR β subunit altogether and is an $\alpha\gamma_2$ complex, as on the transfectants described above, or a β -like molecule that has not yet been identified is taking the place of β in the complex. In fact, data on the detergent sensitivity of Fc ϵ RI complexes support the existence of $\alpha\gamma_2$ complexes. The $\alpha\gamma_2$ complexes are less stable in detergent than $\alpha\beta\gamma_2$ complexes, and as a consequence, the FcR γ_2 homodimer dissociates more easily from the Fc ϵ RI α chain (49). The pattern of detergent instability usually seen with $\alpha\gamma_2$ structure was observed in our laboratory for monocyte-expressed Fc ϵ RI, suggesting that Fc ϵ RI on monocytes are $\alpha\gamma_2$ complexes (D Dombrowicz, J Kinet, unpublished). Although it is still formally possible that $\alpha\gamma_2$ complexes associate with β -like protein, which would not confer the same stability as the FcR β chain, the data, when taken together, are best explained by the existence of $\alpha\gamma_2$ complexes. The capacity of $\alpha\gamma_2$ complexes to be expressed on transfectants without β chains, the absence of detection of FcR β chain transcripts on human Fc ϵ RI-expressing cells, and the detergent sensibility studies provide the experimental basis in support of the existence of $\alpha\gamma_2$ isoforms on human monocytes, Langerhans cells, and dendritic cells. By contrast, mast cells and basophils that express FcR β chain have on their surface the classical $\alpha\beta\gamma_2$ isoform (Figure 1). It is presumed that

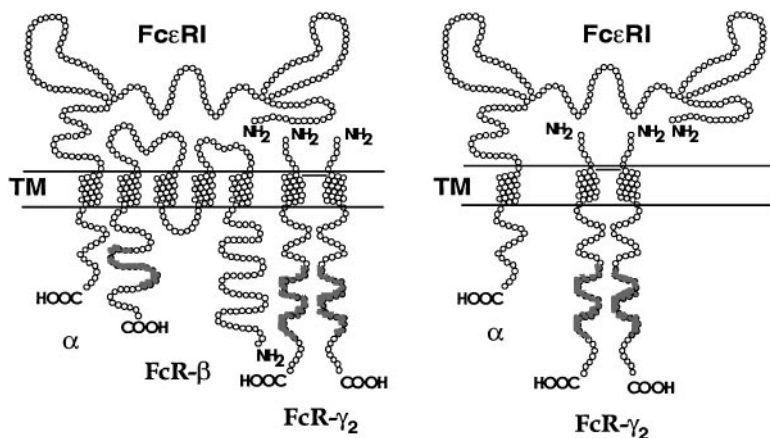


Figure 1 Human Fc ϵ RI isoforms.

eosinophils and platelets also express $\alpha\beta\gamma_2$ complexes, although the existence of FcR β in eosinophils and platelets is based so far on PCR data only. In addition, it has not yet been determined whether human mast cells, basophils, eosinophils, and platelets express only the $\alpha\beta\gamma_2$ isoform or a variable proportion of the two isoforms. In view of the critical importance of the FcR β chain in signal amplification (see below), this question may turn out to be critical in our overall understanding of the human allergic response. In this regard, it is also worth noting that the experimental evidence gathered so far does not eliminate the possibility that the FcR β chain could be expressed on the apparently FcR β chain-negative cells in different circumstances or, perhaps, in pathological conditions. More knowledge on the virtually unknown mechanisms regulating FcR β gene and protein expression will be needed to address these questions.

Fc ϵ RI: A MODULAR UNIT

The extracellular domain of the α chain contains sufficient molecular information to provide the receptor with high affinity binding, whereas the β and γ chains play no apparent role in ligand binding. This point was demonstrated by making a single chain chimera containing the extracellular region of Fc ϵ RI α (50) or, alternatively, by truncation of the extracellular and transmembrane domains of Fc ϵ RI α that results in secretion of a soluble protein (51). The chimera and the soluble Fc ϵ RI α chain both have apparently the same ligand binding characteristics as the tetrameric, plasma-membrane inserted, Fc ϵ RI complex.

In these truncation/transfection experiments, another interesting difference between rodent and humans was detected (51). Soluble α chain is seen only with the human but not with the mouse or rat truncated construct. The rodent constructs result in the ER retention of the corresponding protein, an indication that, as suggested above, the extracellular domain of the rodent Fc ϵ RI α chain contains an additional ER retention signal.

The Fc ϵ RI α chain seems to be entirely devoted to the ligand binding function and, until now, has not been found to contribute any intracellular signals. Truncation of its cytoplasmic tail in a reconstituted tetrameric complex does not seem to affect holo-receptor signaling (52). Thus, in both rodent and human, Fc ϵ RI can be considered as a modular structure with one binding module, the α chain and one signaling module, the $\beta\gamma_2$ or γ_2 complex. The function of the signaling chains is addressed in details below (see signal transduction).

IgE-Fc ϵ RI BINDING CHARACTERISTICS

Fc ϵ RI is specific for the ϵ isotype but is not completely species specific. Rodent IgE binds to both rodent and human Fc ϵ RI whereas human IgE binds only to

human Fc ϵ RI (reviewed in 53). A large body of literature has been generated about the Fc ϵ RI-binding site on IgE (54–60). The consensus reached from these earlier studies was that the Fc ϵ RI α chain binding site on IgE was located within the C ϵ 3 domain (53). Recent attempts have been made to define the residues and domains within C ϵ 3 that are important for binding. Mutations, which negatively affected the binding site, were located in the three putative loops (CD, EF, and FG) of the C ϵ 3 domain (61). A second study, which clearly disagrees with the previous one, implicates the segment 343–353 (located in the AB loop) as being important for IgE binding (62).

The following studies, recently published, took advantage of efficient biophysical methods, such as surface plasmon resonance (SPR) and isothermal titration calorimetry, and used circular dichroism (CD) to verify that the overall structure of the IgE constructs was not affected by the various mutations made to localize the critical residues. One study found that Arginine 334, located at the N terminus of C ϵ 3, is an important contact residue. The absence of involvement of C ϵ 2 was confirmed by thermodynamic measurements that were identical for the whole IgE and for a construct including only C ϵ 3 and C ϵ 4 (63). CD studies showed the conservation of the secondary structures of C ϵ 3, C ϵ 4, and the two Ig-like domains of Fc ϵ RI α in the complex, indicating that complex formation affects only the relative position of these domains (63). This contradicts a previous report of a major conformational rearrangement of IgE upon binding (64). The reason for this contradiction is at present unclear. Incidentally, several of the studies cited here have confirmed the previously shown 1/1 stoichiometry of the IgE/Fc ϵ RI interaction in spite of the fact that there are two potential Fc ϵ RI α -binding sites per molecule of IgE.

The IgE binding site on Fc ϵ RI α is entirely contained in the extracellular portion (EC) of Fc ϵ RI α (51). This EC domain contains two immunoglobulin-like domains (D₁ and D₂), which belong to the truncated C2 subtype of the immunoglobulin superfamily (65, 66). Earlier studies concluded that the carboxy-terminal domain (D₂) contained the IgE-binding site, but that the presence of the amino-terminal domain D₁ was required for high affinity (67–71). Cyclic peptides homologous to the putative C-C' region of D₂ were designed on the basis of the known structure of the second EC of CD2, the prototype for the C2 subtype. These cyclic peptides act as competitive inhibitors of IgE binding, contributing about half of the free energy of binding of the entire EC (72). Lys117 in the C strand was identified as a contact residue (73). These results combined with those regarding the binding site on IgE and the modeling of IgE and Fc ϵ RI α allowed these authors to propose a model of the interaction (73). The crystal structure of Fc ϵ RI α has now been solved and is described in the addendum following this chapter.

BIOLOGICAL FUNCTION OF FcεRI

Control of Parasite-Induced Pathology and Resistance to Parasites

Despite numerous studies on the structure and function of FcεRI, the physiological relevance of FcεRI has remained an open question. FcεRI-deficient mice obtained by targeting specifically the FcεRIα or FcεRIβ gene are essentially normal in all aspects of their development and maturation (47, 74). Because IgE has been traditionally thought to play a role in the immune defense against parasites, the FcεRIα-deficient mice were used to investigate the possible role of FcεRI in the immune defense against *Schistosoma mansoni* (75). The natural resistance to *Schistosoma mansoni* as analyzed by adult worm and tissue egg burdens was similar in wild-type and FcεRIα-deficient mice. Similarly, the helminth-induced initial Th2 response appeared to be the same in both types of mice. However, clear-cut and somewhat unexpected differences in helminth-induced liver pathology were observed between the wild-type and FcεRIα-deficient mice, demonstrating a role for FcεRI in the control of the parasite-induced disease. The volume of granulomas was 25% greater, and the fibrosis 50% increased in the FcεRIα-deficient mice. The mechanism by which FcεRI controls the helminth-induced pathology is still unclear. It does not seem to be due to a variation in the Th2/Th1 balance since *in situ* measurements of various cytokines did not show measurable differences between both types of mice. However, non-B non-T cells from the FcεRIα-deficient showed a complete defect in their capacity to secrete IL-4 in response to anti-IgE or soluble parasite antigen, in contrast to wild-type mice. Whether and how this defect may translate to enhanced liver pathology is not clear. It is possible that IgE-FcεRI dependent secretion of IL-4 from non-B non-T participates in an inflammatory response which is beneficial to the host. In addition, IgE-FcεRI dependent secretion of other inflammatory mediators from mast cells, a function which is clearly abolished in FcεRIα-deficient mice (see below), may also participate in a beneficial inflammatory response and in the control of the helminth-induced pathology. Regardless of the mechanism, this role must be played by mast cells, basophils, or non-B non-T cells because these are the only cell types expressing FcεRI in mice. However, in view of the capacity of the FcεRI-expressing eosinophils and platelets from humans to induce FcεRI-mediated killing of larvae *in vitro* (33, 36), it is tempting to speculate that both eosinophils and platelets, and possibly other FcεRI-expressing cells, may provide an additional protective layer in humans.

As already mentioned, the resistance to infection by *Schistosoma mansoni* is not dependent on an IgE-FcεRI pathway. However, FcεRI may be required to

promote resistance to at least one other type of parasite: *Haemaphysalis longicornis* ticks. Studies on the resistance to cutaneous feeding of larval *Haemaphysalis longicornis* ticks show that mast cell-deficient mice are deficient in their capacity to resist this parasite and that this resistance depends on both the presence of IgEs and of mast cells (76–78). Since IgE interacts with mast cells mainly (and possibly exclusively) through FcεRI, these data suggest strongly that FcεRI plays a role in the mechanism of resistance.

These studies show that IgE/FcεRI-mediated pathways and inflammatory cascade play a beneficial role in our defense system against parasites. However, the overall biological response that operates the control of parasite-induced pathology in one case and the resistance to the infestation in the other is not yet clearly understood and requires further study.

Type I Allergic Reactions

It is beyond dispute that FcεRI, at the cellular level, plays a key role in mediating the IgE-dependent activation and degranulation of mast cell and basophils. The consequent release of mediators such as histamine is associated with the production of type I IgE-dependent allergic reactions. However, there had not been any definite analysis of the role of FcεRI in vivo and of possible redundant mechanisms supporting FcεRI function. These questions have now been addressed genetically by comparing wild-type mice with FcεRI-deficient mice obtained by specific gene targeting of either the α chain gene or of the β chain gene (47, 74). While it is easy to induce local or systemic anaphylactic reactions in wild-type mice after sequential injection of IgEs and the corresponding antigens, these reactions are completely abolished in FcεRI-deficient mice. Therefore, FcεRI is indeed absolutely required to permit IgE-mediated allergic reactions in vivo, and no other mechanisms can compensate, even partially, for its absence.

Antigen Presentation

Because of the difference of cellular expression between mouse and humans, it is obvious that the specifically human function and biological significance of human FcεRI expression on monocytes, Langerhans cells, dendritic cells, eosinophils, and platelets cannot be studied in wild-type mice and FcεRI-deficient mice. To begin addressing this question, a number of functional studies have been done with human monocytes and dendritic cells, and, as already mentioned, with human eosinophils and platelets. FcεRI expressed on monocytes was shown to mediate small calcium fluxes (34) and to promote IgE-mediated antigen presentation (79). This antigen presenting function by FcεRI is even more effective on circulating dendritic cells (35). The

latter cells are efficient stimulators of both primary and Fc ϵ RI-dependent secondary T cell responses, and they therefore may be capable of priming naive T cells to IgE-reactive antigens as well as amplifying established T cell responses.

Monocytes/macrophages, dendritic cells, and Langerhans cells (which are professional antigen presenting cells), all express $\alpha\gamma_2$ complexes. Therefore, it is possible that this $\alpha\gamma_2$ structure serves the antigen presenting function for example by specifically targeting the antigen-IgE-Fc ϵ RI complexes to the intracellular antigen presenting compartment. Furthermore, the antigen presenting function may operate only at a certain level of cell activation. In that case, the absence of FcR β and of its signaling amplification function (see below) may be required to prevent full activation of the signaling machinery and to set the adequate activation level. In any case, the Fc ϵ RI-expressing cells supporting antigen presentation are not seen in rodents and for that reason, it has not been easy to assess the overall biological importance of the Fc ϵ RI-mediated antigen presenting function in host defense or in the development of allergic reactions. The "Fc ϵ RI-humanized" mice described in the section on "Genetic control of the cell specific expression of human Fc ϵ RI" (46, 47) may be a useful model in this regard.

From Parasites to Allergy?

As discussed above, evidence is accumulating in mouse studies that Fc ϵ RI is playing a role in host defense against some parasites and in limiting the pathology induced by other parasites. In other words, the biological function of Fc ϵ RI in these circumstances is beneficial to the host. Therefore the acquisition by the human species of Fc ϵ RI expression on many cells and of the antigen presenting function corresponds probably to the gain of additional advantages to the host (80, 81). Epidemiological studies in Africa and South America support the concept that the IgE-Fc ϵ RI network is protective against *Schistosoma mansoni* (82–84). However, the reason for which this beneficial function has evolved to a function apparently harmful to the host as in the case of allergic diseases is not yet understood. In our "clean" societies, humans are no longer continuously facing parasites, and this situation may have contributed to host sensitization to innocuous antigens and the development of allergies (85). In support for this hypothesis, recent epidemiological studies (86) suggest that there is a tendency for the countries with expected endemic parasitic infestation to have lower incidence of allergic diseases. However, even if this hypothesis is true, it does not provide an explanation for the increase in incidence of allergic diseases in countries with high levels of parasitic diseases.

REGULATION OF Fc ϵ RI BIOLOGICAL FUNCTION*Regulation of Fc ϵ RI Expression*

Until recently, very few studies have addressed directly the question of regulation of expression of Fc ϵ RI. A correlation between the number of IgE molecules bound to basophils and the serum IgE level had been recognized long ago (87). Although the basis for this correlation was not known, it was suggested either that there was a common factor capable of regulating both IgE levels and IgE receptors or that the receptor number was modulated by the serum IgE concentration. These authors thought that the second hypothesis was more likely. Around 1983, two concomitant studies using the mast cell line (RBL-2H3) suggested that the second interpretation might indeed be correct. Incubation of RBL cells with monomeric IgE for 12 to 24 h resulted in an increase in the number of receptors at the cell surface (88, 89). This Fc ϵ RI upregulation by IgE was found to be insensitive to cyclohexamide and was therefore judged to be independent of protein synthesis. Rather, the mechanism of this upregulation was proposed to be the result of a decrease in the degradation rate of Fc ϵ RI "protected" by receptor-bound IgE, as if monomeric IgE was protecting the bound receptors from degradation during receptor recycling.

Recently, Stephen Galli's group in collaboration with us has found that the level of Fc ϵ RI expression on the mast cells of IgE-deficient mice was extremely low (80% reduced). However, the level of surface expression of Fc ϵ RI on mast cells could be upregulated (up to 32-fold) by in vitro incubation of the cells with IgE or injection of IgE in vivo (90). This Fc ϵ RI upregulation can also be observed on mouse basophils (91) and on human mast cells (92) and basophils (93). Therefore this ligand-mediated upregulation of Fc ϵ RI appears to be a general mechanism in both rodent and human. Furthermore, the structure of Fc ϵ RI ($\alpha\beta\gamma_2$ or $\alpha\gamma_2$ complexes) does not seem to matter. The density of Fc ϵ RI expression on monocytes ($\alpha\gamma_2$ complexes) appears higher in atopic subjects when compared to normal (34) and is most likely related to the higher level of circulating IgE (39). Similar observations have been made with eosinophils and Langerhans cells of atopic individuals (39), and treatment of atopic patients with anti-IgE that resulted in substantial decrease of the levels of serum IgE also caused a parallel decrease of Fc ϵ RI expression on basophils (94).

In my view, the mechanism of this upregulation can be explained by the protection against degradation as suggested in one of the 1983 studies. In the recent in vitro study with mouse mast cells, two components of the Fc ϵ RI upregulation were identified: an early cyclohexamide-insensitive phase (confirming earlier studies) followed, a few hours later, by a more sustained component that is highly sensitive to cyclohexamide. Note that transcription of the subunits is unaffected (unpublished). In fact, the existence of these two components

corresponds exactly to what would be expected if a mechanism involving the inhibition of degradation was involved. During the first hours, IgE could upregulate Fc ϵ RI simply by protecting against degradation without being dependent on Fc ϵ RI synthesis. Later, when the pool of available Fc ϵ RI is fully utilized, further accumulation by the same mechanism should become dependent on protein synthesis. Therefore, while it is too early to exclude that other mechanisms could be involved in IgE-mediated Fc ϵ RI upregulation, the data available so far support a simple mechanism of inhibited degradation of the entire receptor complex.

Importantly, this Fc ϵ RI upregulation by IgE results in critical enhancement of effector cell functions (90–92). Both serotonin and cytokine release are substantially enhanced in terms of both the sensitivity and the intensity of the response. Therefore this upregulation mechanism greatly enhances the capacity of Fc ϵ RI-expressing cells to respond to single antigens or to a multiplicity of antigens. This may be a critical mechanism to support host defense against various types and low levels of parasites, but the other side of the coin is that the same mechanism may permit allergic reactions to multiple allergens.

Obviously, the regulation of Fc ϵ RI expression is not entirely dependent on ligand-mediated regulation. The mast cells from IgE-deficient mice still express low levels of Fc ϵ RI (90). This “basal” level could be under the control of cytokines. In mouse mast cells and human basophils generated from cultures of bone marrow in the presence of IL-3 (and in the absence of IgE), Fc ϵ RI expression occurs very early when the cells are still undifferentiated and before intracellular granules can be detected (95). IL-4 plays a positive role on the transcription of Fc ϵ RI α in human mast cells cultured from cord blood mononuclear cells (96) or from fetal liver (97). The rates of transcription, translation, and degradation of individual subunits have not been studied extensively, except to show that transcription of FcR γ chains (98) is enhanced by interferon- γ (22). However, the turnover of the cell surface-expressed tetrameric receptor was analyzed in the rat basophilic leukemia cell (RBL) line. The data from this study showed that surface receptors are internalized and degraded or re-expressed at the surface as a unit (89).

RNA for the three subunits in mast cells is not equally abundant; β transcripts are substantially less abundant than α transcripts, while γ transcripts are the most abundant. This could indicate that the subunits are not coordinately synthesized, assuming that the rate of translation of these mRNAs is similar. Rather than controlling the synthesis of each subunit, the cell could regulate the assembly and surface expression of the receptor by controlling only the limiting subunit, as is apparently the case for the T cell receptor (99). A number of studies suggest that it is the case. Mouse mast cells lacking expression of Fc ϵ RI are deficient in FcR γ but not Fc ϵ RI α and FcR β transcripts (100). Similarly,

expression of Fc ϵ RI ($\alpha\gamma_2$ complexes) on freshly isolated epidermal Langerhans cells appears to correlate with the level of expression of FcR γ chains while the pool of intracellular Fc ϵ RI α is greater than the surface expressed Fc ϵ RI α (101). By contrast, disappearance of the Fc ϵ RI surface expression seen on epidermal Langerhans cells subjected to 24-h culture correlates with the disappearance of Fc ϵ RI α transcripts, an indication that the same cell type may use different mechanisms to regulate the cell surface expression levels of Fc ϵ RI (101).

The FcR β Chain Amplification

A novel potential way to regulate Fc ϵ RI function has been recently identified. In vitro studies from our laboratory addressing the function of FcR β during early Fc ϵ RI signaling demonstrated that FcR β functions to amplify cell activation signals mediated through the FcR γ subunit (49) (see paragraph below on signaling). To assess whether FcR β could influence a host response, an in vivo model had to be constructed. As mentioned above murine $\alpha\gamma_2$ complexes are not transported to the surface and, therefore, a standard homologous recombination to eliminate β chain expression cannot produce a murine experimental system with which to study β function. However, human $\alpha\gamma_2$ (or human α /murine γ_2) complexes are transported to the cell surface. For this reason, the function of FcR was analyzed by comparing the "humanized" transgenics described above with corresponding mice having the same human Fc ϵ RI α cell distribution but expressing only $\alpha\gamma_2$ receptors because of targeted disruption of the FcR β gene (47). The analysis of the Fc ϵ RI-dependent responses in these mice showed that FcR β functions as an amplifier not only of early signaling events but also of later responses such as cell degranulation and cytokine release, with a gain factor ranging from 3 to 5. In addition, FcR β is a critical and substantial amplifier of in vivo anaphylactic responses. Therefore, these data are unequivocal genetic evidence that FcR β has the capacity to modulate an individual's allergic response and that this capacity is due to its function as a net amplifier of effector cell responses. The importance of FcR β as an amplifier of the host response must be considered in the context of recent genetic/epidemiologic studies of atopic human families (see below).

Fc ϵ RI AND HUMAN DISEASES

Allergic Diseases and the FcR β Gene

Many cells and molecules are involved in the production of allergic reactions, including mast cells, basophils, IgE, Fc ϵ RI, and many signal transduction molecules. Obviously, this does not imply that any of these cells or molecules are involved in the pathogenesis of allergic diseases or, in other words, in

discriminating between allergics and nonallergics. However, a number of genetic studies have suggested that the $FcR\beta$ gene may play a role in this regard.

Cookson, Hopkin, and colleagues have accumulated evidence that a gene on chromosome 11 (in the 11q12-13 region) is linked with atopy (102, 103). The linkage between this region of chromosome 11 and atopy and/or bronchial hyperreactivity has been replicated by some but not all groups working with independent sets of probands, suggesting an heterogeneity in the genetic susceptibility to atopy (104–117).

The Cookson and Hopkin group narrowed the critical atopy region to focus on the gene encoding the $FcR\beta$ chain (108). The $FcR\beta$ gene consist of 7 exons, spans about 10 kb, and contains an upstream TATA box. The first exon codes for the 5' untranslated region and a portion of the N-terminal cytoplasmic tail. The first transmembrane domain (TM) is encoded by exons 2 and 3, TM 2 by exons 3 and 4, TM 3 by exon 5, and TM 4 by exon 6. The seventh exon encodes the end of the C-terminal cytoplasmic tail including the $FcR\beta$ ITAM and the 3' untranslated region (118).

Several coding sequence polymorphisms in the $FcR\beta$ gene that are associated with allergic phenotypes were subsequently identified (119–121). The first to be described was in the fourth transmembrane domain of $FcR\beta$, a substitution of a leucine for isoleucine at position 181 (121). However, this polymorphism is uncommon in the general population, with subsequent analyses showing that it is generally found together with a second amino acid change (V183L), and that this double substitution is more strongly associated with atopy than I181L alone (120). Interestingly, the variant sequence Leu181/Leu183 was detected in 72% of the chromosomes analyzed in a Kuwaiti population, indicating a high prevalence in Kuwaiti Arabs compared to British, Australian, and Austrian populations (122). This difference of prevalence among different populations is likely to explain the apparent discrepancies between the studies. In the Kuwaiti Arabs study, the double variant was confirmed to be associated with asthma.

However, the I181LV183L double substitution alone could not account for all of the linkage originally found to the $FcR\beta$ gene (120). A third polymorphism was identified, consisting of a glutamic acid to glycine substitution at position 237 in the β amino acid sequence, which is also strongly linked with atopy, and which is present in 5% of the general population (119). Most intriguingly, this substitution introduces a significant hydrophobicity change just outside of the critical ITAM motif (see signal transduction and the importance of ITAMs) in the β carboxy-terminal cytoplasmic tail. Since the description of the variants described above, a number of additional variants have been found by a number of groups and are currently being investigated (123). In one recent study, a *RsaI* polymorphism (intron 2 and exon 7) was found to be strongly associated with atopic dermatitis (124).

Because they link the development of atopy with a receptor ($\text{Fc}\epsilon\text{RI}$) that can be involved in generating atopic responses, and with a subunit ($\text{FcR}\beta$) of this receptor now known to function to enhance its signaling, these reports provide an intriguing initial step toward understanding a potential immunologic dysregulation that could lead to the development of atopy. The demonstration that $\text{FcR}\beta$ functions as a signal amplifier provides a strong rationale for examination of the described atopy-associated polymorphisms, and possibly others, in terms of their ability to modulate $\text{FcR}\beta$ amplifier function and the effects of such modulation on immunologic and allergic responses *in vivo*.

Autoantibodies and Chronic Urticaria

Chronic urticaria (CU) is a common disorder characterized by recurrent bouts of itchy wheals. In spite of the allergic appearance of the lesions, in most cases no specific allergen is found by provocation tests that could explain skin mast cell degranulation in an IgE - $\text{Fc}\epsilon\text{RI}$ dependent manner. The fact that patient serum can reproduce the lesions when injected intradermally and induce histamine release by normal basophils *in vitro* demonstrates that an endogenous factor present in the serum may be responsible for triggering mast cells (125). Part of this releasing activity could be due to anti- IgE autoAbs binding to IgE molecules normally occupying $\text{Fc}\epsilon\text{RI}$ on basophils and mast cells (126, 127). However, the role of these anti- IgE autoAbs in the pathogenesis of CU is not clear because they can also be detected in the sera of normal individuals and of patients suffering from atopic dermatitis (128, 129). Another potential mechanism for this releasing activity has been provided by recent studies reporting the presence of IgG autoAbs against the $\text{Fc}\epsilon\text{RI}$ α chain in the serum of patients with CU. Among 26 CU patients whose serum caused a wheal when injected intradermally, 4 had a releasing activity contained in the IgG fraction that was inhibited by preincubating the IgG samples with a recombinant protein corresponding to the extracellular part of the α chain of $\text{Fc}\epsilon\text{RI}$ (125). In a subsequent study, Georg Stingl's group found that 12 CU patients among 32 had IgGs capable of recognizing α in a western blot assay (128). Out of the 12 autoAbs, 8 were tested for their functional capacity, 4 of which were able to induce histamine release by normal basophils stripped of IgE , suggesting that this activity was not due to anti- IgE IgG . Unlike anti- IgE autoAbs, the anti- $\text{Fc}\epsilon\text{RI}\alpha$ autoAbs were apparently not observed in normal controls or in patients with atopic dermatitis providing however that the sera be diluted sufficiently. In a later study involving 163 CU patients, 38 (23%) had anti- $\text{Fc}\epsilon\text{RI}\alpha$ autoAbs as assessed by the capacity of soluble recombinant $\text{Fc}\epsilon\text{RI}\alpha$ to inhibit the serum-induced release from basophils or skin mast cells *in vitro* (130). Similar functional results were obtained by Kaplan's group with anti- $\text{Fc}\epsilon\text{RI}\alpha$ autoAbs detected by western blotting (131). From these studies, it appeared that there was a subset

of CU patients with anti-Fc ϵ RI α autoAbs that were able to activate basophils and skin mast cells. However, it was also apparent that both the proportion of patients with the autoAbs and the proportion of autoAbs capable of degranulating basophils varied in the different studies. This variability could be explained in part by the different methods used in the respective studies (serum dilutions, western blotting, or ELISA). In addition, various factors could contribute to the heterogeneity in releasing activity among sera: (a) differences in the releasability of basophils collected from different individuals; (b) differences in the titer of the autoAbs; (c) differences in the affinity of the autoAbs for the α chain; (d) differences in the epitopes recognized on the α chain and in the capacity of the autoAbs to cross-link Fc ϵ RI; (e) differences in the capacity of the Fc fragment of autoAbs to interact with the inhibitory Fc γ RIIB expressed on both mast cells and basophils; after cross-linking of Fc ϵ RI by the autoAbs, the Fc domain of the autoAbs could then bind to Fc γ RIIB, thereby inducing the co-cross-linking of Fc γ RIIB with Fc ϵ RI; this would result in inhibition of the release induced through Fc ϵ RI (132). Recent findings have shed new light on the question of detection of these autoAbs in CU patients. Using an ELISA assay different than the one referred to above, a group of investigators in Switzerland have shown that the anti-Fc ϵ RI α autoAbs can be detected in normal and in CU patients with about the same frequency and that there is no apparent correlation with their detection and the CU disease status (MP Horn, T Gerster, B Ochensberger, T Derer, F Kricek, J-P Kinet, M-H Jouvin, M Vogel, BM Stadler, SM Miescher, submitted). These data are consistent with our own recent results using various types of ELISAs (M-E Jouvin, J-P Kinet, unpublished). Furthermore, the same investigators came fortuitously across an interesting observation when analyzing multi-donor IgG preparations: The anti-Fc ϵ RI α autoAbs have a stronger affinity for a tetanus toxoid antigen (TTd) than for the Fc ϵ RI α antigen (MP Horn, T Gerster, B Ochensberger, T Derer, F Kricek, J-P Kinet, M-H Jouvin, M Vogel, BM Stadler, SM Miescher, submitted). This raises the possibility that the cross-reactive anti-Fc ϵ RI α autoAbs may appear after the TTd vaccination for an unknown reason.

The existence of nonpathogenic anti-Fc ϵ RI α autoAbs in normal human sera raises several important issues. First, discriminating between autoAbs having or not having the capacity to induce cell degranulation is essential. Second, it is clear that a simple ELISA cannot be used to discriminate between pathogenic and nonpathogenic anti-Fc ϵ RI α autoAbs. The identification of specific epitopes potentially associated with the pathogenicity may be required for this distinction. Alternatively, well designed functional tests that do not rely on the variability of the release assays performed with human basophils may be necessary. For example, the use of transfected clonal cell lines may help in the design of well-controlled assays to assess the capacity of anti-Fc ϵ RI α autoAbs to trigger cell degranulation.

SIGNAL TRANSDUCTION MECHANISMS

The Concept of ITAMs

FcRs and other antigen receptors are thought to transduce signals through cytoplasmic tail structures known as immunoreceptor tyrosine-based activation motifs (ITAMs) (133). These activation motifs were first described by Michael Reth in 1989, who recognized an 18-amino acid motif (E/DxxYxxLxxxxxxx-YxxL) present in three copies in the T cell receptor (TCR) ζ subunit, and one copy each in the TCR γ , δ , and ϵ subunits, B cell receptor (BCR) Ig α (MB-29) and Ig β (B1) subunits, and Fc receptor FcR β and FcR γ subunits (134). The ITAMs of FcR β and FcR γ are represented as filled circles in Figure 1. The physiologic importance of this homology was confirmed three years later by three elegant studies in which it was shown that a copy of this motif derived from either the TCR ζ or FcR γ chain could be attached to irrelevant extracellular and transmembrane domains to form chimeric receptors able to function identically as natural antigen receptors (135–138).

An excellent review on FcRs with and without ITAMs has been published recently by Marc Daeron in *Annual Review of Immunology* (139). For that reason, the present review focuses almost exclusively on the signaling properties of Fc ϵ RI and of its signaling chains, the FcR β and FcR γ chains. A special attention is given to the most recent findings on Fc ϵ RI signaling reported over the last two years.

Clustering and Tyrosine Phosphorylation

Signaling through Fc ϵ RI and the resulting cell activation is initiated when binding of specific multivalent antigen to receptor-bound IgE results in receptor clustering. A unique and interesting feature of Fc ϵ RI is its ability to be rapidly engaged with multi-hapten antigen and just as rapidly disengaged through the use of monovalent hapten. Disengagement of Fc ϵ RI results in immediate arrest of cell signaling, demonstrating that continuous receptor engagement is required for maintaining cell activation. The requirements for active receptor clustering in activation of the signaling machinery have been well documented and discussed extensively before in multiple reviews (140–142) and are not discussed here.

For a long time, the initial events following Fc ϵ RI clustering remained unclear. The status of the phosphorylation of FcR β and FcR γ had been analyzed in the early 1980s, but no tyrosine phosphorylation was detected (143). Tyrosine kinase activity was found coprecipitating with Fc ϵ RI as early as 1986 but was considered a contaminating activity (144). In 1990, an important study showed that tyrosine phosphorylation of various substrates including a 72 kDa was induced by Fc ϵ RI clustering, but Fc ϵ RI was not thought to be tyrosine

phosphorylated. In 1991, concomitantly with the discovery of the functionality of the ITAMs, one of our study demonstrated that (a) tyrosine phosphorylation of FcR β and FcR γ was one of the earliest events detectable within 5 s after receptor clustering and (b) Fc ϵ RI tyrosine phosphorylation was a reversible function; as a consequence of Fc ϵ RI disengagement, FcR β and FcR γ dephosphorylate very rapidly (145). In 1992, Joseph Bolen's group discovered that Fc ϵ RI was activating the Lyn tyrosine kinase of the src family shortly after receptor clustering (146). These results were followed by a number of confirmatory studies and by the important demonstration that the Syk tyrosine kinase is also activated though Fc ϵ RI (147–151).

Phosphorylations of ITAMs on the canonical tyrosines after antigen receptor cross-linking are in fact critical for signal transduction, as mutations of these residues abolish signaling (138). In addition, the discovery that tyrosine-phosphorylated ITAMs are binding targets of src homology two (SH2) domains provided a structural basis for the activation properties of these motifs, as an inducible binding site for SH2 containing signaling proteins (152, 153). The two phosphorylated tyrosines within ITAM motifs function as binding sites for tandem SH2 domain-containing tyrosine kinases of the Syk/zap-70 family and possibly a variety of other molecules involved in signal transduction (154–157). In particular, this type of interaction between Syk and Fc ϵ RI was shown to occur and to be functionally important for downstream events (158–164).

FcR β – and FcR γ –ITAMs Signal Differently

The variety of structures found in the antigen receptor superfamily suggests that receptors with different structures would have different functions. Since the identification of the ITAM motif as the key functional signal transduction unit of antigen receptors, much work had been put into identifying differences in the activation properties of motifs from different receptors. Initially, when placed in the context of chimeric receptors, individual ITAM motifs had been found to have cell activation properties equivalent to those of their intact parent receptors and each other, albeit of different efficiencies (135–138, 165). These findings had been interpreted to mean that the multiple ITAMs present in holoreceptors provide parallel but equivalent signaling functions (154). However, this interpretation had left unexplained the impetus for evolution of complex multisubunit structures.

Data from our laboratory and others suggested that the FcR β and γ subunits play different roles in signaling (162, 166–170). One study that used a similar strategy of chimeric molecules as described above showed that the FcR β and FcR γ could function differently in a mast cells context. FcR β unlike FcR γ could be coprecipitated with Lyn but not with Syk, while FcR γ but not FcR β could activate Syk (168). Another critical difference was that FcR β was

incapable of inducing cell degranulation while $\text{FcR}\gamma$ could do it, although with a lesser intensity than did the corresponding endogenous tetrameric $\text{Fc}\epsilon\text{RI}$ (168). Together these data clearly indicated that $\text{FcR}\beta$ and $\text{FcR}\gamma$ were functioning differently.

A Two-Step Activation Model: Lyn Is Upstream of Syk

In addition to the data described in the previous paragraph, another critical and unexpected finding helped us to propose an initial model for $\text{FcR}\beta$ and $\text{FcR}\gamma$ signaling. An $\text{Fc}\epsilon\text{RI}$ -deficient mast cell line was reconstituted by transfection with either the wild-type $\alpha\beta\gamma_2$ $\text{Fc}\epsilon\text{RI}$ complexes or mutated versions. Mutation of the two canonical tyrosines of the $\text{FcR}\gamma$ ITAMs abolished the tyrosine phosphorylation of $\text{FcR}\gamma$ chains but not of $\text{FcR}\beta$ chains, as expected. Surprisingly, the corresponding mutation of the $\text{FcR}\beta$ ITAMs abolished the tyrosine phosphorylation of both $\text{FcR}\beta$ and $\text{FcR}\gamma$, implying that $\text{FcR}\beta$ played a role in $\text{FcR}\gamma$ tyrosine phosphorylation (168). Since $\text{FcR}\beta$ chimeras could bind Lyn (see above), we proposed a model of $\text{Fc}\epsilon\text{RI}$ activation in which $\text{FcR}\beta$ and $\text{FcR}\gamma$ act cooperatively; Lyn is bound to $\text{FcR}\beta$ under resting conditions, Lyn phosphorylates $\text{FcR}\beta$ and $\text{FcR}\gamma$ after receptor engagement with antigen, the phosphorylated $\text{FcR}\gamma$ binds Syk, and $\text{FcR}\gamma$ -bound Syk becomes phosphorylated and activated (168). In support of this model were other data demonstrating that Syk could be activated in vitro by binding phosphorylated $\text{FcR}\gamma$'s ITAMs (159). Studies with inhibitors of Syk that could inhibit cell degranulation but not the phosphorylation of $\text{FcR}\beta$ and $\text{FcR}\gamma$ also supported the position of Lyn and of the phosphorylation of $\text{FcR}\beta$ and $\text{FcR}\gamma$ upstream of the activation of Syk (163, 164). To test this proposed model of activation, a reconstitution system was designed in which the expression of each molecule or a mutated version of it could be controlled (162). Using this system, several features of the proposed model were directly demonstrated including: (a) Lyn phosphorylates both $\text{Fc}\epsilon\text{RI}$ β and γ ITAMs, (b) β and γ ITAM phosphorylation by Lyn causes Syk to bind to $\text{Fc}\epsilon\text{RI}$, and (c) Syk binding to $\text{Fc}\epsilon\text{RI}$ leads to its Lyn-dependent tyrosine phosphorylation and activation (162). In addition, the system of reconstitution was successfully used to reconstruct the dependence of these events on receptor clustering (162). The ability to control the expression levels of Lyn and Syk was critical to this reconstruction, as clustering control was present at levels of Lyn and Syk expression near those seen in hematopoietic cells but was lost at higher levels of expression.

Another approach, overexpression of catalytically inactive molecules, was used for identification of Syk substrates and Syk-dependent signaling pathways (161, 171), and to corroborate data obtained from reconstitution experiments in fibroblasts in a native signaling environment (162). In particular, a Syk-dominant negative could abolish Syk activation, calcium signaling, and cell

degranulation but not the phosphorylation of the FcR β and FcR γ subunits. Thus, these data could confirm, in a mast cell context, the data obtained in the reconstitution system.

Later, a number of genetic studies confirmed this model. In Lyn-deficient mast cells, no phosphorylation of FcR γ and FcR β could be detected, while in Syk-deficient mast cells, the phosphorylation of both FcR γ and FcR β were present after receptor clustering but downstream signals (phosphorylation of PLC- γ , calcium mobilization, and degranulation) were abolished (172–174).

FcR γ Functions as an Independent Activation Module and FcR β as an Amplifier

The above model was paradoxical. It gave a prominent role to FcR β and Lyn even though the FcR γ ITAM-containing chimeras could activate Syk, calcium mobilization and cell degranulation without apparent requirement for the FcR β ITAM. Furthermore, the model could not explain how the newly discovered human $\alpha\gamma_2$ complexes could function. In studies designed to understand the interplay between FcR β and FcR γ subunit function in a holo-receptor context during Fc ϵ RI signaling, we discovered that FcR γ functions as an autonomous activation module, whereas FcR β has the functional properties of an amplifier (49). The concept of FcR β as an amplifier is based on the facts that FcR β (a) does not have autonomous cell activation capability, (b) amplifies the intensity of cell activation signals sent by the FcR γ chain with a gain of five-to seven-fold (as measured by Syk activation and calcium mobilization), and (c) does not qualitatively alter the FcR γ -mediated cell activation signals. Furthermore, the mechanism of FcR β amplification was shown to involve the FcR β ITAM acting as an inducible “anchor” for Lyn, resulting in enhanced Lyn recruitment to the receptor complex after engagement, enhanced Lyn-dependent phosphorylation of FcR γ , enhanced activation of the Syk nonreceptor tyrosine kinase, and enhanced calcium mobilization (49).

The notion that FcR β works as an amplifier was subsequently challenged by binding and coprecipitating studies. FcR β ITAM but not FcR γ ITAM binds in vitro to SH2-containing tyrosine (SHP-1 and SHP-2) and SH2-containing inositol-5'-phosphatase (SHIP) in vitro (175–177). In addition, SHP-2 coprecipitates with Fc ϵ RI after receptor engagement (176). Since these phosphatases have been implicated in negative regulating functions, these data suggested that FcR β might have a negative regulatory function. However, affinities of the phosphorylated FcR β and FcR γ ITAMs were measured recently using surface plasmon resonance under carefully controlled conditions. In this study (178), Syk was confirmed to bind to FcR γ with high affinity ($IC_{50} = 23$ nM) (159) and to FcR β with 18-fold lower affinity, confirming the preference of Syk for FcR γ ITAM. Importantly, no binding of SHP-2 to FcR β or FcR γ ITAMs was

observed even at 100 μM concentrations, suggesting that in physiologic conditions, SHP-2 is unlikely to bind these motifs. More functional studies on the functional role of these phosphatases need to be done to clarify their role in Fc ϵ RI signaling.

That FcR β indeed functions as an amplifier of both early and late responses has now been settled unequivocally by the genetic study described above in the paragraph on FcR β chain amplification (47). However, although overwhelming, the evidence for the FcR β amplification function does not exclude the possibility that FcR β is playing an autonomous specific role in addition to being an amplifier of FcR γ -mediated functions. So far, however, no such specific role for FcR β has been identified functionally.

Molecular Interactions Initiating Lyn and Syk Activation

How is Lyn interacting with FcR β ? In vitro studies have shown that SH2 domains of Lyn bind to FcR β ITAM (158), but our own functional data suggest that the Lyn SH2 domain is not required for Fc ϵ RI phosphorylation (S Lin, J-P Kinet, unpublished). Other studies using the yeast two-hybrid system showed that the FcR β ITAM-containing cytoplasmic domain interacts with the SH4-containing unique domain (179). Overexpression of this domain could inhibit Lyn-dependent tyrosine phosphorylation of Fc ϵ RI, confirming the in vitro binding data (179). However, in another study, overexpression of proline-rich peptides that bind specifically Lyn SH3 domains suppressed the Lyn-mediated phosphorylation of Fc ϵ RI, the membrane translocation of Syk to Fc ϵ RI and calcium signaling (180). One possible interpretation of these results is that both domains are involved in FcR β interaction. Alternatively, the proline-rich peptides binding to the SH3 domain could impair indirectly the SH4-binding function.

How are $\alpha\gamma_2$ complexes signaling in the absence of FcR β ? As mentioned above, FcR γ is able on its own to activate Syk, calcium signaling and degranulation but at a much lower level than $\alpha\beta\gamma_2$ complexes (49, 168). It is critical to note that FcR γ -mediated activation of Syk in the absence of FcR β is still dependent on the presence of the Lyn kinase (49). The latter kinase is necessary to promote the low level (difficult to detect) tyrosine phosphorylation of FcR γ and the activation of Syk. That the Lyn kinase (or another src family kinase) initiates Syk activation has now been established (181). The Lyn kinase initiates the process by phosphorylating tyrosine-518 in the activation loop of Syk, inducing Syk to autophosphorylate and to become fully active. Thus, the absence of FcR β does not preclude Lyn, which is anchored in the inner layer of the plasma membrane by its aminoterminal myristylated glycine, from phosphorylation of FcR γ upon receptor clustering and activation of Syk. However, this process is relatively inefficient, and by anchoring the Lyn kinase, FcR β

provides access to FcR γ and the Syk tyrosine kinase, substantially enhancing the whole process. The domains of Lyn required for a functional interaction with FcR γ and Syk in the absence of FcR β have not yet been analyzed.

How is Lyn activated? The classical view of the activation process of src family kinase is as follows. The csk kinase phosphorylates src family kinases at an autoregulatory c-terminal tyrosine which, when phosphorylated, binds to its own SH2 domain thereby forming an internal loop that is keeping the kinase inactive. The CD45 phosphatase then dephosphorylates this regulatory tyrosine, consequently activating the kinase (182). Evidence that CD45 is involved in the Fc ϵ RI activation has been provided in a few studies. Antibodies against CD45 inhibited Fc ϵ RI-mediated histamine release from human basophils (183). Clustering of reconstituted Fc ϵ RI in a CD45-deficient Jurkat T cell line did not result in phosphorylation of FcR β and FcR γ and calcium mobilization. However, both functions were restored after reconstitution with a chimeric molecule containing the phosphatase domain of CD45 (184). However, in NIH 3T3 cells that lack expression of CD45, clustering of Fc ϵ RI resulted in Lyn-dependent phosphorylation of FcR β and FcR γ , suggesting that CD45 is not required for Lyn activation (49, 162). It is possible that another phosphatase plays the role of CD45 in NIH 3T3 fibroblasts, or that the role of CD45 is to enhance the efficiency of src family kinase activation. In that case, overexpression of Lyn in NIH3T3 may result in a sufficient amount of Lyn in activated form to get the system to work.

Thus, the exact molecular events regulating Lyn activation are not yet fully understood. It is clear, however, that clustering is necessary to initiate the increase in tyrosine phosphorylation of FcR β and FcR γ (145). This process appears to be initiated by the Fc ϵ RI molecules that are adjacent in the cluster to Fc ϵ RI-bound Lyn (185, 186). This transphosphorylation activation process seems to be the only one that would work efficiently with the stoichiometry of Lyn to Fc ϵ RI being substantially lower than 1. In fact, Lyn has been shown to be limiting in an elegant study using two different Fc ϵ RI ligands (187). Activation through the first ligand inhibited subsequent tyrosine phosphorylation of Fc ϵ RI complexes activated via the second ligand. However, the disengagement of Fc ϵ RI from the first ligand induced tyrosine phosphorylation of Fc ϵ RI already clustered by the second ligand, demonstrating that the kinase responsible for Fc ϵ RI phosphorylation cannot phosphorylate efficiently both sets of Fc ϵ RI complexes and is therefore the limiting factor. Retrospectively, it is interesting to note that the NIH3T3 reconstitution of the clustering dependence of tyrosine phosphorylation events initiated by Lyn, was dependent on the level of overexpression of Lyn (162). At high levels of expression, the clustering dependence was lost, suggesting that an intrinsic part of the clustering dependence is a limited amount of Lyn.

Interesting data from Henry Metzger's group have taken the observation that Lyn is limiting a step further (188). The capacity of downstream signaling by an individual Fc ϵ RI is dependent on its capacity to remain in a cluster and is therefore influenced by the ligand affinity. Consequently, low affinity ligands allow the previously engaged receptors to become rapidly disengaged and unproductive in downstream signaling. Furthermore, it appears that an excess of receptors clustered with low affinity ligands is able to sequester Lyn so that a smaller amount of receptors clustered with higher affinity ligand are unable to activate the signaling cascade. The fact that clustered receptors compete for a limited amount of Lyn is proposed to be the basis of the mechanism for ligand antagonism (188).

The Tec Family Kinases: Bruton Tyrosine Kinase (Btk) and Itk

As mentioned above, the earliest events within 5 s of Fc ϵ RI clustering are receptor tyrosine phosphorylation and activation of the Lyn tyrosine kinase. Within 15 s Syk activation follows concomitantly with the initiation of calcium mobilization. Activation of another tyrosine kinase, the Bruton tyrosine kinase (Btk) has also been described after Fc ϵ RI clustering, suggesting a role in mast cell activation. Btk is a member of the recently described Btk/Tec family of tyrosine kinases (189–191). Members of this family are distinguished by the presence of a large unique amino terminal region containing a pleckstrin homology (PH) domain, single SH3 and SH2 domains, and the lack of an autoregulatory C-terminal tyrosine in their requisite kinase domains (191–193). Within the Btk/Tec family, Btk is of particular interest due to its causal role in X-linked agammaglobulinemia in humans (Xla), and in x-linked immunodeficiency in mice (Xid) (189, 194, 195). These related immune deficiencies are associated with an arrest in B cell development at the pre-B cell stage, resulting in low or absent B lineage cell numbers and hypo- or agammaglobulinemia.

Unlike B cell development, mast cell development in Xla is apparently normal. However, Xid mice exhibit an impaired Fc ϵ RI-dependent anaphylactic reaction, with late phase reactions more affected than the early phases (196). Consistent with this, cultured mast cells from both Xid and Btk null mice exhibit mild impairments in Fc ϵ RI-mediated degranulation and more profound defects in Fc ϵ RI-mediated cytokine (TNF- α , IL-2, IL-6, GM-CSF) production (196). In fact, a recent study shows that Btk is required for maximal Fc ϵ RI-mediated IL-2 gene activation and that this regulation occurs through one of the MAP kinases, the c-Jun N-terminal kinase (JNK) (197).

Another Tec family kinase (Itk, also called emt) is expressed in mast cells and is activated through Fc ϵ RI (198). Overexpression of Itk in Btk-deficient mast cells could partially restore the defects of histamine release or cytokine

production but not to the levels obtained with wild-type Btk, indicating at least a partial redundancy of these two Tec kinases (196). From that result, one could have extrapolated that the defects in cell degranulation and cytokine release would probably have been much more profound in the absence of both Tec kinases. In fact, this is exactly the situation in Btk/Itk double deficient mice, where Fc ϵ RI-mediated degranulation of mast cells and anaphylaxis are abolished (Fred Alt, personal communication).

A Novel Connection: Phosphatidylinositol 3 Kinase-Tec Kinases-PLC γ -Sustained Calcium Influx

What function could Tec kinases play in signaling that could explain their biological activity in lymphocytes and mast cells? The beginning of the explanation came from an experiment in the DT40 chicken B cell line in which the gene for Btk was targeted (199). In that cell line, no other Tec kinases are expressed, and the phenotype of the cell line was dramatic: No calcium mobilization and a reduced phosphorylation of PLC- γ 2 were observed after B cell receptor (BCR) clustering. This experiment implicated Btk as a dominant player in the calcium mobilization pathway most likely through an effect, direct or indirect, on PLC- γ 2 activation. However, the molecular mechanism by which Btk could play this function was unclear. In order to understand how Btk could regulate calcium mobilization, it was critical to first understand how Tec kinases are activated.

Data from B cell studies and from a reconstitution system demonstrate that the basic mechanisms of activation of the Syk and Tec kinases are similar and are initiated by Lyn (181, 200), which phosphorylates a regulatory tyrosine in the activation loop of Syk (tyrosine-518) and Btk (tyrosine-551). However, for this to happen, membrane targeting of the cytoplasmic kinase is required. Membrane targeting of Syk to the phosphorylated FcR γ ITAMs is well grasped. However, the molecular interactions required for the membrane targeting of Btk were not well understood until recently. In vitro binding studies with the SH2, SH3, and PH domains of Tec kinases had identified a number of potential membrane targets. In particular, the PH domain of Btk was shown to be capable of interacting with phosphatidyl-3,4,5,-trisphosphate (PtdIns-3,4,5-P3), a product of the activation of phosphatidylinositol 3 kinases (PI3 kinases) (201, 202).

Numerous receptors have been shown to activate PI3 kinases (reviewed in 203, 204). To demonstrate the effect of the activation of PI3 kinases, most studies have used wortmannin or similar inhibitors of its enzymatic activity. The activation of PI3 kinase through Fc ϵ RI was demonstrated to occur in mast cell lines using such inhibitors. Inhibition of PI3 kinase caused the inhibition of Fc ϵ RI-mediated cell degranulation but did not influence the activation of

Lyn or Syk kinases (205–207). However, wortmannin was shown to inhibit the formation of the second messenger inositol-1,4,5-P₃ (IP₃), itself the product of the action of phospholipase C isoenzymes (207).

All of these observations about Tec kinases and PI3 kinases fell into place when a novel pathway linking PdtIns-3,4,5-P₃ to calcium signaling was described. Using a B cell line and a reconstitution system, the direct demonstration was made that PdtIns-3,4,5-P₃ interacting with the PH domain serves as the membrane targeting signal and upstream activation signal of Tec kinases (208, 209). The lyn-initiated activation of membrane-targeted Tec kinases results in Tec-kinase activation and in Tec-kinase dependent PLC γ tyrosine phosphorylation (this function requires the Tec SH2 domain), which induces enhanced IP₃ production. In turn, this PdtIns-3,4,5-P₃-dependent IP₃ production appears to be critical for maintaining the sustained calcium influx (210, 211), probably by keeping empty the specific intracellular stores that regulate the opening of the calcium regulated activated channels (CRAC) (209, 212).

The capacity of the PI3 kinase-Tec-PLC γ to regulate intracellular calcium levels allows this pathway to regulate later events of the signaling cascade. For example, the peak and sustained phases of the intracellular calcium increases are required for induction of the mitogen-activated protein kinase (MAPK), JNK and p38 cascades and the calcium dependent transcription factor NF-AT and NF- κ B (213, 214). A minireview on the PI3 kinase-Tec-PLC γ pathway and the mechanisms by which it may regulate the openings of the calcium regulated activated channels (CRAC) has been recently published in *Cell* (209); because of space limitations, we do not discuss any more details here except to say that this pathway is blocked when a SHIP-dependent inhibitory receptor such as Fc γ RIIb is engaged. Coengagement of Fc γ RIIb with Fc ϵ RI efficiently inhibits cell degranulation (132). The inhibitory function of Fc γ RIIb in B cells or mast cells is mediated by SHIP (see the accompanying chapter on Inhibitory Receptors by Eric Long), but how SHIP was producing its inhibition had remained unclear. In fact, in a B cell line and a reconstitution system, SHIP hydrolyzes Pdt Ins-3,4,5-P₃ and in so doing inhibits Btk activation, PLC γ phosphorylation, IP₃ production, and the sustained calcium influx (210, 211).

Where is Syk in all of this? In mast cells and B cells, the absolute requirement for Syk in calcium signaling and many downstream functions has been demonstrated in many studies (158–164, 171, 173, 174). At the same time, Btk-deficient DT40 B cells (which are in fact Tec kinases deficient) do not flux calcium either, indicating a crucial role for Btk in calcium signaling as well. How could both Btk and Syk be required?

The mechanism of activation of PI3 kinases has been thought to result from an interaction of the p85 subunit of PI3 kinase with src family kinases (215). We revisited recently this question by using the Syk-deficient DT40 cell line

and demonstrated that the BCR-dependent production of PtdIns-3,4,5-P3 is abolished. In the RBL mast cell line and in another B cell line A20, we could also confirm that Syk is indeed upstream of the activation of PI3-kinases (LO Beitz, DA Fruman, AM Scharenberg, T Kurosaki, LC Cantley, J-P Kinet, unpublished). Therefore, these data support a model in which Syk is upstream of PI3 kinase, which is itself upstream of Btk.

Sphingosine Kinases

Knowledge of the diverse array of biological activities mediated by sphingolipid metabolites has exploded in the past few years (216–218). One of these metabolites, sphingosine-1-phosphate (S1P), is a calcium mobilizing second messenger (219). S1P is generated by the activity of sphingosine kinase (SK), an enzyme described approximately 20 years ago, which until recently has been difficult to adequately purify (220). SK is thought to work directly on available plasma membrane sphingosine to produce S1P (221). S1P mediates calcium efflux from microsome preparations, and an S1P gated calcium channel has been described in microsomes (222–224). Rapid production of S1P has now been proposed to be the mechanism by which the platelet derived growth factor (PDGF) receptor mobilizes calcium (219).

A study of RBL-2H3 cells transfected with a G-protein coupled receptor (the m1 muscarinic receptor) had produced a paradoxical result which was not consistent with PLC γ /IP $_3$ as the sole mechanism of Fc ϵ RI-mediated calcium mobilization. Stimulation of transfected m1 receptors with carbachol produces a calcium flux similar in magnitude to that of Fc ϵ RI, but which is associated with more than 10 times the IP $_3$ release seen with Fc ϵ RI stimulation (225, 226). This Fc ϵ RI “IP $_3$ gap” suggested that Fc ϵ RI may also utilize another second messenger such as S1P to mobilize calcium. This possibility was tested by using a sphingosine kinase inhibitor, DL-threo-dihydrosphingosine (DHS) (227), and measuring its effect on calcium mobilization through Fc ϵ RI and through carbachol in m1-transfected RBL-2H3 cells (228). At a given concentration, DHS almost completely suppressed calcium mobilization through Fc ϵ RI, and essentially left unaffected calcium mobilization through m1 muscarinic receptors, suggesting that Fc ϵ RI mobilizes calcium primarily via activation of an SK. Fc ϵ RI-mediated SK activation was then directly addressed by measurement of the production of S1P and of the SK activity after engagement of Fc ϵ RI with antigen. These data showed that stimulation through Fc ϵ RI produced S1P and an approximate two-fold enhancement of SK activity, nearly identical to that described for the PDGF receptor (219). Finally, the S1P applied to cells induced an EGTA-insensitive calcium mobilization, an indication that it was the result of a release from intracellular stores. Together these data suggested that Fc ϵ RI utilizes a SK pathway (228).

A number of studies led to a reappraisal of our initial interpretation of these data. First, studies described the presence on the cell surface of G-protein-coupled receptor for S1P, which is pertussis toxin sensitive and capable of mobilization of calcium via IP₃ (218, 229–231). In light of these studies, the experiment of S1P-mediated calcium was repeated in presence of pertussis toxin. The toxin could abolish the S1P-mediated calcium mobilization, indicating that it was using the G-protein receptor (O Choi, J-P Kinet, unpublished). Because the FcεRI-dependent pathway is pertussis-toxin insensitive (232–234), it is clear that the S1P/G-protein receptor pathway is not involved in FcεRI-dependent calcium mobilization. However, S1P could have a dual role and could act as a second messenger also (218). Second, by analyzing multiple RBL cells in single cell calcium mobilization studies, it became clear (E Donnadieu, J-P Kinet, unpublished) that the m1 receptor-mediated calcium signal was synchronous while the FcεRI-mediated calcium response was very asynchronous. This difference of synchronicity using the same clonal cell line was unexpected and could account, at least in part, for the IP₃ gap described above. Third, the high affinity IgG receptor FcγRI, which is signaling by using FcRγ, is also capable of activating SK and producing S1P, and this S1P production appears to be linked to calcium mobilization as in the case of FcεRI. However, the activation of SK is the result of the activation of phospholipase D, itself being activated through tyrosine kinases (235). Fourth, Reinhold Penner's group has recently demonstrated that sphingosine, unlike S1P, is a very potent inhibitor of CRAC current (ICRAC) (236). At the end of this paper, the authors speculate that "Sphingosine might be involved in the activation mechanism of ICRAC. In the resting state, sphingosine could act as a blocker of ICRAC. Upon depletion of internal stores, metabolism of sphingosine, possibly by conversion to sphingosine-1-phosphate by sphingosine kinase, could lower the sphingosine levels and lead to the disinhibition of ICRAC."

At present, one is left with a number of data that support the notion that sphingosine kinase is involved in calcium mobilization. Sphingosine kinase is activated and S1P is produced by two FcRγ-containing receptors, FcεRI and FcγRI. However, the effect of this activation on later events such as calcium mobilization is so far entirely based on studies with inhibitors (with the exception of Reinhold Penner's data) and is therefore subject to unsuspected problems or artifacts. Because the activation of ICRAC is so intimately linked to the release of special stores, a mechanism which is itself not completely understood, it is difficult to discriminate between the possible effects of SK activation on the stores and on ICRAC. For these reasons, it is likely that a better understanding of the role of SK in FcεRI signaling will await molecular characterization of SK that will permit genetic mapping of the system.

Other Signaling Molecules

What is the role of the serine/threonine phosphorylation of Fc ϵ RI? Engagement of Fc ϵ RI induces the serine phosphorylation of FcR β and threonine phosphorylation of FcR γ (145). The phosphorylated residues are in the ITAMs of FcR β and FcR γ (237). The threonine phosphorylation of FcR γ appears to be mediated by protein kinase C- δ (PKC- δ) and to correlate with receptor endocytosis (238). Other PKC isoforms are also activated through Fc ϵ RI. PKC- β and PKC- ϵ seem to play a role in the expression of c-fos and c-jun (238a). PKC- α and PKC- ϵ act as negative regulators of phospholipase A2 (PLA2) activity, and PKC- β as a positive modulator of secretion, PLA2 activity, and cytokine production (239). How these pathways are connected to the tyrosine kinase pathways is at present unclear.

What is the role of phosphatases? The possible role of CD45 has already been discussed. Other phosphatases that are probably implicated are SHP-1, SHP-2, and SHIP (175, 177), but their precise role remains undefined. They could play a role in balancing the activation of the tyrosine kinases and/or be operating in the rapid uncoupling of Fc ϵ RI complexes following receptor disengagement with haptens. What is known, however, is that the phosphatase activity responsible for receptor disengagement is not cytoplasmic but is associated with the plasma membrane (240).

A number of studies implicate various adapter molecules in Fc ϵ RI-mediated pathways whose phosphorylation depends on Syk: (a) "shc" which may be involved in the activation of mitogen-activated protein kinase (MAPK) (171) (241), (b) "vav" (163, 242), which seems to associate with Grb2, Raf-1, and MAPK (243) and may be involved in Rac1-dependent JNK activation (241), (c) "SLP-76" (244) is also tyrosine phosphorylated by Syk (245) and is implicated in the activation of PLC- γ (246). Its gene targeting demonstrates that it is essential for T cell development and function (247, 248), and (d) "cbl" plays also a role, but it remains to be defined (249).

Many more signaling molecules have been described to be involved in Fc ϵ RI signaling pathways, but their precise molecular roles and functions remain unclear. Because of space limitation and for the purpose of clarity, this review does not include all papers published on all molecules involved in Fc ϵ RI and other immunoreceptors pathways. Of the large and complex puzzle of the Fc ϵ RI-dependent pathways, only a few very small pieces have started to fit together. Our future task is to find a place for each of these molecules and to integrate them in the complex puzzle of Fc ϵ RI biological function.

Updated Model of Fc ϵ RI Signaling

Upon clustering of receptor-bound IgE by multivalent antigens, Lyn which is bound to FcR β prior to receptor engagement, transphosphorylates adjacent

receptors on FcR β and FcR γ . This tyrosine phosphorylation of FcR β ITAM recruits more Lyn, and the transphosphorylation of more adjacent receptors in the cluster goes on. The two phosphorylated tyrosines of FcR γ ITAM serve as the membrane targeting signal for the tandem-SH2 of Syk, which is now in the proximity of FcR β -attached Lyn. Lyn phosphorylates Syk on tyrosine-518, and this results in Syk autophosphorylation at other sites and activation of other adjacent Syk molecules. In Fc ϵ RI $\alpha\gamma$ 2 complexes where FcR β is not present, the membrane-attached Lyn operates the same function of phosphorylating FcR γ and Syk but at a much lower level.

Syk then phosphorylates a number of substrates (shc, vav, SLP-76, cbl ...) and activates PI3 kinase. PI3 kinase generates PtdIns-3,4,5-P3 in the vicinity of the cluster. Then PtdIns-3,4,5-P3 becomes the membrane targeting signal for the PH domain of Tec kinases. This allows Lyn to phosphorylate tyrosine-551 in the activation loop of Btk (or other Tec kinases). Membrane attached Tec kinases autophosphorylate at other sites and become fully active. Tec kinases serve at least two functions: the function of adapter molecules via SH2 domains (additional adapters are most likely necessary) and the kinase function. As a result, PLC- γ is membrane targeted and becomes phosphorylated by both Tec kinases and Syk. IP3 is generated and the CRAC controlling stores release intracellular calcium and activate ICRAC. This store-operated function and the opening of ICRAC may involve additional controls (sphingosine and S1P?).

Visit the *Annual Reviews* home page at
<http://www.AnnualReviews.org>

See Addendum following, page 973: The Crystal Structure of the High-Affinity IgE Receptor (Fc ϵ RI α).

Literature Cited

1. Metzger H, Alcaez G, Hohman R, Kinet JP, Pribluda V, Quarto R. 1986. The receptor with high affinity for immunoglobulin E. *Annu. Rev. Immunol.* 4:419-70
2. Ravetch JV, Kinet JP. 1991. Fc receptors. *Annu. Rev. Immunol.* 9:457-92
3. Ra C, Jouvin MH, Blank U, Kinet JP. 1989. A macrophage Fc gamma receptor and the mast cell receptor for IgE share an identical subunit. *Nature* 341:752-54
4. Hibbs ML, Selvaraj P, Carpen O, Springer TA, Kuster H, Jouvin MH, Kinet JP. 1989. Mechanisms for regulating expression of membrane isoforms of Fc gamma RIII (CD16). *Science* 246:1608-11
5. Kurosaki T, Ravetch JV. 1989. A single amino acid in the glycosyl phosphatidylinositol attachment domain determines the membrane topology of Fc gamma RIII [published erratum appears in *Nature* 1990, Jan. 25; 343 (6256):390]. *Nature* 342:805-7
6. Orloff DG, Ra CS, Frank SJ, Klausner RD, Kinet JP. 1990. Family of disulphide-linked dimers containing the zeta and eta chains of the T-cell receptor and the gamma chain of Fc receptors. *Nature* 347:189-91
7. Blank U, Ra C, Miller L, White K, Metzger H, Kinet JP. 1989. Complete structure and expression in transfected cells of high affinity IgE receptor. *Nature* 337:187-89
8. Ra C, Jouvin MH, Kinet JP. 1989.

- Complete structure of the mouse mast cell receptor for IgE (Fc epsilon RI) and surface expression of chimeric receptors (rat-mouse-human) on transfected cells. *J. Biol. Chem.* 264:15323-27
9. Kinet JP, Alcaraz G, Leonard A, Wank S, Metzger H. 1985. Dissociation of the receptor for immunoglobulin E in mild detergents. *Biochemistry* 24:4117-24
 10. Kinet JP, Quarto R, Perez-Montfort R, Metzger H. 1985. Noncovalently and covalently bound lipid on the receptor for immunoglobulin E. *Biochemistry* 24:7342-48
 11. Kinet JP, Metzger H, Hakimi J, Kochan J. 1987. A cDNA presumptively coding for the alpha subunit of the receptor with high affinity for immunoglobulin E [published erratum appears in *Biochemistry* 1988 Nov. 15; 27(23):8694]. *Biochemistry* 26:4605-10
 12. Shimizu A, Tepler I, Benfey PN, Berenstein EH, Siraganian RP, Leder P. 1988. Human and rat mast cell high-affinity immunoglobulin E receptors: characterization of putative alpha-chain gene products. *Proc. Natl. Acad. Sci. USA* 85:1907-11
 13. Kochan J, Pettine LF, Hakimi J, Kishi K, Kinet JP. 1988. Isolation of the gene coding for the alpha subunit of the human high affinity IgE receptor. *Nucleic Acids Res.* 16:3584
 14. Letourneur O, Sechi S, Willette-Brown J, Robertson MW, Kinet JP. 1995. Glycosylation of human truncated Fc epsilon RI alpha chain is necessary for efficient folding in the endoplasmic reticulum. *J. Biol. Chem.* 270:8249-56
 15. Kinet JP, Blank U, Ra C, White K, Metzger H, Kochan J. 1988. Isolation and characterization of cDNAs coding for the beta subunit of the high-affinity receptor for immunoglobulin E. *Proc. Natl. Acad. Sci. USA* 85:6483-7
 16. Kurosaki T, Gander I, Wirthmueller U, Ravetch JV. 1992. The beta subunit of the Fc epsilon RI is associated with the Fc gamma RIII on mast cells. *J. Exp. Med.* 175:447-51
 17. Lanier LL, Yu G, Phillips JH. 1989. Co-association of CD3zeta with a receptor (CD16) for IgG Fc on human natural killer cells. *Nature* 342:803-5
 18. Anderson P, Caligiuri M, Ritz J, Schlossman SF. 1989. CD3-negative natural killer cells express zeta TCR as part of a novel molecular complex. *Nature* 341:159-62
 19. Anderson P, Caligiuri M, O'Brien C, Manley T, Ritz J, Schlossman SF. 1990. Fc gamma receptor type III (CD16) is included in the zeta NK receptor complex expressed by human natural killer cells. *Proc. Natl. Acad. Sci. USA* 87:2274-78
 20. Kinet JP. 1992. The gamma-zeta dimers of Fc receptors as connectors to signal transduction. *Curr. Opin. Immunol.* 4:43-48
 21. Ernst LK, Duchemin AM, Anderson CL. 1993. Association of the high-affinity receptor for IgG (Fc gamma RI) with the gamma subunit of the IgE receptor. *Proc. Natl. Acad. Sci. USA* 90:6023-27
 22. Scholl PR, Geha RS. 1993. Physical association between the high-affinity IgG receptor (Fc gamma RI) and the gamma subunit of the high-affinity IgE receptor (Fc epsilon RI gamma). *Proc. Natl. Acad. Sci. USA* 90:8847-50
 23. Pfefferkorn LC, Yeaman GR. 1994. Association of IgA-Fc receptors (Fc alpha R) with Fc epsilon RI gamma 2 subunits in U937 cells. Aggregation induces the tyrosine phosphorylation of gamma 2. *J. Immunol.* 153:3228-36
 24. Morton HC, van den Herik-Oudijk IE, Vossebeld PA, Snijders A, Verhoeven AJ, Capel PJ, van de Winkel JG. 1995. Functional association between the human myeloid immunoglobulin A Fc receptor (CD89) and FcR gamma chain. Molecular basis for CD89/FcR gamma chain association. *J. Biol. Chem.* 270:29781-87
 25. Gibbins J, Asselin J, Farndale R, Barnes M, Law CL, Watson SP. 1996. Tyrosine phosphorylation of the Fc receptor gamma-chain in collagen-stimulated platelets. *J. Biol. Chem.* 271:18095-99
 26. Gibbins JM, Okuma M, Farndale R, Barnes M, Watson SP. 1997. Glycoprotein VI is the collagen receptor in platelets which underlies tyrosine phosphorylation of the Fc receptor gamma-chain. *FEBS Lett.* 413:255-59
 27. Tsuji M, Ezumi Y, Arai M, Takayama H. 1997. A novel association of Fc receptor gamma-chain with glycoprotein VI and their co-expression as a collagen receptor in human platelets. *J. Biol. Chem.* 272:23528-31
 28. Letourneur F, Hennecke S, Demolliere C, Cosson P. 1995. Steric masking of a dilysine endoplasmic reticulum retention motif during assembly of the human high affinity receptor for immunoglobulin E. *J. Cell Biol.* 129:971-78
 29. Miller L, Blank U, Metzger H, Kinet JP. 1989. Expression of high-affinity binding of human immunoglobulin E by transfected cells. *Science* 244:33437

30. Kuster H, Thompson H, Kinet JP. 1990. Characterization and expression of the gene for the human Fc receptor gamma subunit. Definition of a new gene family. *J. Biol. Chem.* 265:6448–52
31. Wang B, Rieger A, Kilgus O, Ochiai K, Maurer D, Fodinger D, Kinet JP, Stingl G. 1992. Epidermal Langerhans cells from normal human skin bind monomeric IgE via Fc epsilon RI. *J. Exp. Med.* 175:1353–65
32. Bieber T, de la Salle H, Wollenberg A, Hakimi J, Chizzonite R, Ring J, Hanau D, de la Salle C. 1992. Human epidermal Langerhans cells express the high affinity receptor for immunoglobulin E (Fc epsilon RI). *J. Exp. Med.* 175:1285–90
33. Gounni AS, Lamkhoui B, Ochiai K, Tanaka Y, Delaporte E, Capron A, Kinet JP, Capron M. 1994. High-affinity IgE receptor on eosinophils is involved in defence against parasites. *Nature* 367:183–6
34. Maurer D, Fiebiger E, Reininger B, Wolff-Winiski B, Jouvin MH, Kilgus O, Kinet JP, Stingl G. 1994. Expression of functional high affinity immunoglobulin E receptors (Fc epsilon RI) on monocytes of atopic individuals. *J. Exp. Med.* 179:745–50
35. Maurer D, Fiebiger S, Ebner C, Reininger B, Fischer G, Wichlas S, Jouvin MH, Schmitt-Egenolf M, Kraft D, Kinet JP, Stingl G. 1996. Peripheral blood dendritic cells express Fc epsilon RI as a complex composed of Fc epsilon RI alpha- and Fc epsilon RI gamma-chains and can use this receptor for IgE-mediated allergen presentation. *J. Immunol.* 157:607–16
36. Joseph M, Gounni AS, Kusnier JP, Vorng H, Sarfati M, Kinet JP, Tonnel AB, Capron A, Capron M. 1997. Expression and functions of the high-affinity IgE receptor on human platelets and megakaryocyte precursors. *Eur. J. Immunol.* 27:2212–18
37. Tanaka Y, Takenaka M, Matsunaga Y, Okada S, Anan S, Yoshida H, Ra C. 1995. High affinity IgE receptor (Fc epsilon RI) expression on eosinophils infiltrating the lesions and mite patchtested sites in atopic dermatitis. *Arch. Dermatol. Res.* 287:712–17
38. Humbert M, Grant JA, Taborda-Barata L, Durham SR, Pfister R, G Menz, Barkans J, Ying S, Kay AB. 1996. High-affinity IgE receptor (Fc epsilon RI)-bearing cells in bronchial biopsies from atopic and nonatopic asthma. *Am. J. Respir. Crit. Care Med.* 153:1931–37
39. Sihra BS, Kon OM, Grant JA, Kay AB. 1997. Expression of high-affinity IgE receptors (Fc epsilon RI) on peripheral blood basophils, monocytes, and eosinophils in atopic and nonatopic subjects: relationship to total serum IgE concentrations. *J. Allergy Clin. Immunol.* 99:699–706
40. Rajakulasingam K, Durham SR, O'Brien F, Humbert M, Barata LT, Reece L, Kay AB, Grant JA. 1997. Enhanced expression of high-affinity IgE receptor (Fc epsilon RI) alpha chain in human allergen-induced rhinitis with co-localization to mast cells, macrophages, eosinophils, and dendritic cells. *J. Allergy Clin. Immunol.* 100:78–86
41. Ying S, Barata LT, Meng Q, Grant JA, Barkans J, Durham SR, Kay AB. 1998. High-affinity immunoglobulin E receptor (Fc epsilon RI)-bearing eosinophils, mast cells, macrophages and Langerhans' cells in allergen-induced late-phase cutaneous reactions in atopic subjects. *Immunology* 93:281–88
42. Riske F, Hakimi J, Mallamaci M, Griffin M, Pilson B, Tobkes N, Lin P, Danho W, Kochan J, Chizzonite R. 1991. High affinity human IgE receptor (Fc epsilon RI). Analysis of functional domains of the alpha-subunit with monoclonal antibodies. *J. Biol. Chem.* 266:11245–51
43. Tepler I, Shimizu A, Leder P. 1989. The gene for the rat mast cell high affinity IgE receptor alpha chain. Structure and alternative mRNA splicing patterns. *J. Biol. Chem.* 264:5912–15
44. Ye ZS, Kinet JP, Paul WE. 1992. Structure of the gene for the alpha-chain of the mouse high affinity receptor for IgE (Fc epsilon RI). *J. Immunol.* 49:897–900
45. Pang J, Taylor GR, Munroe DG, Ishaque A, Fung-Leung WP, Lau CY, Liu FT, Zhou L. 1993. Characterization of the gene for the human high affinity IgE receptor (Fc epsilon RI) alpha-chain. *J. Immunol.* 151:6166–74
46. Dombrowicz D, Brini AT, Flamand V, Hicks E, Snouwaert JN, Kinet JP, Koller BH. 1996. Anaphylaxis mediated through a humanized high affinity IgE receptor. *J. Immunol.* 157:1645–51
47. Dombrowicz D, Lin S, Flamand V, Brini AT, Koller BH, Kinet JP. 1998. Allergy-associated FcR beta is a molecular amplifier of IgE- and IgG-mediated in vivo responses. *Immunity* 8:517–29
48. Fung-Leung WP, De Sousa-Hitzler J, Ishaque A, Zhou L, Pang J, Ngo K, Panakos JA, Chourmouzis E, Liu FT,

- Lau CY. 1996. Transgenic mice expressing the human high-affinity immunoglobulin (Ig) E receptor alpha chain respond to human IgE in mast cell degranulation and in allergic reactions. *J. Exp. Med.* 183:49–56
49. Lin S, Cicala C, Scharenberg AM, Kinet JP. 1996. The Fc(epsilon)RI beta subunit functions as an amplifier of Fc(epsilon)RI gamma-mediated cell activation signals. *Cell* 85:985–95
 50. Hakimi J, Seals C, Kondas JA, Pettine L, Danho W, Kochan J. 1990. The alpha subunit of the human IgE receptor (FcERI) is sufficient for high affinity IgE binding. *J. Biol. Chem.* 265:22079–81
 51. Blank U, Ra CS, Kinet JP. 1991. Characterization of truncated alpha chain products from human, rat, and mouse high affinity receptor for immunoglobulin E. *J. Biol. Chem.* 266:2639–46
 52. Alber G, Miller L, Jelsema CL, Varin-Blank N, Metzger H. 1991. Structure-function relationships in the mast cell high affinity receptor for IgE. Role of the cytoplasmic domains and of the beta subunit. *J. Biol. Chem.* 266:22613–20
 53. Sutton BJ, Gould HJ. 1993. The human IgE network. *Nature* 366:421–28
 54. Helm B, Marsh P, Vercelli D, Padlan E, Gould HJ, Geha R. 1988. The mast cell binding site on human immunoglobulin E. *Nature* 331:180–83
 55. Helm B, Kebo D, Vercelli D, Glover MM, Gould H, Ishizaka K, Geha R, Ishizaka T. 1989. Blocking of passive sensitization of human mast cells and basophil granulocytes with IgE antibodies by a recombinant human epsilon-chain fragment of 76 amino acids. *Proc. Natl. Acad. Sci. USA* 86:9465–69
 56. Schwarzbaum S, Nissim A, Alkalay I, Ghozi MC, Schindler DG, Bergman Y, Eshhar Z. 1989. Mapping of murine IgE epitopes involved in IgE-Fcε receptor interactions. *Eur. J. Immunol.* 19:1015–23
 57. Weetall M, Shopes B, Holowka D, Baird B. 1990. Mapping the site of interaction between murine IgE and its high affinity receptor with chimeric Ig. *J. Immunol.* 145:3849–54
 58. Nissim A, Jouvin M-H, Eshhar Z. 1991. Mapping of the high affinity Fcε receptor binding site to the third constant region domain of IgE. *EMBO J.* 10:101–7
 59. Basu M, Hakimi J, Dharm E, Kondas JA, Tsien W-H, Pilson RS, Lin P, Gilfillan A, Haring P, Braswell EH, Nettleton MY, Kochan JP. 1993. Purification and characterization of human recombinant IgE-Fc fragments that bind to the human high affinity IgE receptor. *J. Biol. Chem.* 268:13118–27
 60. Nissim A, Schwarzbaum S, Siraganian R, Eshhar Z. 1993. Fine specificity of the IgE interaction with the low and high affinity Fc receptor. *J. Immunol.* 150:1365–74
 61. Presta L, Shields R, O'Connell L, Lahr S, Porter J, Gorman C, Jardiou P. 1994. The binding site on human immunoglobulin E for its high affinity receptor. *J. Biol. Chem.* 269:26368–73
 62. Helm BA, Sayers I, Higginbottom A, Machado DC, Ling Y, Ahmad K, Padlan EA, Wilson AP. 1996. Identification of the high affinity receptor binding region in human immunoglobulin E. *J. Biol. Chem.* 271:7494–7500
 63. Keown MB, Henry AJ, Ghirlando R, Sutton BJ, Gould HJ. 1998. Thermodynamics of the interaction of human immunoglobulin E with its high-affinity receptor Fc epsilon RI. *Biochemistry* 37:8863–69
 64. Sechi S, Roller PP, Willette-Brown J, Kinet JP. 1996. A conformational rearrangement upon binding of IgE to its high affinity receptor. *J. Biol. Chem.* 271:19256–63
 65. Williams AF, Davis SJ, He Q, Barclay AN. 1989. Structural diversity in domains of the immunoglobulin superfamily. *Cold Spring Harbor Symp. Quant. Bio.* 54:637
 66. Padlan EA, Helm BA. 1992. A modeling study of the α-subunit of human high-affinity receptor for immunoglobulin E receptor. 2:129–43
 67. Hogarth PM, Hulett MD, Ierino FL, Tate B, Powell MS, Brinkworth RI. 1992. Identification of the immunoglobulin binding regions (IBR) of FcγRII and FcεRI. *Immunol. Rev.* 125:21–35
 68. Hulett MD, McKenzie IFC, Hogarth PM. 1993. Chimeric Fc receptors identify immunoglobulin-binding regions in human FcγRII and FcεRI. *Eur. J. Immunol.* 23:640–45
 69. Mallamaci MA, Chizzonite R, Griffin M, Nettleton M, Hakimi J, Tsien W-H, Kochan JP. 1993. Identification of sites on the human FcεRIα subunit which are involved in binding human and rat IgE. *J. Biol. Chem.* 268:22076–83
 70. Robertson MW. 1993. Phage and *Escherichia coli* expression of the human high affinity immunoglobulin E receptor α-subunit ectodomain. Domain localization of the IgE-binding site. *J. Biol. Chem.* 268:12736–43
 71. Scarselli E, Esposito G, Traboni C. 1993.

- Receptor phage: display of functional domains of the high affinity IgE receptor on the M13 phage surface. *FEBS Lett.* 329:223–26
72. McDonnell JM, Beavil AJ, Mackay GA, Jameson BA, Korngold R, Gould HJ, Sutton BJ. 1996. Structure based design and characterization of peptides that inhibit IgE binding to its high-affinity receptor. *Nature Struct. Biol.* 3:419–26
 73. Cook JP, Henry AJ, McDonnell JM, Owens RJ, Sutton BJ, Gould HJ. 1997. Identification of contact residues in the IgE binding site of human Fc epsilon RI alpha. *Biochemistry* 36:15579–88
 74. Dombrowicz D, Flamand V, Brigman KK, Koller BH, Kinet JP. 1993. Abolition of anaphylaxis by targeted disruption of the high affinity immunoglobulin E receptor alpha chain gene. *Cell* 75:969–76
 75. Jankovic D, Kullberg MC, Dombrowicz D, Barbieri S, Caspar P, Wynn TA, Paul WE, Cheever AW, Kinet JP, Sher A. 1997. Fc epsilon RI-deficient mice infected with *Schistosoma mansoni* mount normal Th2-type responses while displaying enhanced liver pathology. *J. Immunol.* 159:1868–75
 76. Matsuda H, Watanabe N, Kiso Y, Hirota S, Ushio H, Kannan Y, Azuma M, Koyama H, Kitamura Y. 1990. Necessity of IgE antibodies and mast cells for manifestation of resistance against larval *Haemaphysalis longicornis* ticks in mice. *J. Immunol.* 144:259–62
 77. Matsuda H, Fukui K, Kiso Y, Kitamura Y. 1985. Inability of genetically mast cell-deficient W/Wv mice to acquire resistance against larval *Haemaphysalis longicornis* ticks. *J. Parasitol.* 71:443–48
 78. Matsuda H, Nakano T, Kiso Y, Kitamura Y. 1987. Normalization of anti-tick response of mast cell-deficient W/Wv mice by intracutaneous injection of cultured mast cells. *J. Parasitol.* 73:155–60
 79. Maurer D, Ebner C, Reininger B, Fiebigler E, Kraft D, Kinet JP, Stingl G. 1995. The high affinity IgE receptor (Fc epsilon RI) mediates IgE-dependent allergen presentation. *J. Immunol.* 154:6285–90
 80. Gounni AS, Lamkhouiou B, Delaporte E, Dubost A, Kinet JP, Capron A, Capron M. 1994. The high-affinity IgE receptor on eosinophils: from allergy to parasites or from parasites to allergy? *J. Allergy Clin. Immunol.* 94:1214–16
 81. Pritchard DI, Hewitt C, Moqbel R. 1997. The relationship between immunological responsiveness controlled by T-helper 2 lymphocytes and infections with parasitic helminths. *Parasitology* 115:S33–44
 82. Dunne DW, Butterworth AE, Fulford AJ, Kariuki HC, Langley JG, Ouma JH, Capron A, Pierce RJ, Sturrock RF. 1992. Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection. *Eur. J. Immunol.* 22:1483–94
 83. Hagan P, Blumenthal UJ, Dunn D, Simpson AJ, Wilkins HA. 1991. Human IgE, IgG4 and resistance to reinfection with *Schistosoma haematobium*. *Nature* 349:243–45
 84. Rihet P, Demeure CE, Bourgois A, Prata A, Dessein AJ. 1991. Evidence for an association between human resistance to *Schistosoma mansoni* and high anti-larval IgE levels. *Eur. J. Immunol.* 21:2679–86
 85. Allen JE, Maizels RM. 1996. Immunology of human helminth infection. *Int. Arch. Allergy Immunol.* 109:3–10
 86. ISAAC Steering Committee. 1998. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee [see comments]. *Lancet* 351:1225–32
 87. Malveaux FJ, Conroy MC, Adkinson NF Jr, Lichtenstein LM. 1978. IgE receptors on human basophils. Relationship to serum IgE concentration. *J. Clin. Invest.* 62:176–81
 88. Furuichi K, Rivera J, Isersky C. 1985. The receptor for immunoglobulin E on rat basophilic leukemia cells: effect of ligand binding on receptor expression. *Proc. Natl. Acad. Sci. USA* 82:1522–25
 89. Quarto R, Kinet JP, Metzger H. 1985. Coordinate synthesis and degradation of the alpha-, beta- and gamma-subunits of the receptor for immunoglobulin E. *Mol. Immunol.* 22:1045–51
 90. Yamaguchi M, Lantz CS, Oettgen HC, Katona IM, Fleming T, Miyajima I, Kinet JP, Galli SJ. 1997. IgE enhances mouse mast cell Fc(epsilon)RI expression in vitro and in vivo: evidence for a novel amplification mechanism in IgE-dependent reactions. *J. Exp. Med.* 185:663–72
 91. Lantz CS, Yamaguchi M, Oettgen HC, Katona IM, Miyajima I, Kinet JP, Galli SJ. 1997. IgE regulates mouse basophil

- Fc epsilon RI expression in vivo. *J. Immunol.* 158:2517-21
92. Yano K, Yamaguchi M, de Mora F, Lantz CS, Butterfield JH, Costa JJ, Galli SJ. 1997. Production of macrophage inflammatory protein-1 alpha by human mast cells: increased anti-IgE-dependent secretion after IgE-dependent enhancement of mast cell IgE-binding ability. *Lab. Invest.* 77:185-93
 93. MacGlashan D Jr, McKenzie-White J, Chichester K, Bochner BS, Davis FM, Schroeder JT, Lichtenstein LM. 1998. In vitro regulation of Fc epsilon RI alpha expression on human basophils by IgE antibody. *Blood* 91:1633-43
 94. MacGlashan DW Jr, Bochner BS, Adelman DC, Jardieu PM, Togias A, McKenzie-White J, Sterbinsky SA, Hamilton RG, Lichtenstein LM. 1997. Down-regulation of Fc(epsilon)RI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. *J. Immunol.* 158:1438-45
 95. Rottem M, Barbieri S, Kinet JP, Metcalfe DD. 1992. Kinetics of the appearance of Fc epsilon RI-bearing cells in interleukin-3-dependent mouse bone marrow cultures: correlation with histamine content and mast cell maturation. *Blood* 79:972-80
 96. Toru H, Ra C, Nonoyama S, Suzuki K, Yata J, Nakahata T. 1996. Induction of the high-affinity IgE receptor (Fc epsilon RI) on human mast cells by IL-4. *Int. Immunol.* 8:1367-73
 97. Xia HZ, Du Z, Craig S, Klisch G, Noben-Trauth N, Kochan JP, Huff TH, Irani AM, Schwartz LB. 1997. Effect of recombinant human IL-4 on tryptase, chymase, and Fc epsilon receptor type I expression in recombinant human stem cell factor-dependent fetal liver-derived human mast cells. *J. Immunol.* 159:2911-21
 98. Brini AT, Lee GM, Kinet JP. 1993. Involvement of Alu sequences in the cell-specific regulation of transcription of the gamma chain of Fc and T cell receptors. *J. Biol. Chem.* 268:1355-61
 99. Klausner RD, Lippincott-Schwartz J, Bonifacio JS. 1990. The T cell antigen receptor: insights into organelle biology. *Annu. Rev. Cell Biol.* 6:403-31
 100. Ryan JJ, Kinzer CA, Paul WE. 1995. Mast cells lacking the high affinity immunoglobulin E receptor are deficient in Fc epsilon RI gamma messenger RNA. *J. Exp. Med.* 182:567-74
 101. Kraft S, Wessendorf JH, Hanau D, Bieber T. 1998. Regulation of the high affinity receptor for IgE on human epidermal Langerhans cells. *J. Immunol.* 161:1000-6
 102. Cookson WO, Sharp PA, Faux JA, Hopkin JM. 1989. Linkage between immunoglobulin E responses underlying asthma and rhinitis and chromosome 11q. *Lancet* 1:1292-95
 103. Young RP, Sharp PA, Lynch Jr, Faux JA, Lathrop GM, Cookson WO, Hopkin JM. 1992. Confirmation of genetic linkage between atopic IgE responses and chromosome 11q13. *J. Med. Genet.* 29:236-38
 104. Marsh DG, Meyers DA. 1992. A major gene for allergy-factor fancy? [news]. *Nat. Genet.* 2:252-54
 105. Lympay P, Welsh K, MacCochrane G, Kemeny DM, Lee TH. 1992. Genetic analysis using DNA polymorphism of the linkage between chromosome 11q13 and atopy and bronchial hyperresponsiveness to methacholine. *J. Allergy Clin. Immunol.* 89:619-28
 106. Amelung PJ, Panhuysen CI, Postma DS, Levitt RC, Koeter GH, Francomano CA, Bleecker ER, Meyers DA. 1992. Atopy and bronchial hyperresponsiveness: exclusion of linkage to markers on chromosomes 11q and 6p. *Clin. Exp. Allergy* 22:1077-84
 107. Rich SS, Roitman-Johnson B, Greenberg B, Roberts S, Blumenthal MN. 1992. Genetic analysis of atopy in three large kindreds: no evidence of linkage to D11S97. *Clin. Exp. Allergy* 22:1070-76
 108. Sandford AJ, Shirakawa T, Moffatt MF, Daniels SE, Ra C, Faux JA, Young RP, Nakamura Y, Lathrop GM, Cookson WO, et al. 1993. Localisation of atopy and beta subunit of high-affinity IgE receptor (Fc epsilon RI) on chromosome 11q [see comments]. *Lancet* 341:332-34
 109. Shirakawa T, Hashimoto T, Furuyama J, Takeshita T, Morimoto K. 1994. Linkage between severe atopy and chromosome 11q13 in Japanese families. *Clin. Genet.* 46:228-32
 110. Brereton HM, Ruffin RE, Thompson PJ, Turner DR. 1994. Familial atopy in Australian pedigrees: adventitious linkage to chromosome 8 is not confirmed nor is there evidence of linkage to the high affinity IgE receptor. *Clin. Exp. Allergy* 24:868-77
 111. Watson M, Lawrence S, Collins A, Beasley R, Doull I, Begishvili B, Lampe F, Holgate ST, Morton NE. 1995. Exclusion from proximal 11q of a common

- gene with megaphenic effect on atopy. *Ann. Hum. Genet.* 59:403–11
112. van Herwerden L, Harrap SB, Wong ZY, Abramson MJ, Kutin JJ, Forbes AB, Raven J, Lanigan A, Walters EH. 1995. Linkage of high-affinity IgE receptor gene with bronchial hyperreactivity, even in absence of atopy [see comments]. *Lancet* 346:1262–65
 113. Doull IJ, Lawrence S, Watson M, Begishvili T, Beasley RW, Lampe F, Holgate T, Morton NE. 1996. Allelic association of gene markers on chromosomes 5q and 11q with atopy and bronchial-hyperresponsiveness. *Am. J. Respir. Crit. Care Med.* 153:1280–84
 114. Wong ZY, Tsonis D, van Herwerden L, Raven J, Forbes A, Abramson MJ, Walters EH, Harrap SB. 1997. Linkage analysis of bronchial hyperreactivity and atopy with chromosome 11q13. *Electrophoresis* 18:1641–45
 115. Thomas NS, Wilkinson J, Holgate ST. 1997. The candidate region approach to the genetics of asthma and allergy. *Am. J. Respir. Crit. Care Med.* 156:S144–51
 116. Folster-Holst R, Moises HW, Yang L, Fritsch W, Weissenbach J, Christophers E. 1998. Linkage between atopy and the IgE high-affinity receptor gene at 11q13 in atopic dermatitis families. *Hum. Genet.* 102:236–39
 117. Amelung PJ, Postma DS, Xu J, Meyers DA, Bleeker ER. 1998. Exclusion of chromosome 11q and the Fc epsilon RI-beta gene as aetiological factors in allergy and asthma in a population of Dutch asthmatic families. *Clin. Exp. Allergy* 28:397–403. In press
 118. Kuster H, Zhang L, Brini AT, MacGlashan DW, JP Kinet. 1992. The gene and cDNA for the human high affinity immunoglobulin E receptor beta chain and expression of the complete human receptor. *J. Biol. Chem.* 267:12782–87
 119. Hill MR, Cookson WO. 1996. A new variant of the beta subunit of the high-affinity receptor for immunoglobulin E (Fc epsilon RI-beta E237G): associations with measures of atopy and bronchial hyper-responsiveness. *Hum. Mol. Genet.* 5:959–62
 120. Hill MR, James AL, Faux JA, Ryan G, Hopkin JM, le Souef P, Musk AW, Cookson WO. 1995. Fc epsilon RI-beta polymorphism and risk of atopy in a general population sample [see comments] [published erratum appears in *Br. Med. J.* 1995 Nov 4; 311(7014):1196]. *Br. Med. J.* 311:776–79
 121. Shirakawa T, Li A, Dubowitz M, Dekker JW, Shaw AE, Faux JA, Ra C, Cookson WO, Hopkin JM. 1994. Association between atopy and variants of the beta subunit of the high-affinity immunoglobulin E receptor [see comments]. *Nat. Genet.* 7:125–29
 122. Hijazi Z, Haider MZ, Khan MR, Al-Dowaisan AA. 1998. High frequency of IgE receptor Fc epsilon RI beta variant (Leu181/Leu183) in Kuwaiti Arabs and its association with asthma. *Clin. Genet.* 53:149–52. In press
 123. Palmer LJ, Pare PD, Faux JA, Moffatt MF, Daniels SE, LeSouef PN, Brenner PR, Mockford E, Gracey M, Spargo R, Musk AW, Cookson WO. 1997. Fc epsilon RI-beta polymorphism and total serum IgE levels in endemically parasitized Australian aborigines. *Am. J. Hum. Genet.* 61:182–88
 124. Cox HE, Moffatt MF, Faux JA, Walley AJ, Coleman R, Trembath RC, Cookson WO, Harper JJ. 1998. Association of atopic dermatitis to the beta subunit of the high affinity immunoglobulin E receptor. *Br. J. Dermatol.* 138:182–87
 125. Hide M, Francis DM, Grattan CE, Hakimi J, Kochan JP, Greaves MW. 1993. Autoantibodies against the high-affinity IgE receptor as a cause of histamine release in chronic urticaria [see comments]. *N. Engl. J. Med.* 328:1599–1604
 126. Gruber BL, Baeza ML, Marchese MJ, Agnello V, Kaplan AP. 1988. Prevalence and functional role of anti-IgE autoantibodies in urticarial syndromes. *J. Invest. Dermatol.* 90:213–17
 127. Grattan CE, Francis DM, Hide M, Greaves MW. 1991. Detection of circulating histamine releasing autoantibodies with functional properties of anti-IgE in chronic urticaria. *Clin. Exp. Allergy* 21:695–704
 128. Fiebigier E, Maurer D, Holub H, Reininger B, Hartmann G, Woisetschlager M, Kinet JP, Stingl G. 1995. Serum IgG autoantibodies directed against the alpha chain of Fc epsilon RI: a selective marker and pathogenetic factor for a distinct subset of chronic urticaria patients? *J. Clin. Invest.* 96:2606–12
 129. Marone G, Casolaro V, Paganelli R, Quinti I. 1989. IgG anti-IgE from atopic dermatitis induces mediator release from basophils and mast cells. *J. Invest. Dermatol.* 93:246–52
 130. Niimi N, Francis DM, Kermani F, O'Donnell BF, Hide M, Kobza-Black

- A, Winkelman RK, Greaves MW, Barr RM. 1996. Dermal mast cell activation by autoantibodies against the high affinity IgE receptor in chronic urticaria. *J. Invest. Dermatol.* 106:1001-6
131. Tong LJ, Balakrishnan G, Kochan JP, Kinet JP, Kaplan AP. 1997. Assessment of autoimmunity in patients with chronic urticaria. *J. Allergy Clin. Immunol.* 99:461-65
 132. Daeron M, Latour S, Malbec O, Espinosa E, Pina P, Pasmans S, Fridman WH. 1995. The same tyrosine-based inhibition motif, in the intracytoplasmic domain of Fc gamma R1b, regulates negatively BCR-, TCR-, and FcR- dependent cell activation. *Immunity* 3:635-46
 133. Cambier JC. 1995. New nomenclature for the Reth motif (orARH1/TAM/ARAM/YXXL) [letter]. *Immunol. Today.* 16:110
 134. Reth M. 1989. Antigen receptor tail clue [letter]. *Nature* 338:383-84
 135. Irving BA, Weiss A. 1991. The cytoplasmic domain of the T cell receptor zeta chain is sufficient to couple to receptor-associated signal transduction pathways. *Cell* 64:891-901
 136. Romeo C, Seed B. 1991. Cellular immunity to HIV activated by CD4 fused to T cell or Fc receptor polypeptides. *Cell* 64:1037-46
 137. Letourneur F, Klausner RD. 1991. T-cell and basophil activation through the cytoplasmic tail of T-cell-receptor zeta family proteins. *Proc. Natl. Acad. Sci. USA* 88:8905-9
 138. Romeo C, Amiot M, Seed B. 1992. Sequence requirements for induction of cytolysis by the T cell antigen/Fc receptor zeta chain. *Cell* 68:889-97
 139. Daeron M. 1997. Fc receptor biology. *Annu. Rev. Immunol.* 15:203-34
 140. Holowka D, Baird B. 1996. Antigen-mediated IGE receptor aggregation and signaling: a window on cell surface structure and dynamics. *Annu. Rev. Biophys. Biomol. Struct.* 25:79-112
 141. Metzger H, Goldstein B, Kent U, Mao SY, Pribluda C, Pribluda V, Wofsy C, Yamashita T. 1994. Quantitative aspects of receptor aggregation. *Adv. Exp. Med. Biol.* 365:175-83
 142. Metzger H. 1992. The receptor with high affinity for IgE. *Immunol. Rev.* 125:37-48
 143. Perez-Montfort R, Fewtrell C, Metzger H. 1983. Changes in the receptor for immunoglobulin E coincident with receptor-mediated stimulation of basophilic leukemia cells. *Biochemistry* 22:5733-37
 144. Quarto R, Metzger H. 1986. The receptor for immunoglobulin E: examination for kinase activity and as a substrate for kinases. *Mol. Immunol.* 23:1215-23
 145. Paolini R, Jouvin MH, Kinet JP. 1991. Phosphorylation and dephosphorylation of the high-affinity receptor for immunoglobulin E immediately after receptor engagement and disengagement. *Nature* 353:855-58
 146. Eiseman E, Bolen JB. 1992. Engagement of the high-affinity IgE receptor activates src protein-related tyrosine kinases. *Nature* 355:78-80
 147. Benhamou M, Stephan V, Robbins KC, Siraganian RP. 1992. High-affinity IgE receptor-mediated stimulation of rat basophilic leukemia (RBL-2H3) cells induces early and late protein-tyrosine phosphorylations. *J. Biol. Chem.* 267:7310-14
 148. Stephan V, Benhamou M, Gutkind JS, Robbins KC, Siraganian RP. 1992. Fc epsilon RI-induced protein tyrosine phosphorylation of pp72 in rat basophilic leukemia cells (RBL-2H3). Evidence for a novel signal transduction pathway unrelated to G protein activation and phosphatidylinositol hydrolysis. *J. Biol. Chem.* 267:5434-41
 149. Hutchcroft JE, Geahlen RL, Deanin GG, Oliver JM. 1992. Fc epsilon RI-mediated tyrosine phosphorylation and activation of the 72-kDa protein-tyrosine kinase, PTK72, in RBL-2H3 rat tumor mast cells. *Proc. Natl. Acad. Sci. USA* 89:9107-11
 150. Pribluda VS, Metzger H. 1992. Transmembrane signaling by the high-affinity IgE receptor on membrane preparations. *Proc. Natl. Acad. Sci. USA* 89:11446-50
 151. Benhamou M, Ryba NJ, Kihara H, Nishikata H, Siraganian RP. 1993. Protein-tyrosine kinase p72syk in high affinity IgE receptor signaling. Identification as a component of pp72 and association with the receptor gamma chain after receptor aggregation. *J. Biol. Chem.* 268:23318-24
 152. Moran MF, Koch CA, Anderson D, Ellis C, England L, Martin GS, Pawson T. 1990. Src homology region 2 domains direct protein-protein interactions in signal transduction. *Proc. Natl. Acad. Sci. USA* 87:8622-26
 153. Zhou S, Cantley LC. 1995. SH2 domain specificity determination using oriented phosphopeptide library. *Meth. Enzymol.* 254:523-35

154. Weiss A, Littman DR. 1994. Signal transduction by lymphocyte antigen receptors. *Cell* 76:263-74
155. Bu JY, Shaw AS, Chan AC. 1995. Analysis of the interaction of ZAP-70 and syk protein-tyrosine kinases with the T-cell antigen receptor by plasmon resonance. *Proc. Natl. Acad. Sci. USA* 92:5106-10
156. Cambier JC. 1995. Antigen and Fc receptor signaling. The awesome power of the immunoreceptor tyrosine-based activation motif (ITAM). *J. Immunol.* 155:3281-85
157. Chen T, Repetto B, Chizzonite R, Pullar C, Burghardt C, Dharm E, Zhao Z, Carroll R, Nunes P, Basu M, Danho W, Visnick M, Kochan J, Waugh D, Gillfillan AM. 1996. Interaction of phosphorylated Fc epsilon RI gamma immunoglobulin receptor tyrosine activation motif-based peptides with dual and single SH2 domains of p72syk. Assessment of binding parameters and real time binding kinetics. *J. Biol. Chem.* 271:25308-15
158. Kihara H, Siraganian RP. 1994. Src homology 2 domains of Syk and Lyn bind to tyrosine-phosphorylated subunits of the high affinity IgE receptor. *J. Biol. Chem.* 269:22427-32
159. Shiue L, Zoller MJ, Brugge JS. 1995. Syk is activated by phosphotyrosine-containing peptides representing the tyrosine-based activation motifs of the high affinity receptor for IgE. *J. Biol. Chem.* 270:10498-502
160. Rivera VM, Brugge JS. 1995. Clustering of Syk is sufficient to induce tyrosine phosphorylation and release of allergic mediators from rat basophilic leukemia cells. *Mol. Cell. Biol.* 15:1582-90
161. Hirasawa N, Scharenberg A, Yamamura H, Beaven MA, Kinet JP. 1995. A requirement for Syk in the activation of the microtubule-associated protein kinase/phospholipase A2 pathway by Fc epsilon RI is not shared by a G protein-coupled receptor. *J. Biol. Chem.* 270:10960-67
162. Scharenberg AM, Lin S, Cuenod B, Yamamura H, Kinet JP. 1995. Reconstitution of interactions between tyrosine kinases and the high affinity IgE receptor which are controlled by receptor clustering. *Embo J.* 14:3385-94
163. Oliver JM, Burg DL, Wilson BS, McLaughlin JL, Geahlen RL. 1994. Inhibition of mast cell Fc epsilon RI-mediated signaling and effector function by the Syk-selective inhibitor, piceatannol. *J. Biol. Chem.* 269:29697-703
164. Valle A, Kinet JP. 1995. N-acetyl-L-cysteine inhibits antigen-mediated Syk, but not Lyn tyrosine kinase activation in mast cells. *FEBS Lett.* 357:41-44
165. Letourneur F, Klausner RD. 1992. Activation of T cells by a tyrosine kinase activation domain in the cytoplasmic tail of CD3 epsilon. *Science* 255:79-82
166. Adamczewski M, Paolini R, Kinet JP. 1992. Evidence for two distinct phosphorylation pathways activated by high affinity immunoglobulin E receptors. *J. Biol. Chem.* 267:18126-32
167. Eiseman E, Bolen JB. 1992. Signal transduction by the cytoplasmic domains of Fc epsilon RI-gamma and TCR-zeta in rat basophilic leukemia cells. *J. Biol. Chem.* 267:21027-32
168. Jouvain MH, Adamczewski M, Numerof R, Letourneur O, Valle A, Kinet JP. 1994. Differential control of the tyrosine kinases Lyn and Syk by the two signaling chains of the high affinity immunoglobulin E receptor. *J. Biol. Chem.* 269:5918-25
169. Wilson BS, Kapp N, Lee RJ, Pfeiffer Jr, Martinez AM, Platt Y, Letourneur F, Oliver JM. 1995. Distinct functions of the Fc epsilon RI gamma and beta subunits in the control of Fc epsilon RI-mediated tyrosine kinase activation and signaling responses in RBL-2H3 mast cells. *J. Biol. Chem.* 270:4013-22
170. Shiue L, Green J, Green OM, Karas JL, Morgenstern JP, Ram MK, Taylor MK, Zoller MJ, Zydowsky LD, Bolen JB, et al. 1995. Interaction of p72syk with the gamma and beta subunits of the high-affinity receptor for immunoglobulin E, Fc epsilon RI. *Mol. Cell. Biol.* 15:272-81
171. Jabril-Cuenod B, Zhang C, Scharenberg AM, Paolini R, Numerof R, Beaven MA, Kinet JP. 1996. Syk-dependent phosphorylation of Shc. A potential link between Fc epsilon RI and the Ras/mitogen-activated protein kinase signaling pathway through SOS and Grb2. *J. Biol. Chem.* 271:16268-72
172. Nishizumi H, Yamamoto T. 1997. Impaired tyrosine phosphorylation and Ca²⁺ mobilization, but not degranulation, in lyn-deficient bone marrow-derived mast cells. *J. Immunol.* 158:2350-55
173. Costello PS, Turner M, Walters AE, Cunningham CN, Bauer PH, Downward J, Tybulewicz VL. 1996. Critical role for the tyrosine kinase Syk in signalling through the high affinity IgE receptor of mast cells. *Oncogene* 13:2595-605
174. Zhang J, Berenstein EH, Evans RL,

- Siraganian RP. 1996. Transfection of Syk protein tyrosine kinase reconstitutes high affinity IgE receptor-mediated degranulation in a Syk-negative variant of rat basophilic leukemia RBL-2H3 cells. *J. Exp. Med.* 184:71-9
175. Osborne MA, Zenner G, Lubinus M, Zhang X, Songyang Z, Cantley LC, Majerus P, Burn P, Kochan JP. 1996. The inositol 5'-phosphatase SHIP binds to immunoreceptor signaling motifs and responds to high affinity IgE receptor aggregation. *J. Biol. Chem.* 271:29271-78
 176. Kimura T, Zhang J, Sagawa K, Sakaguchi K, Appella E, Siraganian RP. 1997. Syk-independent tyrosine phosphorylation and association of the protein tyrosine phosphatases SHP-1 and SHP-2 with the high affinity IgE receptor. *J. Immunol.* 159:4426-34
 177. Kimura T, Sakamoto H, Appella E, Siraganian RP. 1997. The negative signaling molecule SH2 domain-containing inositol-polyphosphate 5-phosphatase (SHIP) binds to thertyrosine-phosphorylated beta subunit of the high affinity IgE receptor. *J. Biol. Chem.* 272:13991-96
 178. Ottinger EA, Botfield MC, Shoelson SE. 1998. Tandem SH2 domains confer high specificity in tyrosine kinase signaling. *J. Biol. Chem.* 273:729-35
 179. Vonakis BM, Chen H, Haleem-Smith H, Metzger H. 1997. The unique domain as the site on Lyn kinase for its constitutive association with the high affinity receptor for IgE. *J. Biol. Chem.* 272:24072-80
 180. Stauffer TP, Martenson CH, Rider JE, Kay BK, Meyer T. 1997. Inhibition of Lyn function in mast cell activation by SH3 domain binding peptides. *Biochemistry* 36:9388-94
 181. El-Hillal O, Kurosaki T, Yamamura H, Kinet JP, Scharenberg AM. 1997. Syk kinase activation by a src kinase-initiated activation loop phosphorylation chain reaction. *Proc. Natl. Acad. Sci. USA* 94:1919-24
 182. Trowbridge IS, Thomas ML. 1994. CD45: an emerging role as a protein tyrosine phosphatase required for lymphocyte activation and development. *Annu. Rev. Immunol.* 12:85-116
 183. Hook WA, Berenstein EH, Zinsser FU, Fischler C, Siraganian RP. 1991. Monoclonal antibodies to the leukocyte common antigen (CD45) inhibit IgE-mediated histamine release from human basophils. *J. Immunol.* 147:2670-76
 184. Adamczewski M, Numerof RP, Koretzky GA, Kinet JP. 1995. Regulation by CD45 of the tyrosine phosphorylation of high affinity IgE receptor beta- and gamma-chains. *J. Immunol.* 154:3047-55
 185. Pribluda VS, Pribluda C, Metzger H. 1994. Transphosphorylation as the mechanism by which the high-affinity receptor for IgE is phosphorylated upon aggregation. *Proc. Natl. Acad. Sci. USA* 91:11246-50
 186. Yamashita T, Mao SY, Metzger H. 1994. Aggregation of the high-affinity IgE receptor and enhanced activity of p53/56 lyn protein-tyrosine kinase. *Proc. Natl. Acad. Sci. USA* 91:11251-55
 187. Torigoe C, Goldstein B, Wofsy C, Metzger H. 1997. Shuttling of initiating kinase between discrete aggregates of the high affinity receptor for IgE regulates the cellular response. *Proc. Natl. Acad. Sci. USA* 94:1372-77
 188. Torigoe C, Inman JK, Metzger H. 1998. An unusual mechanism for ligand antagonism. *Science* 281:568-72
 189. Tsukada S, Saffran DC, Rawlings DJ, Parolini O, Allen RC, Klisak I, Sparkes RS, Kubagawa H, Mohandas T, Quan S, et al. 1993. Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. *Cell* 72:279-90
 190. Vihinen M, Vetrie D, Maniar HS, Ochs HD, Zhu Q, Vorechovsky I, Webster AD, Notarangelo LD, Nilsson L, Sowadski JM, et al. 1994. Structural basis for chromosome X-linked agammaglobulinemia: a tyrosine kinase disease. *Proc. Natl. Acad. Sci. USA* 91:12803-7
 191. Desiderio S, Siliciano JD. 1994. The Itk/Btk/Tec family of protein-tyrosine kinases. *Chem. Immunol.* 59:191-210
 192. Tsukada S, Rawlings DJ, Witte ON. 1994. Role of Bruton's tyrosine kinase in immunodeficiency. *Curr. Opin. Immunol.* 6:623-30
 193. Rawlings DJ, Witte ON. 1994. Bruton's tyrosine kinase is a key regulator in B-cell development. *Immunol. Rev.* 138:105-19
 194. Rawlings DJ, Saffran DC, Tsukada S, Largaespada DA, Grimaldi JC, Cohen L, Mohr RN, Bazan JF, Howard M, Copeland NG, et al. 1993. Mutation of unique region of Bruton's tyrosinekinase in immunodeficient XID mice. *Science* 261:358-61
 195. Thomas JD, Sideras P, Smith CI, Vorechovsky I, Chapman V, Paul WE. 1993. Colocalization of X-linked agammaglobulinemia and X-linked immunodeficiency genes. *Science* 261:355-68

196. Hata D, Kawakami Y, Inagaki N, Lantz CS, Kitamura T, Khan WN, Maeda-Yamamoto M, Miura T, Han W, Hartman SE, Yao L, Nagai H, Goldfeld AE, Alt FW, Galli SJ, Witte ON, Kawakami T. 1998. Involvement of Bruton's tyrosine kinase in Fc epsilon RI-dependent mast cell degranulation and cytokine production. *J. Exp. Med.* 187:1235-47
197. Hata D, Kitaura J, Hartman SE, Kawakami Y, Yokota T, Kawakami T. 1998. Bruton's tyrosine kinase-mediated interleukin-2 gene activation in mast cells. Dependence on the c-Jun N-terminal kinase activation pathway. *J. Biol. Chem.* 273:10979-87
198. Kawakami Y, Yao L, Miura T, Tsukada S, Witte ON, Kawakami T. 1994. Tyrosine phosphorylation and activation of Bruton tyrosine kinase upon Fc epsilon RI cross-linking. *Mol. Cell. Biol.* 14:5108-13
199. Takata M, Kurosaki T. 1996. A role for Bruton's tyrosine kinase in B cell antigen receptor-mediated activation of phospholipase C-gamma 2. *J. Exp. Med.* 184:31-40
200. Rawlings DJ, Scharenberg AM, Park H, Wahl MI, Lin S, Kato RM, Fluckiger AC, Witte ON, Kinet JP. 1996. Activation of BTK by a phosphorylation mechanism initiated by SRC family kinases. *Science* 271:822-25
201. Salim K, Bottomley MJ, Querfurth E, Zvelebil MJ, Gout I, Scaife R, Margolis RL, Gigg R, Smith CI, Driscoll PC, Waterfield MD, Panayotou G. 1996. Distinct specificity in the recognition of phosphoinositides by the pleckstrin homology domains of dynamin and Bruton's tyrosine kinase. *Embo J.* 15:6241-50
202. Rameh LE, Arvidsson A, Carraway KL 3rd, Couvillon AD, Rathbun G, Crompton A, Van Renterghem B, Czech MP, Ravichandran KS, Burakoff SJ, Wang DS, Chen CS, Cantley LC. 1997. A comparative analysis of the phosphoinositide binding specificity of pleckstrin homology domains. *J. Biol. Chem.* 272:22059-66
203. Carpenter CL, Cantley LC. 1996. Phosphoinositide kinases. *Curr. Opin. Cell Biol.* 8:153-58
204. Vanhaesebroeck B, Leevers SJ, Panayotou G, Waterfield MD. 1997. Phosphoinositide 3-kinases: a conserved family of signal transducers. *Trends Biochem. Sci.* 22:267-72
205. Yano H, Nakanishi S, Kimura K, Hanai N, Saitoh Y, Fukui Y, Nonomura Y, Matsuda Y. 1993. Inhibition of histamine secretion by wortmannin through the blockade of phosphatidylinositol 3-kinase in RBL 2H3 cells. *J. Biol. Chem.* 268:25846-56
206. Yano H, Agatsuma T, Nakanishi S, Saitoh Y, Fukui Y, Nonomura Y, Matsuda Y. 1995. Biochemical and pharmacological studies with KT7692 and LY294002 on the role of phosphatidylinositol 3-kinase in Fc epsilon RI-mediated signal transduction. *Biochem J.* 312:145-50
207. Barker SA, Caldwell KK, Hall A, Martinez AM, Pfeiffer Jr, Oliver JM, Wilson BS. 1995. Wortmannin blocks lipid and protein kinase activities associated with PI 3-kinase and inhibits a subset of responses induced by Fc epsilon R1 cross-linking. *Mol. Cell. Biol.* 106:45-58
208. Bolland S, Pearce RN, Kurosaki T, Ravetch JV. 1998. SHIP modulates immune receptor responses by regulating membrane association of Btk. *Immunity* 8:509-16
209. Scharenberg AM, Kinet JP. 1998. PtdIns-3,4,5-P3: a regulatory nexus between tyrosine kinases and sustained calcium signals. *Cell* 94:5-8. In press
210. Scharenberg AM, El-Hillal O, Fruman DA, Beitz LO, Li Z, Lin S, Gout I, Cantley LC, Rawlings DJ, Kinet JP. 1998. Phosphatidylinositol-3,4,5-trisphosphate (PtdIns-3,4,5-P3)/Teckinase-dependent calcium signaling pathway: a target for SHIP-mediated inhibitory signals. *Embo J.* 17:1961-72
211. Fluckiger AC, Li Z, Kato RM, Wahl MI, Ochs HD, Longnecker R, Kinet JP, Witte ON, Scharenberg AM, Rawlings DJ. 1998. Btk/Teckinases regulate sustained increases in intracellular Ca2+ following B-cell receptor activation. *Embo J.* 17:1973-85
212. Parekh AB, Fleig A, Penner R. 1997. The store-operated calcium current I(CRAC): nonlinear activation by InsP3 and dissociation from calcium release. *Cell* 89:973-80
213. Dolmetsch RE, Lewis RS, Goodnow CC, Healy JI. 1997. Differential activation of transcription factors induced by Ca2+ response amplitude and duration [see comments] [published erratum appears in *Nature* 1997 Jul. 17; 388 (6639):308]. *Nature* 386:855-58
214. Healy JI, Dolmetsch RE, Timmerman LA, Cyster JG, Thomas ML, Crabtree GR, Lewis RS, Goodnow CC. 1997. Different nuclear signals are activated by the B cell receptor during positive

- versus negative signaling. *Immunity* 6: 419–28
215. Pleiman CM, Hertz WM, Cambier JC. 1994. Activation of phosphatidylinositol-3' kinase by Src-family kinase SH3 binding to the p85 subunit. *Science* 263:1609–12
 216. Spiegel S, Merrill AH Jr. 1996. Sphingolipid metabolism and cell growth regulation. *Faseb J.* 10:1388–97
 217. Spiegel S, Cuvillier O, Edsall LC, Kohama T, Menzeleev R, Olah Z, Olivera A, Pirianov G, Thomas DM, Tu Z, Van Brocklyn Jr, Wang F. 1998. Sphingosine-1-phosphate in cell growth and cell death. *Ann. N. Y. Acad. Sci.* 845:11–18
 218. Van Brocklyn Jr, Lee MJ, Menzeleev R, Olivera A, Edsall L, Cuvillier O, Thomas DM, Coopman PJP, Thangada S, Liu CH, Hla T, Spiegel S. 1998. Dual actions of sphingosine-1-phosphate: extracellular through the Gi-coupled receptor edg-1 and intracellular to regulate proliferation and survival. *J. Cell. Biol.* 142:229–40
 219. Olivera A, Spiegel S. 1993. Sphingosine-1-phosphate as second messenger in cell proliferation induced by PDGF and FCS mitogens. *Nature* 365:557–60
 220. Olivera A, Kohama T, Tu Z, Milstien S, Spiegel S. 1998. Purification and characterization of rat kidney sphingosinekinase. *J. Biol. Chem.* 273:12576–83
 221. Buehrer BM, Bell RM. 1993. Sphingosine kinase: properties and cellular functions. *Adv. Lipid Res.* 26:59–67
 222. Ghosh TK, Bian J, Gill DL. 1994. Sphingosine 1-phosphate generated in the endoplasmic reticulum membrane activates release of stored calcium. *J. Biol. Chem.* 269:22628–35
 223. Kim S, Lakhani V, Costa DJ, Sharara AI, Fitz JG, Huang LW, Peters KG, Kindman LA. 1995. Sphingolipid-gated Ca²⁺ release from intracellular stores of endothelial cells is mediated by a novel Ca(2+)-permeable channel. *J. Biol. Chem.* 270:5266–69
 224. Mattie M, Brooker G, Spiegel S. 1994. Sphingosine-1-phosphate, a putative second messenger, mobilizes calcium from internal stores via an inositoltrisphosphate-independent pathway. *J. Biol. Chem.* 269:3181–88
 225. Choi OH, Lee JH, Kassessinoff T, Cunha-Melo Jr, Jones SV, Beaven MA. 1993. Antigen and carbachol mobilize calcium by similar mechanisms in a transfected mast cell line (RBL-2H3 cells) that expresses ml muscarinic receptors. *J. Immunol.* 151:5586–95
 226. Jones SV, Choi OH, Beaven MA. 1991. Carbachol induces secretion in a mast cell line (RBL-2H3) transfected with the ml muscarinic receptor gene. *FEBS Lett.* 289:47–50
 227. Buehrer BM, Bell RM. 1992. Inhibition of sphingosine kinase in vitro and in platelets. Implications for signal transduction pathways. *J. Biol. Chem.* 267:3154–59
 228. Choi OH, Kim JH, Kinet JP. 1996. Calcium mobilization via sphingosine kinase in signalling by the Fc epsilon RI antigen receptor. *Nature* 380:634–36
 229. Wu J, Spiegel S, Sturgill TW. 1995. Sphingosine-1-phosphate rapidly activates the mitogen-activated protein kinase pathway by a G protein-dependent mechanism. *J. Biol. Chem.* 270:11484–88
 230. Goodemote KA, Mattie ME, Berger A, Spiegel S. 1995. Involvement of a pertussis toxin-sensitive G protein in the mitogenic signaling pathways of sphingosine 1-phosphate. *J. Biol. Chem.* 270:10272–77
 231. Lee MJ, Van Brocklyn Jr, Thangada S, Liu CH, Hand AR, Menzeleev R, Spiegel S, Hla T. 1998. Sphingosine-1-phosphate as a ligand for the G protein-coupled receptor EDG-1. *Science* 279:1552–55
 232. Matthews G, Neher E, Penner R. 1989. Second messenger-activated calcium influx in rat peritoneal mast cells. *J. Physiol.* 418:105–30
 233. Meyer T, Holowka D, Stryer L. 1988. Highly cooperative opening of calcium channels by inositol 1,4,5-trisphosphate. *Science* 240:653–56
 234. Saito H, Ishizaka K, Ishizaka T. 1989. Effect of nonhydrolyzable guanosine phosphate on IgE-mediated activation of phospholipase C and histamine release from rodent mast cells. *J. Immunol.* 143:250–58
 235. Melendez A, Floto RA, Gillooly DJ, Harnett MM, Allen JM. 1998. Fc gammaRI coupling to phospholipase D initiates sphingosine kinase-mediated calcium mobilization and vesicular trafficking. *J. Biol. Chem.* 273:9393–402
 236. Mathes C, Fleig A, Penner R. 1998. Calcium-release-activated calcium current (ICRAC) is a direct target for sphingosine. *J. Biol. Chem.* 273:25020–30
 237. Pribluda VS, Pribluda C, Metzger H. 1997. Biochemical evidence that the phosphorylated tyrosines, serines, and threonines on the aggregated high affinity receptor for IgE are in the immunore-

- ceptor tyrosine-based activation motifs. *J. Biol. Chem.* 272:11185–92
238. Germano P, Gomez J, Kazanietz MG, Blumberg PM, Rivera J. 1994. Phosphorylation of the gamma chain of the high affinity receptor for immunoglobulin E by receptor-associated protein kinase C-delta. *J. Biol. Chem.* 269:23102–7
 - 238a. Razin E, Szallasi Z, Kazanietz MG, Blumberg PM, Rivera J. 1994. *Proc. Natl. Acad. Sci. USA* 91:7722–26
 239. Chang EY, Szallasi Z, Acs P, Raizada V, Wolfe PC, Fewtrell C, Blumberg PM, Rivera J. 1997. Functional effects of overexpression of protein kinase C-alpha, -beta, -delta, -epsilon, and -eta in the mast cell line RBL-2H3. *J. Immunol.* 159:2624–32
 240. Mao SY, Metzger H. 1997. Characterization of protein-tyrosine phosphatases that dephosphorylate the high affinity IgE receptor [published erratum appears in *J. Biol. Chem.* 1997 Sep. 19; 272(38):24096]. *J. Biol. Chem.* 272:14067–73
 241. Teramoto H, Salem P, Robbins KC, Bustelo XR, Gutkind JS. 1997. Tyrosine phosphorylation of the vav proto-oncogene product links Fc epsilon RI to the Rac1-JNK pathway. *J. Biol. Chem.* 272:10751–55
 242. Margolis B, Hu P, Katzav S, Li W, Oliver JM, Ullrich A, Weiss A, Schlessinger J. 1992. Tyrosine phosphorylation of vav proto-oncogene product containing SH2 domain and transcription factor motifs [see comments]. *Nature* 356:71–74
 243. Song JS, Gomez J, Stancato LF, Rivera J. 1996. Association of a p95 Vav-containing signaling complex with the Fc epsilon RI gamma chain in the RBL-2H3 mast cell line. Evidence for a constitutive in vivo association of Vav with Grb2, Raf-1, and ERK2 in an active complex. *J. Biol. Chem.* 271:26962–70
 244. Jackman JK, Motto DG, Sun Q, Tanemoto M, Turck CW, Peltz GA, Koretzky GA, Findell PR. 1995. Molecular cloning of SLP-76, a 76-kDa tyrosine phosphoprotein associated with Grb2 in T cells. *J. Biol. Chem.* 270:7029–32
 245. Hendricks-Taylor LR, Motto DG, Zhang J, Siraganian RP, Koretzky GA. 1997. SLP-76 is a substrate of the high affinity IgE receptor-stimulated protein tyrosine kinases in rat basophilic leukemia cells. *J. Biol. Chem.* 272:1363–67
 246. Yablonski D, Kuhne MR, Kadlecsek T, Weiss A. 1998. Uncoupling of nonreceptor tyrosine kinases from PLC-gamma 1 in an SLP-76-deficient T cell. *Science* 281:413–16
 247. Clements JL, Yang B, Ross-Barta SE, Eliason SL, Hrstka RF, Williamson RA, Koretzky GA. 1998. Requirement for the leukocyte-specific adapter protein SLP-76 for normal T cell development. *Science* 281:416–19
 248. Pivniouk V, Tsitsikov E, Swinton P, Rathbun G, Alt FW, Geha RS. 1998. Impaired viability and profound block in thymocyte development in mice lacking the adaptor protein SLP-76. *Cell* 94:229–38
 249. Ota Y, Beitz LO, Scharenberg AM, Donovan JA, Kinet JP, Samelson LE. 1996. Characterization of Cbl tyrosine phosphorylation and a Cbl-Syk complex in RBL-2H3 cells. *J. Exp. Med.* 184:1713–23



CONTENTS

Discovering the Origins of Immunological Competence, <i>Jacques F. A. P. Miller</i>	1
Multifaceted Regulation of IL-15 Expression and Its Role in NK Cell Differentiation & Host Response to Intracellular Pathogens, <i>T. A. Waldmann, Y. Tagaya</i>	19
Immunodominance in Major Histocompatibility Complex Class I-Restricted T Lymphocyte Responses, <i>Jonathan W. Yewdell, Jack R. Bennink</i>	51
Integration of TCR-Dependent Signaling Pathways by Adapter Proteins, <i>James L. Clements, Nancy J. Boerth, Jong Ran Lee, Gary A. Koretzky</i>	89
Evolution of Antigen Binding Receptors, <i>Gary W. Litman, Michele K. Anderson, Jonathan P. Rast</i>	109
Transcriptional Regulation of T Lymphocyte Development and Function, <i>Chay T. Kuo, Jeffrey M. Leiden</i>	149
Natural Killer Cells in Antiviral Defense: Function and Regulation by Innate Cytokines, <i>Christine A. Biron, Khuong B. Nguyen, Gary C. Pien, Leslie P. Cousens, Thais P. Salazar-Mather</i>	189
Mature T Lymphocyte Apoptosis--Immune Regulation in a Dynamic and Unpredictable Antigenic Environment, <i>Michael Lenardo, Francis Ka-Ming Chan, Felicita Hornung, Hugh McFarland, Richard Siegel, Jin Wang, Lixin Zheng</i>	221
Immunologic Basis of Antigen-Induced Airway Hyperresponsiveness, <i>Marsha Wills-Karp</i>	255
Regulation of T Cell Fate by Notch, <i>Ellen Robey</i>	283
The CD1 System: Antigen Presenting Molecules for T Cell Recognition of Lipids and Glycolipids, <i>Steven A. Porcelli, Robert L. Modlin</i>	297
Tumor Necrosis Factor Receptor and Fas Signaling Mechanisms, <i>D. Wallach, E. E. Varfolomeev, N. L. Malinin, Yuri V. Goltsev, A. V. Kovalenko, M. P. Boldin</i>	331
Structural Basis of T Cell Recognition, <i>K. Christopher Garcia, Luc Teyton, Ian A. Wilson</i>	369
Development and Maturation of Secondary Lymphoid Tissues, <i>Yang-Xin Fu, David D. Chaplin</i>	399
The Structural Basis of T Cell Activation by Superantigens, <i>Hongmin Li, Andrea Llera, Emilio L. Malchiodi, Roy A. Mariuzza</i>	435
The Dynamics of T Cell Receptor Signaling: Complex Orchestration and the Key Roles of Tempo and Cooperation, <i>Ronald N. Germain, Irena Stefanová</i>	467
The Regulation of CD4 and CD8 Coreceptor Gene Expression During T Cell Development, <i>Wilfried Ellmeier, Shinichiro Sawada, Dan R. Littman</i>	523
Genetic Analysis of B Cell Antigen Receptor Signaling, <i>Tomohiro Kurosaki</i>	555
Mechanisms of Phagocytosis in Macrophages, <i>Alan Aderem, David M. Underhill</i>	593
Population Biology of HIV-1 Infection: Viral and CD4 ⁺ T Cell Demographics and Dynamics in Lymphatic Tissues, <i>A. T. Haase</i>	625

Chemokine Receptors as HIV-1 Coreceptors: Roles in Viral Entry, Tropism, and Disease, <i>Edward A. Berger, Philip M. Murphy, Joshua M. Farber</i>	657
The IL-4 Receptor: Signaling Mechanisms and Biologic Functions, <i>Keats Nelms, Achsah D. Keegan, José Zamorano, John J. Ryan, William E. Paul</i>	701
Degradation of Cell Proteins and the Generation of MHC Class I-Presented Peptides, <i>Kenneth L. Rock, Alfred L. Goldberg</i>	739
The Central Effectors of Cell Death in the Immune System, <i>Jeffrey C. Rathmell, Craig B. Thompson</i>	781
Selection of the T Cell Repertoire, <i>Eric Sebzda, Sanjeev Mariathasan, Toshiaki Ohteki, Russell Jones, Martin F. Bachmann, Pamela S. Ohashi</i>	829
Regulation of Immune Responses Through Inhibitory Receptors, <i>Eric O. Long</i>	875
The Wiskott-Aldrich Syndrome Protein (WASP): Roles in Signaling and Cytoskeletal Organization, <i>Scott B. Snapper, Fred S. Rosen</i>	905
The High Affinity IgE Receptor (Fc Epsilon RI): From Physiology to Pathology, <i>Jean-Pierre Kinet</i>	931
THE CRYSTAL STRUCTURE OF THE HUMAN HIGH-AFFINITY IgE RECEPTOR (Fc epsilon RI alpha), <i>Scott C. Garman, Jean-Pierre Kinet, Theodore S. Jardetzky</i>	973