Immunologically mediated signaling in basophils and mast cells: finding therapeutic targets for allergic diseases in the human FcεR1 signaling pathway

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Oliver JM, Kepley, CL, Ortega E, Wilson BS. Immunologically mediated signaling in basophils and mast cells: finding therapeutic targets for allergic diseases in the human FcεR1 signaling pathway. *Immunopharmacology* 2000; 48(3):269-81.

Made available courtesy of Elsevier: https://doi.org/10.1016/S0162-3109(00)00224-1

Abstract:

The high affinity IgE receptor, FcεRI, plays key roles in an array of acute and chronic human allergic reactions including asthma, allergic rhinitis, atopic dermatitis, urticaria and anaphylaxis. In humans and rodents, this receptor is found at high levels on basophils and mast cells where its activation by IgE and multivalent antigen produces mediators and cytokines responsible for FcεRI-dependent acute inflammation. Mast cells can additionally contribute to sustained inflammatory responses by internalizing antigen bound to IgE-FcεRI complexes for processing to peptides and presentation to T cells. In humans, the FcεRI is also expressed, at lower density, on monocytes, macrophages and dendritic cells (DC) where its likely functions again include both signaling to mediator and cytokine production and antigen presentation. Our laboratories have focused on defining the earliest steps in the FcεRI signaling cascade in basophils and mast cells and on developing new routes to control allergic inflammation based on inhibiting these events. Here, we describe novel strategies to limit antigen-stimulated FcεRI signaling by: (1) sequestering the FcεRI-associated protein-tyrosine kinase, Lyn, that initiates FcεRI signaling; (2) eliminating; or (3) inactivating the protein-tyrosine kinase, Syk, that propagates FcεRI signaling; and (4) establishing inhibitory crosstalk between FcεRI and a co-expressed receptor, FcγRII, that again limits FcεRI-mediated Syk activation. These strategies may form the basis for new therapies for allergic inflammation.

Keywords: Basophil | Mast cell | IgE | FcεRI | IgG | FcγRII | Allergy | Inflammation | Asthma | Tyrosine kinase | Lyn | Syk | Antigen-presenting cells | ITAM | ITIM | Anti-IgE | Secretion | Degranulation | Cytokine production | Piceatannol | Immunotherapy

Article:

1. Introduction: the distribution and function of the high affinity IgE receptor, FcεRI

1.1. Basophils and mast cells

Mammalian basophils and mast cells express high levels of the tetrameric ($\alpha\beta\gamma_2$) high affinity IgE receptor, FcεRI (Fig. 1) implicated in an array of acute and chronic allergic reactions including asthma, allergic rhinitis, atopic dermatitis, urticaria and anaphylaxis. IgE-mediated allergic inflammation is stimulated by a two-step process in which basophils and mast cells are first primed by the binding of allergen-specific IgE to the α subunit of the FcεRI and then activated by allergen that crosslinks adjacent IgE-FcεRI complexes. Crosslinking FcεRI in the cell membrane activates two cytoplasmic tyrosine kinases, Lyn and Syk, that in turn phosphorylate and activate enzymes (PLCγ isoforms, PI 3-kinase isoforms, PKC isoforms, Akt, BTK, MEK, ERK1/ERK2, JNK and others), adaptors (Cbl, Grb2, SLP-76, LAT and others) and GTP exchange factors/GTPases (Ras, Rho, Vav, Sos and others) and induce the mobilization of stored and extracellular Ca^{2+}. These and other biochemical and ionic events lead within seconds to minutes to the secretion of inflammatory mediators such as histamine that induce airway constriction, mucous production and other acute allergic symptoms. They also rapidly induce actin polymerization, membrane ruffling, the assembly of actin plaques at sites of cell-substrate interaction and the upregulation of VLA-4-mediated adhesion. These responses are likely to be involved in basophil recruitment from blood to inflamed tissues and in the migration of both basophils and mast cells within and between tissues. Several hours after FcεRI crosslinking, basophils and mast cells additionally begin the production of cytokines, including IL-4 and IL-13, strongly implicated in the activation of the TH2 (pro-inflammatory) subset of T cells and in the stimulation of B cells to produce more IgE.

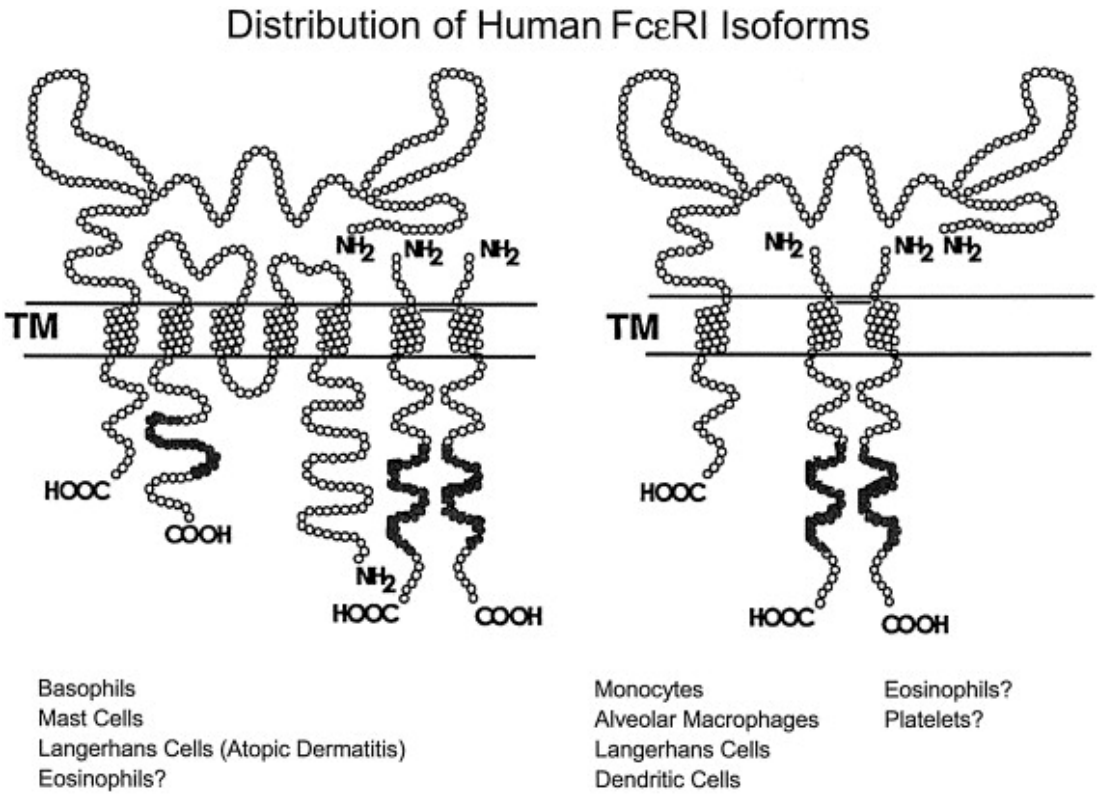

Fig. 1. The structure and distribution of human FcεRI. The left panel shows the heterotetrameric ($\alpha\beta\gamma_2$) FcεRI found primarily on basophils and mast cells. The heterotrimeric variant of the FcεRI expressed mainly on antigen-presenting cells is shown in the right panel. The figure is modified from Kinet (1999).

Fig. 2 shows a sampling of the FcεRI-coupled biochemical pathways and physiological responses of basophils and mast cells. All of the events depicted have been reported in rodent mast cells. Many have been extended to human peripheral blood basophils and/or cord blood-derived mast cells. The antigen-induced membrane ruffling response of human basophils is demonstrated here for the first time in Fig. 3. These and other FcεRI-mediated signaling responses have recently been described in chapters contributed by many groups, including our own, to books edited by Hamawy (1997) and Razin and Rivera (1999).

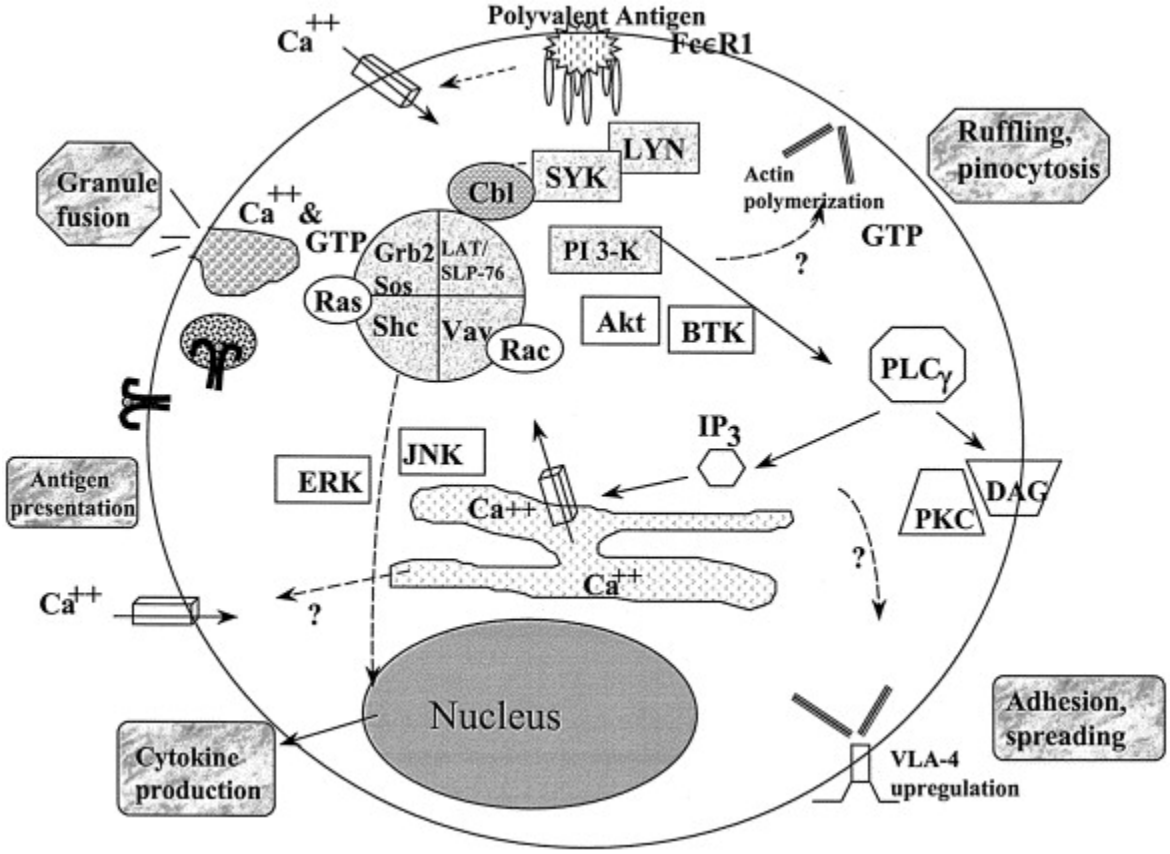

Fig. 2. An overview of FcεRI signaling. Boxes outside the "cell" list the principal physiological responses induced by FcεRI crosslinking in basophils and mast cells. A subset of the biochemical and ionic events linking FcεRI crosslinking to these physiological responses are depicted inside the "cell".

Mecheri and colleagues have reported that IgE-primed mast cells can function additionally as antigen-presenting cells (APC), focusing and internalizing allergen into vesicular compartments for processing to small peptides that form complexes with MHC class II and can be re-expressed on the cell surface both by constitutive vesicular trafficking and by a more abrupt delivery induced by a second round of antigen-induced FcεRI crosslinking Raposo et al., 1997, Tkaczyk et al., 1999. This newly recognized physiological function of the mast cell FcεRI, also shown in Fig. 2, implicates the mast cell in T cell activation not only via cytokine production but also through antigen presentation.

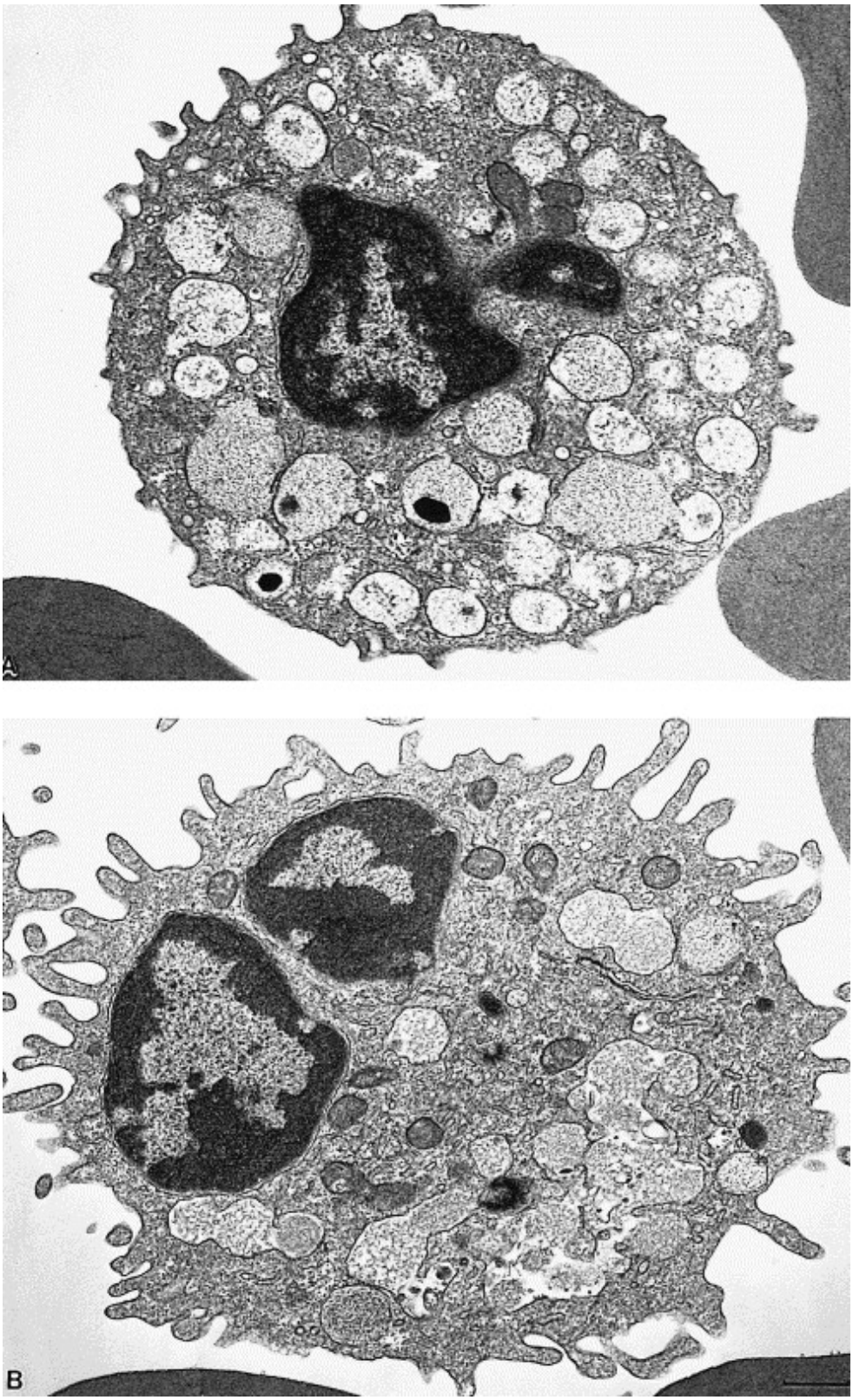

Fig. 3. Human basophil ultrastructure. IgE-primed human basophils were incubated without (A) or with (B) multivalent antigen for 5 min, then fixed and processed for TEM. The resting cell has a densely granulated cytoplasm and a fairly rounded shape. The activated cell has abundant surface ruffles and shows evidence of intracytoplasmic granule fusion. Bar=1 μM.

1.2. Of mouse and man

In rodents, only basophils and mast cells express FcεRI and it occurs only in the tetrameric $\alpha\beta\gamma_2$ form. In contrast, humans express a trimeric variant of the FcεRI, lacking the α subunit, in monocytes, macrophages, a range of dendritic cells (DC) including those in blood, lung and dermis, epidermal Langerhans cells and DC differentiated from cord blood (Fig. 1). FcεRI expression levels on these various APC are typically between<1–10% of the levels measured on basophils and mast cells, with higher levels being characteristically found on cells from donors with higher levels of circulating IgE and with various allergic diseases. The consequences for IgE binding affinity of expressing FcεRI in the $\alpha\gamma_2$ rather than the $\alpha\beta\gamma_2$form are not substantial. However, there is evidence that crosslinking the $\alpha\gamma_2$ FcεRI isoform activates Syk, PLCγ and PI 3-kinase and mobilizes Ca^{2+}, all properties shared with the $\alpha\beta\gamma_2$ FcεRI. Importantly, there is growing evidence that the $\alpha\gamma_2$ FcεRI on monocytes, macrophages and DC are critically involved in allergen-focusing, internalization and processing as described above for mast cells. There is also limited evidence linking atopic dermatitis to the expression of $\alpha\beta\gamma_2$ rather than $\alpha\gamma_2$ FcεRI in Langerhans cells and there are reports of low levels of expression of the FcεRI ($\alpha\beta\gamma_2$ in some reports; $\alpha\gamma_2$ in others) on human eosinophils and platelets. Thus, in man, the FcεRI may promote allergic inflammation via effects on a range of hematopoietic cells in addition to basophils and mast cells. Work in this area was recently reviewed by Bieber (1996), Stingl and Maurer (1997) and Kinet (1999).

2. Therapeutic targets for allergic diseases in the human FcεRI signaling pathway

2.1. Rationale

Many investigators have sought inhibitors of human FcεRI signaling on the premise that inhibiting basophil and mast cell degranulation should block the symptoms, if not necessarily the underlying immunological cause, of IgE-mediated allergic inflammation. As described above, we now know that FcεRI-positive basophils, mast cells and DC can additionally generate pro-inflammatory cytokines and present antigen to T cells. These new data suggest that reliable blockade of FcεRI signaling is likely not only to relieve allergic symptoms but also to arrest the cycle of immunological events associated with disease progression.

Until recently, studies of human FcεRI signaling were quite difficult because FcεRI-positive cells were simply not available in sufficient numbers. Recent advances have improved the yield and speed of basophil isolation from human blood Bochner et al., 1997, Kepley et al., 1998a and have provided protocols to differentiate basophils and mast cells from cord blood Saito et al., 1995, Kepley et al., 1998b. Human DC and monocytes are available from blood and macrophages can be obtained from bronchial alveolar lavage fluid. At least for human basophils, the antigen-activated FcεRI signaling pathway is comfortingly similar, although not identical, to the well-characterized rodent pathway (Kepley et al., 1998a). It is finally possible to search for new therapeutic targets for allergic diseases in the human FcεR1 signaling pathway.

2.2. Strategies

Fig. 4 shows our current understanding of the sequence of events linking FcεRI crosslinking to Syk activation and downstream signaling. The earliest known FcεRI-induced response is the Lyn-mediated phosphorylation of tyrosines located in the immunoreceptor tyrosine-based activation motifs (ITAMs) of the β and γ subunits of the tetrameric ($\alpha\beta\gamma_2$) FcεRI. The FcεRI γ subunit has a typical ITAM consisting of two YXXL sequences separated by approximately 10 amino acids. The FcεRI β ITAM has two YXXL motifs and a third tyrosine in the intervening sequence. Work in the Metzger laboratory at NIH has emphasized the role of Lyn's interaction with FcεRI β subunits in the process of β and γ subunit phosphorylation (reviewed in Metzger, 1999). Brugge and others have shown the critical role of the dually phosphorylated γ subunit ITAM in the recruitment and activation of Syk (Rivera and Brugge, 1995). Linking these studies, our recent work, described below, has identified Lyn dissociation from fully phosphorylated FcεRI as a new regulatory event required for Syk-phospho-ITAM interaction and signal propagation (Ortega et al., 1999).

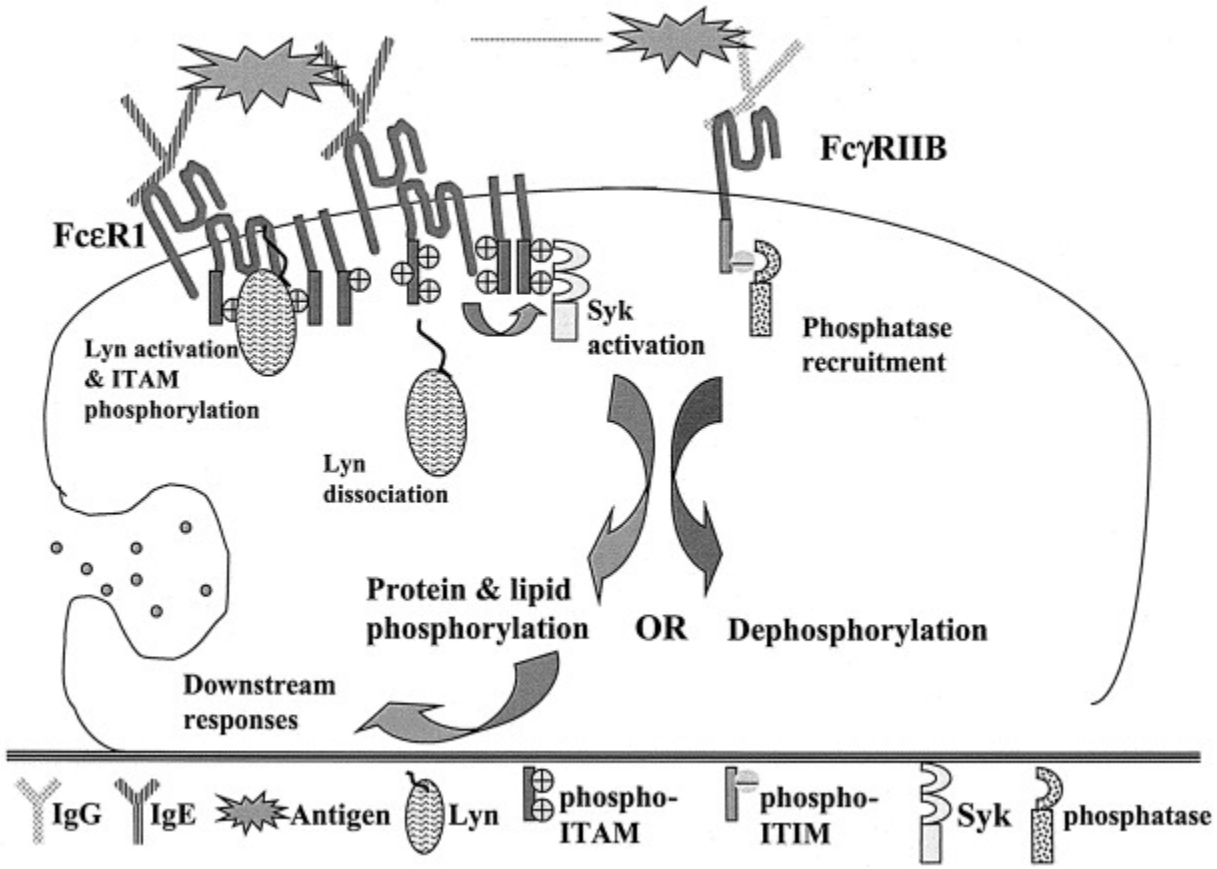

Fig. 4. Modeling FcεRI signal initiation. FcεRI signaling is initiated by the Lyn-mediated tyrosine phosphorylation of ITAMs in the β and γ subunits of the heterotrimeric ($\alpha\beta\gamma_2$) FcεRI. Subsequent Lyn displacement reveals phospho-ITAM binding sites for Syk recruitment and activation. FcεRI signaling can be antagonized by phosphatases presented by co-crosslinked FcγRII. Therapeutic interventions proposed here to block these initiating events include treatments that: (1) block IgE-receptor interaction; (2) prevent the dissociation of Lyn from phosphorylated receptor subunits; (3) impair either the expression or activation of Syk; and (4) recruit FcγRII and its cargo of phosphatases to the FcεRI signaling complex.

The most direct way to block FcεRI signaling is to prevent IgE binding to the receptor, thereby preventing FcεRI crosslinking and signal initiation. The work of P. Jardieu and colleagues at

Genentech, with laboratory and clinical collaborators at many institutions, has demonstrated remarkable new results in this area with a recombinant humanized anti-IgE Ab, E25. RhumAb E25 binds circulating IgE at the site that normally interacts with FcεRI α, forming immune complexes that cannot bind to FcεRI. In humans, biweekly subcutaneous administration of E25 causes a substantial reduction in allergic rhinitic and asthma symptoms. Symptomatic improvement is accompanied by dramatically reduced serum IgE levels and basophil FcεRI expression levels. To date, almost no complications associated with drug treatment or withdrawal have been encountered (Heusser and Jardieu, 1997, Saini et al., 1999; see also the paper by P. Jardieu in this volume). Thus, current concern about this treatment focuses largely on the expense and inconvenience of bimonthly injections of recombinant protein. The Genentech study establishes a central role for IgE in human allergic inflammation. It focuses attention on the FcεRI signaling pathway as a target for the development of new therapeutics.

The second obvious way to block FcεRI signaling is to prevent the propagation of signals from crosslinked receptors. Targets immediately downstream of FcεRI crosslinking include Lyn activation, the dissociation of Lyn from phosphorylated receptor subunits, and the recruitment and activation of Syk. Here we describe strategies that may ultimately generate new therapies for allergic inflammation based on the specific inhibition of the earliest events in the FcεRI-mediated signaling cascade.

2.3. “Lyn-sequestering, signal-curtailing” FcεRI dimers block mast cell activation

Collaborative studies between our laboratories in New Mexico and in Mexico City of the differences in signaling competence between three anti-FcεRI α mAbs originally produced in the Pecht laboratory at the Weizmann Institute (Ortega et al., 1988) led to our first model for rational allergy and asthma therapy through the blockade of FcεRI signal propagation. These mAbs bind competitively with each other and with IgE to the FcεRI of RBL-2H3 rat tumor mast cells (Ortega et al., 1988). Nevertheless, mAb H10 is a poor secretagogue in comparison with mAbs F4 and J17. We demonstrated that the mAb H10-FcεRI interaction also induces very little Ins(1,4,5)P_3 production, Ca^{2+} mobilization or cytoskeletal reorganization in comparison with multivalent Ag or with anti-FcεRI mAbs, F4 and J17. Little or no internalization of mAb H10-FcεRI complexes was detectable on RBL-2H3 cells (Ortega et al., 1999). In contrast, FcεRI complexes with multivalent crosslinkers redistribute to coated pits and are cleared rapidly from the mast cell surface.

Although mAb H10 was severely signaling-impaired, H10-induced Lyn phosphorylation, the first known step in FcεRI signaling, was apparently normal. Furthermore, H10-receptor complexes supported more FcεRI β and γ subunit phosphorylation than signaling-competent crosslinkers in anti-phosphotyrosine immune complex kinase assays. However, mAb H10 was a poor Syk activator, suggesting that FcεRI signaling proceeds efficiently from Lyn to receptor subunits but inefficiently from subunit phosphorylation to Syk when FcεRI crosslinking is induced with mAb H10.

Further studies showed that Lyn immune complexes from H10-treated cells contained substantial amounts of phosphorylated FcεRI β and γ subunit. In contrast, only very small amounts of β and γ subunits could be found associated with Lyn in complexes from cells treated either with

multivalent ligand or with signaling-competent anti-FcεRI mAbs. We also found that the principal phospho-β species induced by mAb H10 in intact cells was a less completely phosphorylated isoform than the principal phospho-β species in antigen-treated cells. It was suggested that the failure of Lyn dissociation and signal propagation in mAb H10-treated cells may reflect differences in conformation or orientation between FcεRI dimers induced by mAb H10 and FcεRI dimers and multimers induced by signaling-competent crosslinkers. Supporting this hypothesis, multivalent complexes of mAb H10 with F(ab)′$_2$ rabbit anti-mouse IgG activate a normal sequence of Lyn recruitment and displacement and of Syk activation, presumably because a signaling-competent configuration of crosslinked receptors had been achieved between co-crosslinked dimers.

From these and other data, we proposed Lyn dissociation from optimally oriented and phosphorylated FcεRI β subunits as a previously unknown rate-limiting step required for Syk activation and signal propagation (Ortega et al., 1999). We speculated that receptor orientation and subunit phosphorylation may be key regulators of signal initiation not only for FcεR1 but also for other key immune system receptors. Supporting this, there is now evidence in T cells linking the poor signaling activity of some altered peptide ligands (APLs) to incomplete phosphorylation of the TCR ζ subunit (Kersh et al., 1998) and independent evidence linking the poor signaling activity of some cytokines to the induction of cytokine receptor dimers with unfavorable orientations (reviewed in Jiang and Hunter, 1998).

Importantly, Wofsy and colleagues have established that Lyn is rate-limiting for FcεRI signaling Wofsy et al., 1997, Wofsy et al., 1999. Based on their analyses, we proposed further that crosslinkers like mAb H10, that sequester Lyn through the formation of stable complexes between Lyn and receptor dimers, should be strong inhibitors of allergen-induced FcεRI signaling. One current goal is to generate and test “Lyn-sequestering, signal-curtailing” crosslinkers of the human FcεRI as novel therapeutic agents for allergic inflammation.

The micrographs in Fig. 5 provide direct support for a model in which productive signaling involves a “kiss-and-run” interaction of Lyn with crosslinked receptor and in which reagents like mAb H10 that bring crosslinked FcεRI into a stably Lyn-bound conformation (“kiss-and-stick”) will inhibit Syk recruitment and signal propagation. In these images, RBL-2H3 cells were activated with multivalent antigen (1 μg/ml DNP-BSA) for 2 min or with 70 nM mAb H10 for 5 min, times shown previously to give the strongest peaks, respectively, of antigen- and mAb-induced tyrosine phosphorylation (Ortega et al., 1999). Membrane sheets were prepared by a modification of the method of Sanan and Anderson (1991) and labeled with colloidal gold particles conjugated to Abs specific for FcεRI β (the 3 nm particles) or for Lyn (the 5 nm particles). In the absence of crosslinking, FcεRI β and Lyn appeared to be loosely co-localized with each other but not with clathrin lattices (Fig. 5A). Following 2 min of crosslinking with DNP-BSA, most of the FcεRI β is in clusters (Fig. 5B). Many of the smaller FcεRI β clusters also contain Lyn (circles). The larger FcεRI β clusters (boxed) typically contain little or no Lyn. These clusters are, however, surrounded by Lyn-containing membrane as might occur if receptor and kinase had first interacted and then segregated in the plane of the membrane. One of the two large clusters of FcεRI β in Fig. 5B is closely associated with two clathrin-coated pits. In contrast, FcεRI β is concentrated in small clusters that include Lyn and show no interaction with clathrin-coated membrane when crosslinking is induced by the signal-curtailing mAb H10 (Fig.

5C). These typical images are compatible with our biochemical evidence (above): that mAb H10 induces the formation of stable complexes of Lyn with receptor whereas, Lyn-receptor complexes are transient in cells treated with signaling-competent antigen. The clear exclusion of mAb H10-receptor complexes from clathrin-coated membrane supports other evidence that H10-like therapeutics should have a long half-life on the cell surface.

2.4. Syk deficiency in "non-releaser" basophils

Studies of the failure of FcεRI-mediated secretion and cytokine synthesis that occurred in basophils from five of 40 healthy non-allergic donors (and has previously been observed in 10–20% of non-allergic donors screened by other groups; see for example the chapter by D. MacGlashan in Razin and Rivera, 1999) led to a second model for rational therapy based on the blockade of FcεRI signal initiation (Kepley et al., 1999a). "Non-releaser" basophils express normal levels of FcεRI and show normal secretion and cytokine synthesis responses to Ca^{2+} ionophore, phorbol ester and formyl peptide, all stimuli that bypass the FcεRI. Their signaling and secretory machinery thus seems intact. The cells are morphologically normal, although they fail to ruffle in response to FcεRI crosslinking. Further analysis showed that Syk protein is absent from the basophils of all five donors. Although basophils lack Syk protein, B cells, eosinophils and neutrophils from non-releaser donors express normal levels of this kinase. Furthermore, Syk mRNA has been detected at variable levels in highly purified basophils from all five non-releaser donors. Basophils from one non-releaser converted to the releaser phenotype, accompanied by Syk protein expression, and reverted back to the non-releaser phenotype in the 6-month course of our studies. Basophils from all five non-releasers expressed Syk and showed modest degranulation after incubation for 4 days in medium with IL-3. These data suggest that basophil Syk expression is manipulatable both in vivo and in vitro.

These results show a clear relationship between the lineage-specific lack of Syk expression and the lack of FcεRI-mediated signaling activity in human non-releaser basophils. Our challenge is to determine the mechanism by which basophils from some donors selectively suppress Syk expression and to apply this knowledge to suppress Syk expression in the basophils and mast cells of allergy sufferers. Our current effort is focused on the product of the Cbl proto-oncogene. Cbl has been identified as a binding partner and negative regulator of Syk that reduces both Syk phosphorylation and Syk levels (Lupher et al., 1999). Remarkably, the RING finger domain of Cbl has ubiquitin-protein ligase activity (Joazeiro et al., 1991). Thus Cbl overexpression or excessive catalytic activity could promote the degradation of Syk protein in non-releaser basophils.

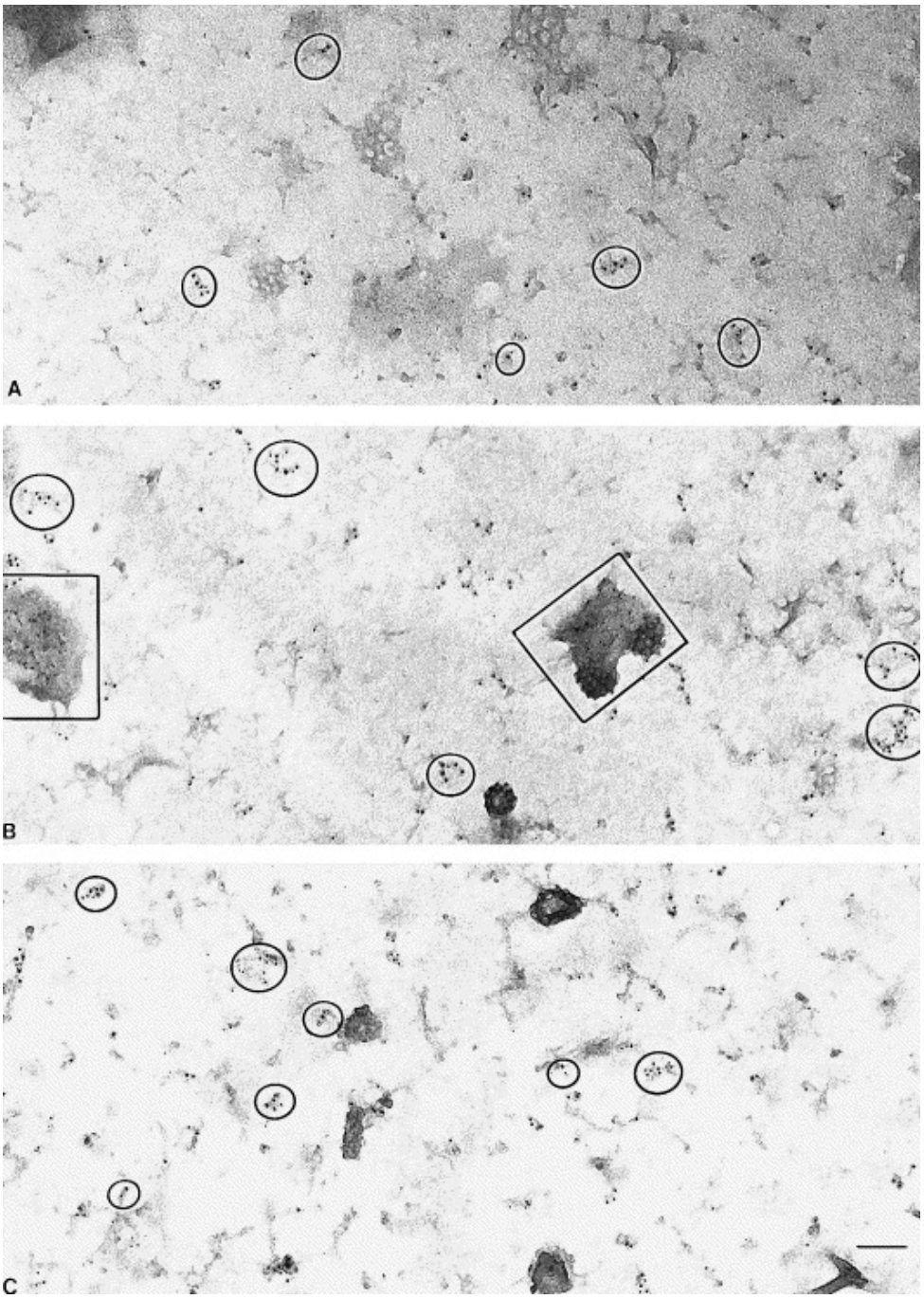

Fig. 5. Observing Lyn-receptor interaction induced by signaling-competent and signal-curtailing FcεRI crosslinkers. Membrane sheets were produced from the upper surface of variously activated RBL-2H3 cells by a modification of the method of Sanan and Anderson (1991). The inner membrane was fixed lightly and labeled with gold-conjugated antibody specific for FcεRI β subunits (the 3 nm gold particles) and for Lyn (the 5 nm gold particles). The three panels show the distributions of FcεRI β and Lyn on the inner membranes of unstimulated cells (A), of cells incubated for 2 min with multivalent antigen (B), and of cells incubated for 5 min with mAb H10, respectively. In unstimulated cells (A), FcεRI β and Lyn appear to be loosely associated in dispersed clusters over the inner cell membrane (circles). Neither marker co-localizes with clathrin lattices. In antigen-stimulated cells (B), FcεRI β occurs in small clusters with Lyn (circles) and also in larger clusters that largely exclude Lyn (boxes). A pair of coated vesicles appears to be budding from membrane adjacent to one of these larger clusters. In mAb H10-stimulated cells (C), FcεRI β is concentrated in small clusters that typically include Lyn and do not associate with coated pits (circles). Bar=0.1 μM.

2.5. Selective inhibition of FcεRI-mediated Syk activation

Collaborative studies between our laboratories in New Mexico and the Geahlen laboratory at Purdue University were the first to localize Syk to the FcεRI signaling complex of RBL-2H3 mast cells (Hutchcroft et al., 1992) and to identify the novel stilbene derivative, piceatannol, as a Syk-selective inhibitor capable of blocking all known signaling responses downstream of Syk in both RBL-2H3 cells (Oliver et al., 1994) and human basophils (Kepley et al., 1998a). Although Syk expression is limited to hematopoietic cells, this kinase is nevertheless a key component of the signaling pathways activated through both antigen and adhesion receptors on B cells, NK cells, monocytes, macrophages and platelets. Thus the therapeutic potential of piceatannol for allergic inflammation is not high without additional targeting to limit its interaction to only basophils and mast cells. It may be possible to provide this targeting specificity by conjugating piceatannol to humanized Abs or ligands for basophil and mast cell-specific co-receptors that could internalize the drug by receptor-mediated endocytosis. Ligands for co-receptors with C-terminal immunoreceptor tyrosine-based inhibitory motifs (ITIMs), typically consisting of 13 amino acid sequences including a single YXXL motif, are particularly attractive candidates since occupying these receptors does not induce degranulation and can in fact reduce degranulation induced by FcεRI crosslinking (see below). Obvious targets include the putative human analog of MAFA, a C-type lectin found primarily on rat mast cells (Guthman et al., 1995) and HM18, recently identified as the human analog of gp49B1 found primarily on mouse mast cells (see the chapter by H. Katz in Razin and Rivera, 1999).

The search for Syk inhibitors that target basophils and mast cells may be advanced by additionally learning more about the Syk-phospho-ITAM interaction that activates Syk. Moriya et al. (1997) demonstrated that the novel acridone-related compound, ER-27319, inhibits Syk tyrosine phosphorylation and downstream signaling in RBL-2H3 mast cells not by directly inactivating Syk but by blocking its association with the FcεRI γ subunit phospho-ITAM, a prerequisite for Syk activation in vivo. Remarkably, Syk activation induced by its binding to the Ig β ITAM of the B cell receptor is not inhibited by ER-27319 despite the substantial structural similarity that exists between the ITAMs of different receptors. Of course, ER-27319 can be expected to inhibit signaling through other receptors that use the FcεRI γ subunit, including FcRγI, FcγRIII, some T cell receptor isoforms and the platelet collagen receptor Daeron, 1997, Watson and Gibbins, 1998. Nevertheless, ER-27319 may serve as a parent compound for the development of inhibitors of human mast cell responses that specifically inhibit interactions between Syk and the FcεRI γ subunit while sparing Syk-dependent functions of other critical immune cells.

2.6. FcεRI/FcγRII co-crosslinking blocks FcεRI-mediated basophil and mast cell activation

Studies of crosstalk between FcγRII and FcεRI on human basophils have potential to provide an independent method to block FcεRI-mediated signal propagation in human cells. We (Kepley et al., 1999b) confirmed published evidence that human basophils and mast cells express only the low affinity IgG receptor, FcγRII and not FcγRI or FcγRIII (Anselmino et al., 1989). In collaboration with P. Morel at the University of Pittsburgh, we found that the principal FcγRII species in peripheral blood basophils and cord blood-derived mast cells is the ITIM-containing

FcγRIIB isoform. Basophils also contain mRNA for the ITAM-containing FcγRIIA isoform but not for FcγRIIC. Importantly, we demonstrated that FcεRI-mediated secretion, IL-4 production, Ca^{2+}mobilization and Syk phosphorylation are all significantly reduced when FcεRI and FcγRII are primed with anti-DNP IgE and anti-DNP IgG, respectively, and co-crosslinked with the same antigen.

These results resembled published data from B cells, where co-crosslinking the BCR to FcγRIIB inhibits BCR-mediated cell activation (reviewed in Cambier, 1997, Daeron, 1997). In B cells, the mechanism of inhibition most likely involves the Lyn-mediated tyrosine phosphorylation of the ITIM sequence found in the FcγRIIB cytoplasmic tail, creating phosphotyrosine binding sites for the SH2 domain-containing phospholipase, phosphatidylinositol 5-phosphatase (SHIP) and for the SH2 domain-containing protein tyrosine phosphatases, SHP-1 and SHP-2. Following this lead, we showed that human basophils express SHP-1, SHP-2 and SHIP; demonstrated that phosphorylated FcγRIIB ITIM peptides recruit SHP-1 from basophil lysates; and finally, established that SHP-1 translocates to the basophil membrane in response to FcγRII crosslinking. These data support the hypothesis that negative signaling from FcγRII to the FcεR1 of human basophils occurs at least in part by the recruitment of SHP-1 to the FcγRIIB ITIM, positioning it to block or reverse the phosphorylation and activation of Syk needed to couple FcεR1 crosslinking to downstream responses. It remains possible that the less abundant SHIP also contributes to the inhibition of FcεR1 signaling by co-crosslinked FcγRII in human basophils and that more sentitive detection methods will reveal its involvement.

The therapeutic potential of these studies may already have been applied in clinical practice. Physicians have used immunotherapy, the injection of increasing doses of substances that induce allergic responses in patients with severe allergies, for most of the 20th century. Successful immunotherapy, defined by reduced responsiveness to particular antigens, has been linked to increased levels of allergen-specific IgG, sometimes accompanied by increased levels of IgE specific for the same allergen. It is generally assumed that the clinical improvement results from the neutralization and clearance of circulating allergen by the newly synthesized IgG. Extending a suggestion made earlier by Daeron (1997), we hypothesize that the efficacy of immunotherapy in some allergy patients may result additionally from allergen-mediated FcγRII/FcεRI co-crosslinking on basophils and mast cells, resulting in the FcγRIIB-mediated recruitment of signal-terminating phosphatases to the FcεRI signaling complex in vivo. This co-crosslinking could occur in at least three ways. The first is by the binding of allergen to dually (IgG plus IgE)-primed basophils and mast cells, just as in our in vitro model. However, FcγRII is a low affinity receptor that prefers to bind IgG aggregates (hence our considerable search for an anti-DNP IgG for basophil priming). Thus, a second, and perhaps more likely, route to FcγRII/FcεRI co-crosslinking in vivo would involve the simultaneous binding of allergen-IgG complexes to IgE-primed FcεRI and to unprimed FcγRII on basophils and mast cells. Co-crosslinking could also occur if the circulating immune complexes contained IgE as well as IgG and allergen and the complexes bound simultaneously to unprimed FcγRII and to unprimed FcεRI on basophils and mast cells. In all cases, individuals with little or no basophil and mast cell FcγRIIB, and thus little ability to draw phosphatases into the FcεRI signaling complex, would not be expected to benefit from immunotherapy. Thus it may be possible in the future to predict the success or failure of immunotherapy based on the heterogeneity in FcγRII isoform expression between individuals.

3. Summary

The success of the Genentech-sponsored studies of anti-IgE immunotherapy, that have so far provided symptomatic relief to over 90% of patients enrolled in trials, strongly supports the use of strategies to block human FcεRI signaling as a general approach to limit or even prevent the symptoms of allergic inflammation (see the review by P. Jardieu in this volume). Because FcεRI-positive cells are increasingly implicated in the cycle of immunological events (cytokine production, cell migration, antigen presentation) responsible for disease progression, it is likely that inhibiting FcεRI signaling will also help to correct the immunological imbalance that maintains the allergic phenotype.

This paper summarizes four alternative strategies to inhibit early events regulating FcεRI signal propagation. The strategies we describe have potential to complement other new therapies based on the neutralization of IgE or of cytokines as well as established therapies with antihistamines and steroids.

In the first study, FcεR1 crosslinkers that activate Lyn but fail to release Lyn from phosphorylated FcεR1 subunits, a prerequisite for Syk recruitment and signal propagation were used to prevent FcεR1 signal propagation (Ortega et al., 1999). These reagents are so far available only for the rodent FcεR1. Analogous "Lyn-sequestering, signal-curtailing" ligands could be effective inhibitors of Syk activation and signal propagation through the human FcεRI.

The second study showed that the 10% or so of "non-releaser" human donors lack basophil Syk protein expression even though Syk levels are normal in co-purified B cells (Kepley et al., 1999a). Understanding mechanisms regulating this lineage-specific Syk expression may yield strategies to selectively inhibit its expression in FcεRI-positive cells from asthmatics.

The third approach was by the pharmacological inhibition of Syk. Our work has identified piceatannol as a Syk-selective drug that still requires further engineering, most obviously by its conjugation to ligands for mast cell specific co-receptors, for uptake specifically into basophils and mast cells (Oliver et al., 1994). Complementary work by the group at Esai in Japan, in collaboration with the J. Rivera laboratory at NIH, has identified ER-27319 as a drug that inhibits the association of Syk with the phospho-ITAMs of FcεR1γ, but not of other members of the multichain immunoregulatory receptors (Moriya et al., 1997). Because the FcεR1 γ subunit is a component of several immune receptors, ER-27319 also requires further engineering for targeting specifically to basophils and mast cells. Nevertheless, this evidence that Syk may interact differently with different phospho-ITAMs encourages the continued search for therapeutic agents that inactivate Syk in human basophils and mast cells while sparing other Syk-dependent cells.

The final study shows that human FcεRI signaling is inhibited in vitro by phosphatases presented to FcεRI by co-crosslinked FcγRII (Kepley et al., 1999b). In this case the therapy may have preceded the science. Immunotherapy, practiced in the clinic since the mid-1930's, may work in part by stimulating the production of allergen-specific IgG in patients who are already producing allergen-specific IgE. We hypothesize that the simultaneous interactions of these IgE and IgE

antibodies with common allergen but distinct receptors on basophils and mast cells may co-crosslink FcεRI and FcγRII, blocking FcεRI signal propagation in vivo through the recruitment of FcγRII-associated phosphatases to the FcεRI signaling complex and contributing to the reduction of allergy symptoms. Studies are in progress to explore the heterogeneity of FcγRII isoform expression between individuals. We speculate that only patients expressing relatively high levels of the ITIM-containing FcγRIIB isoform in their basophils and mast cells will show a good response to immunotherapy.

Fig. 2 shows many other potential targets for inhibitors of FcεRI-mediated signaling. We expect that the further refinement of strategies to block human FcεRI signal initiation or propagation through the heterotetrameric FcεRI of basophils and mast cells and the heterotrimeric FcεRI of antigen-presenting and other cells will form the basis of other new and useful therapies for allergic inflammation.

Acknowledgements

This work was supported in part by NIH grants RO1 GM49814, R29 GM50562, RO3 TW00440 and P50 HL56384 and by a pilot project award from the UNM Asthma Research Center. We thank Janet Pfeiffer for the electron micrographs presented here and Lama Youssef, Ron Andrews, Marina Martinez, Martha Lara, Irene Lee, Carla Santana and Penelope Morel for contributions to the data summarized above. We also thank Israel Pecht for generous access to mAbs produced in his laboratory and Mary Lipscomb, Mark Schuyler and other members of the UNM Asthma SCOR for constructive criticism and consistent support.

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