

Rethinking the Role of Src Family Protein Tyrosine Kinases in the Allergic Response

New Insights on the Functional Coupling of the High Affinity IgE Receptor

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Abstract

Antigen-induced cross-linking of immunoglobulin E (IgE) antibodies bound to the high-affinity IgE receptor (FcεRI), on mast cells results in the release of mediators that initiate an inflammatory response. This normal immune response has been abducted by immunological adaptation, through the production of IgE antibodies to normally innocuous substances, to cause allergic disease. Therefore, understanding the molecular requirements in IgE-dependent mast-cell activation holds promise for therapeutic intervention in disease. Recent investigation on the functional coupling of FcεRI to the intracellular signaling apparatus has provided paradigm-altering insights on the importance and

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function of Src family protein tyrosine kinases (Src PTK) in mast-cell activation. In this synopsis, we review the current knowledge on the role of the Src PTKs, Fyn and Lyn, in mast-cell activation and discuss the implications of our findings on allergic disease.

Introduction

The principal mode of mast-cell activation is dependent on recognition of an antigen by cell surface-bound IgE antibody, which initiates immune-complex formation and concurrent aggregation of Fc ϵ RI. The aggregation of Fc ϵ RI is both necessary and sufficient to elicit the biochemical steps that result in an activated mast cell, which releases preformed and *de novo* synthesized mediators that collectively contribute to the allergic response (1). The Fc ϵ RI can exist as a trimeric (humans) or tetrameric (humans and rodents) receptor; the two forms being distinguished by exclusion or inclusion of a tetraspanning β subunit that contains an immunoreceptor tyrosine-based activation motif (ITAM), which functions to amplify the generated intracellular signals. Thus, the Fc ϵ RI β influences the extent of a mast cell's effector response (reviewed in ref. [2]). The α subunit binds the IgE antibody, whereas a disulfide-linked γ subunit dimer provides signaling competence to this receptor through its encoded ITAMs.

More than a decade ago it was recognized that phosphorylation of the ITAMs occurred upon Fc ϵ RI aggregation (3). It was later observed that the Src PTK Lyn co-immunoprecipitated with Fc ϵ RI (4) and through an aggregation-dependent transphosphorylation (5), which requires an appropriate lipid environment (6), Lyn phosphorylated the Fc ϵ RI ITAMs. Once phosphorylated, the ITAMs become a docking site for activation and/or coordination of other signaling proteins, like Syk kinase, whose activity and function are

essential for sustaining the biochemical signals that drive the cells response (reviewed in ref. [2]). Because of the central role of Lyn kinase in the initial step of Fc ϵ RI phosphorylation and the demonstrated association of Lyn and the Fc ϵ RI, it has generally been accepted that Lyn kinase functions to positively regulate mast-cell activation and the release of inflammatory mediators. Studies in which generic or Src- and Syk-selective tyrosine kinase inhibitors were used to treat mast cells showed that IgE-dependent degranulation and cytokine production were inhibited (7–10). Thus, the notion that Lyn kinase might serve as a reasonable therapeutic target in allergic disease has been understandably proposed (11).

However, in recent years several studies conducted primarily by the use of genetically altered mice have cast doubt (and caused considerable confusion) on the importance of Lyn kinase in mast-cell degranulation and cytokine production. Mast cells derived from mice with a homozygous-null mutation of the *Lyn* gene were not impaired in degranulation nor in the production of cytokines (12,13). This brought into question the previous report of a defective passive cutaneous anaphylaxis response observed in *Lyn*^{-/-} mice (14), which had suggested a critical need for Lyn kinase in mast-cell degranulation. Over the last several years, we and others have investigated the role of Src PTKs and adapter proteins in mast-cell activation and responses. Evidence of a complementary pathway, initiated by the Src PTK Fyn and organized through the adapter function of Gab2, has emerged (15,16). These new

findings have changed our view on how the FcεRI couples to the signaling apparatus. Moreover, a differential role for Src PTKs in the positive and negative regulation of mast-cell responses is now evident. A new picture is emerging that brings into question the concept of Lyn kinase as a positive effector in allergic responses and its suitability as a therapeutic target for intervention in allergic disease.

Activation and Regulation of Src Family Kinases

Src family kinases have a characteristic four-domain structure. The unique domain at the N-terminus is comprised of the first 70–90 amino acids and contains sequences important for the post-translational acylation that targets most family members to membranes. The dual modification of myristoylation of glycine 2 and palmitoylation of cysteines 3, 5, or 6, is critical for inclusion of Src PTKs in cholesterol-enriched membrane microdomains, commonly referred to as lipid rafts. These kinases also contain the prototypic protein–protein interaction domains, src homology region 2 (SH2) and 3 (SH3), which evolved to recognize tyrosine phosphorylated sequences, like ITAMs (YXXL), and poly-proline type II helices, respectively. The catalytic domain of Src PTKs (like Lyn and Fyn) has the characteristic tyrosine kinase two-lobe structure with a conserved positive regulatory tyrosine (Y397/417) in the activation loop, which is followed by a short tail that contains a conserved negative regulatory tyrosine (Y508/528). The activity of Src kinases must be tightly regulated because of their oncogenic potential (17). This is accomplished through intrinsic and extrinsic regulation: (1) intramolecular interactions of the SH2 domain with the phosphorylated negative regulatory tyrosine at the C-terminus (Y508/528), and of the SH3 domain with a poly-proline rich sequence in the linker region (between the SH2

and catalytic domains) creates a “closed” inactive conformation of the kinase. (2) The inactive “closed” conformation is promoted by C-terminal Src kinase (Csk) (18), which phosphorylates the C-terminus (Y508/528). In contrast, the active “open” conformation is facilitated by dephosphorylation of this C-terminal tyrosine by the CD45 phosphatase (19). (3) The active “open” conformation promotes a conformational change in the activation loop of the kinase that increases its specific activity. This state is stabilized by autophosphorylation of a positive regulatory tyrosine (Y397/417) within it. It should be noted, however, that an active state can occur where both regulatory tyrosines are phosphorylated but an “open” conformation is promoted by interaction with other proteins (20).

Association of Src PTKs With FcεRI

As mentioned earlier, Lyn kinase was demonstrated to co-immunoprecipitate with FcεRI (4). A considerable amount of data is now available on the interaction of Lyn kinase with FcεRI, which was demonstrated to occur via the β subunit (21,22). Chemical cross-linking experiments unequivocally established that Lyn kinase bound to FcεRI, prior to stimulation of this receptor, and was further recruited upon stimulation (23). Additionally, the interaction of Lyn with FcεRIβ in resting cells was weak and required the acylation signal-containing unique domain of Lyn (24,25). In contrast, the interaction with FcεRIβ in stimulated cells is primarily through SH2 domain and phospho-ITAM interactions (22) that require the canonical tyrosine(s) (YF and JR, unpublished observation). Strikingly, FcεRI-associated Lyn kinase does not increase its specific activity upon stimulation of this receptor and appears to be active in resting cells (5,23). Nonetheless, phosphorylation of FcεRI requires the aggregation of this recep-

tor by IgE-antigen complexes, demonstrating that transphosphorylation is required (5). As alluded to earlier, there is considerable evidence that a lipid-raft environment, where most of Lyn kinase resides, may function as a site where the initial or continued interaction of Lyn with Fc ϵ RI and/or Fc ϵ RI phosphorylation may occur, because this receptor is recruited to these domains upon aggregation (6,26,27). However, there is also considerable, and apparently contradictory, evidence demonstrating that Lyn kinase interaction with Fc ϵ RI is independent of aggregation of this receptor or of the inclusion of Lyn in the coalesced lipid rafts that occur after Fc ϵ RI aggregation (24,25,28,29). The recent report that lipid raft-resident Lyn kinase in resting cells is in an active state (30) and the previous finding of active Lyn kinase associating with Fc ϵ RI (5,23) suggests that in resting cells the Fc ϵ RI-associated Lyn kinase may come from the same pool of active lipid raft-localized Lyn. Thus, it is possible that in resting cells some receptors may interact with Lyn kinase because of their inclusion in a micro-lipid-raft environment that cannot be isolated or identified by current biochemical or immunological techniques. If true, this would provide a unifying explanation for the apparent conflict in prior observations.

More recently, it was recognized that Fyn kinase also co-immunoprecipitates with Fc ϵ RI in a specific manner analogous to Lyn (16). However, very little is known about Fc ϵ RI and Fyn interactions. Based on studies in T-cells, the acylation of Fyn is required for membrane targeting and association with the T-cell receptor (TCR) ζ chain through SH2 domain interactions (31). Thus, strong similarities are evident in the mechanism of Lyn and Fyn interactions with antigen receptors. Additionally, preliminary experiments in which the tyrosine residues in the ITAM of the Fc ϵ RI β were mutated showed that normal Fyn activation

requires an intact Fc ϵ RI β ITAM (YF and JR, unpublished observation). Unlike Lyn, Fyn activation requires Fc ϵ RI stimulation (16) resulting in an increase of its total specific activity by 2.5–4-fold (32). Only a modest fraction of Fyn can be found in lipid rafts in resting cells (whereas the major fraction of Lyn is in these domains), although it is further recruited into lipid rafts upon Fc ϵ RI stimulation ([16]; MK and JR, unpublished observation). Interestingly, it does not appear that there is considerable redundancy of Fyn or of other Src PTKs in the phosphorylation of Fc ϵ RI, because in the absence of Lyn the peak phosphorylation of the Fc ϵ RI ITAMs (usually seen at 3 min of stimulation) was impaired (29). In preliminary experiments, Fc ϵ RI phosphorylation is inhibited by 50–90% even up to 27 min poststimulation (SO and JR, unpublished observation). Thus, although Fyn co-immunoprecipitates with Fc ϵ RI, it does not appear to effectively target the receptor ITAMs as a substrate.

Signaling Pathways Initiated by Lyn and Fyn

As proximal Fc ϵ RI kinases, Lyn and Fyn function to initiate the cascade of signals that govern a mast cell's response to IgE-antigen engagement. The events following Lyn-dependent phosphorylation of Fc ϵ RI have been well-delineated and elegantly reviewed (33). Briefly, because of its role in receptor phosphorylation Lyn is required for full activation of Syk kinase through its interaction with phospho-ITAMs. This results in the subsequent phosphorylation of the adapter LAT by Syk. LAT scaffolds a macromolecular signaling complex (because of phosphorylation of its multiple tyrosine residues, which allows SH2 domain interactions) that includes PLC γ and the adapters Grb2 and Gads, and thus associates with their binding partners as well

(34–36). In mast cells, like in T cells, the LAT scaffolded complex is critically important for normal calcium responses through its ability to regulate the activation of PLC γ , which is required for the inositol-3, 4, 5-trisphosphate-mediated release of calcium from endoplasmic reticular stores (37). Thus, Lyn plays an important role in mast-cell calcium responses. This view is clearly supported by the observation of defective Syk and LAT phosphorylation as well as defective calcium responses in *Lyn*^{−/−} mast cells (13,16). In contrast, Fyn does not appear to play a major role in the regulation of intracellular calcium because *Fyn*^{−/−} mast cells have normal or slightly elevated calcium responses ([16]; GG and JR, unpublished observation). Fyn kinase activity is important in the phosphorylation of the adapter Gab2 (16), although it is unclear whether Gab2 serves as a substrate for Fyn or whether the requirement for Fyn activity is indirect. Gab2 and Fyn were demonstrated to interact (16) and Gab2 was found to be critical to the activation of phosphatidylinositol 3-OH kinase (PI3K) in mast cells (15). Consistent with the view that Fyn and Gab2 are in the same pathway, *Fyn*^{−/−} mast cells also showed a defect in PI3K activity and both *Fyn*^{−/−} and *Gab2*^{−/−} mast cells failed to elicit normal Akt phosphorylation, which requires a functional PI3K-dependent kinase 1 (PDK1) (15,16). Importantly, it appears that the Lyn/LAT and Fyn/Gab2 pathways function in parallel. In the absence of Lyn, Fyn is functional and Gab2 is phosphorylated (16,32) and PI3K activity is intact. In the absence of Fyn, LAT is phosphorylated and calcium signals are not impaired (16). Thus, Fyn initiated signals can be generated in the absence of Lyn and vice versa. Nonetheless, there is cross-talk between these pathways as both of them are necessary for normal mast-cell activation (16).

However, we have come to realize that there are differences in the sensitivity of Fc ϵ RI coupling to these distinct signaling pathways. Our recent study demonstrates that stimulating mast cells with low amounts of an antigen or low occupancy of Fc ϵ RI with IgE (where only 10–15% of receptors are occupied) caused the preferential (but not exclusive) induction of signals through activation of the Fyn/Gab2 axis (38). As might be expected, given the importance of Fyn and Gab2 to PI3K activation (15,16), this weak stimulation favored the activation of Akt and interestingly also favored the activation of p38MAPK (38). In contrast, the phosphorylation of LAT and of ERK2 required a strong stimulus provided by large amounts of antigen or high Fc ϵ RI occupancy with IgE-antigen. Because both Lyn and Fyn are proximal to Fc ϵ RI, the finding of preferential Fyn activation under weak stimulation provides suggestive evidence for divergence in the molecular machinery downstream of the receptor proximal kinases. This view is supported by high-resolution electron microscopic studies, which showed Fc ϵ RI to be localized in plasma membrane regions that included Gab2 but excluded LAT (39). LAT was found in a distinctly different membrane region that seemingly coalesced with Fc ϵ RI-containing domains after a strong stimulus. Thus, it appears that receptor proximity is a critical determinant for increased sensitivity in initiation of signals.

Lyn Kinase as a Negative Regulator of Mast-Cell Signaling and Responses

Beyond its role in phosphorylation of Fc ϵ RI, little was known about the role of Lyn kinase in mast cells and in the allergic response. Interestingly, some biochemical and electron microscopic studies have shown that Lyn dissociates from Fc ϵ RI after its stimula-

tion (40) and that receptors that are signaling-competent are devoid of Lyn and complexed with Syk (41). This suggests a need for the dissociation of Lyn from FcεRI after its phosphorylation to promote the appropriate interactions that elicit cellular signaling. This is further supported by the demonstration that receptors stably associated with Lyn were less capable of promoting signals (41). Implied is the view that Lyn may function as a negative regulator if it remains stably associated with FcεRI. Interestingly, this view of Lyn as a negative regulator is supported by studies of Lyn function in B cells and monocytes. Although some of the signaling capability of Lyn-deficient B cells and monocytes is impaired, some responses like immunoglobulin production and sensitivity to cytokine stimulation are enhanced (14,42). Moreover, it is clear that Lyn is required for negative regulation of B-cell activation and monocyte activation through its role in membrane targeting and activating phosphatases (42,43). Given the strong evidence for the negative regulatory role of Lyn downstream of the B-cell antigen receptor (BCR) and cytokine receptors, clarification of its role in mast cell-mediated allergic responses was needed.

As mentioned earlier, Lyn-deficient mast cells degranulate. Thus, a reasonable conclusion is that Lyn kinase is not the driving force for mast-cell degranulation downstream of FcεRI stimulation. However, what was striking is that mast cells derived from Lyn-deficient mice showed greatly enhanced degranulation and cytokine production (13,16; C. G.-E. and JR, unpublished observation). This suggested the release of Lyn-dependent negative regulatory constraints on these responses. A recent series of experiments demonstrate this to be the case and define a mechanism by which Lyn kinase controls mast-cell degranulation (32). As detailed previously, Src PTK activity can be regulated

through Csk. This regulation requires the targeting of Csk (18) to the plasma membrane through its interaction with the adapter Cbp/PAG (reviewed in ref. [44]). Lyn-deficiency was found to cause defective phosphorylation of Cbp/PAG and thus decreased membrane targeting of Csk. The consequence is a selective and constitutive elevation of Fyn kinase activity in Lyn-deficient mast cells (32). Our previous demonstration of Fyn kinase involvement in mast-cell degranulation (16) led us to explore whether the enhanced Fyn kinase activity in Lyn-deficient mast cells was contributory to the hyper-degranulation phenotype of these cells. We postulated that if Fyn is responsible for the hyper-degranulation of Lyn-deficient mast cells, two requirements must be met: (1) introduction of Lyn kinase in Lyn-deficient mast cells should reverse the enhanced Fyn activity and the enhanced degranulation, and (2) removing Fyn from Lyn-deficient mast cells (*Lyn/Fyn*^{-/-}-double deficient) should ablate degranulation if Fyn kinase is driving this response. Reconstitution of Lyn showed that this kinase is a negative regulator of Fyn activation through the phosphorylation of Cbp/PAG and Csk, because Fyn kinase activity was reduced and the phosphorylation and interaction of Csk with Cbp/PAG was restored along with FcεRI-control of Fyn kinase activation. Moreover, *Lyn/Fyn*^{-/-}-double deficient mast cells were impaired in their degranulation with 80–90% inhibition when compared to wild-type cells. Therefore, the findings demonstrate that Lyn kinase functions to negatively regulate the activity of Fyn kinase and thus the extent and occurrence of the degranulation response (see next section).

Importantly, the role of Lyn as a negative regulator of the allergic response transcends its function in mast cells. We and others (45) have recognized an important role for Lyn kinase in B cell-mediated IgE production.

Thus, as will be outlined later, in the absence of Lyn kinase an apparent increased allergic susceptibility is evident *in vivo*. This argues for a broad physiological role of Lyn kinase in negative regulation of the allergic response.

Fyn Kinase as a Positive Regulator of Mast-Cell Degranulation

In contrast to Lyn, Fyn kinase seemingly plays an important positive regulatory role in regulating mast-cell degranulation downstream of Fc ϵ RI. The demonstration that *Fyn*^{-/-} mast cells are defective in degranulation (16) and the more recent finding that *Lyn/Fyn*^{-/-}-double deficient mast cells (but not *Lyn*^{-/-} mast cells) also have a defective degranulation response (32) clearly links Fyn kinase to the positive regulation of this event. An important insight from our prior work (16) is the finding of an apparent defect in PI3K activity and in phosphorylation of downstream targets, like PDK1 and Akt, in *Fyn*^{-/-} mast cells. This suggests a possible connection between PI3K and mast-cell degranulation. This view is supported by our studies and those of others demonstrating inhibition of mast-cell degranulation by PI3K inhibitors (16,46). Of note is that Lyn-deficient mast cells were equally or slightly more sensitive to inhibition of degranulation by the PI3K inhibitor LY294002 than wild-type mast cells (16). This demonstrates that Lyn kinase is not essential for the PI3K activity that contributes to mast-cell degranulation. It also suggests that Fyn kinase controls the majority of PI3K activity, which is consistent with the idea that Gab2 is downstream of Fyn and is responsible for the majority of PI3K activity in mast cells (15,16).

Although there is minimal evidence on how Fyn functions to support mast-cell degranulation, some clues exist. Considering that mast cells require both calcium and PKC activation

for the degranulation response (reviewed in ref. [47]), the defect in PDK1 activation in the absence of Fyn may be critically important. A threonine residue in the activation loop of PKC isoforms must be phosphorylated by PDK1 to achieve full PKC activation and all tested isoforms of PKC could also form a complex with PDK1 (48). PDK1 is highly dependent on PI3K activity for its activation and Fyn-deficient mast cells have a defective PI3K response. Therefore, Fyn-deficiency causes the loss of PKC activity because the PDK1-specific Thr 505 in the activation loop is not effectively phosphorylated (as demonstrated for the novel calcium-independent PKC δ) (16). The phosphorylation of the conserved activation loop site (Thr 500) has also been demonstrated as essential for the activation of the classical calcium-dependent PKC β (49). Because both PKC β and δ were demonstrated to specifically control mast-cell degranulation (50), the loss of activation loop phosphorylation may serve as the critical block of degranulation in Fyn-deficient mast cells. This would suggest that total PKC activity is reduced in Fyn-deficient mast cells when compared to wild-type cells (because this site is conserved among various PKC isoforms), this point remains to be demonstrated. However a greater degree of regulatory complexity is likely, given the recent realization that phospholipase D (PLD)-generated diacylglycerol (DAG) and phosphatidic acid (PtdOH) are essential for the localization and activation of certain PKC isoforms in mast cells (51) and that Src PTKs, like c-Src and Fyn, can phosphorylate the plasma membrane-localized PLD2 isoform (52). Thus, Fyn may be required for two distinct events critical for PKC activation, namely, by influencing the phosphorylation of the activation loop of PKC and by generating DAG/PtdOH, which serves to translocate and activate PKC. It should be noted that the calcium response remains intact

in Fyn-deficient mast cells. Therefore, the PKC activation defect provides the most reasonable molecular explanation for the degranulation defect (see prior review in ref. [47]) and thus merits further investigation.

An Emerging View on the (Patho)physiological Role of Lyn and Fyn

The *in vivo* consequence of Lyn- or Fyn-deficiency is the development of multiple disorders. The most pronounced phenotype of Lyn-deficient mice is an autoimmune disease with features of systemic lupus erythematosus (SLE) (14,53). Some of these mice die because of renal failure owing to massive immune-complex deposition in the kidney, a feature of lupus nephritis. Although peripheral B-cell numbers are decreased, Lyn-deficient mice show enhanced IgM antibody production (14) and also seem to have enhanced B-cell class switching in response to IL-4 (45). The autoimmune phenotype is most evident with increased age (more than 16 wk) but some mice as young as 8 wk show early markers of disease, such as production of autoantibodies (54). It should be noted that these mice also display myelomonocytic infiltration of the spleen leading to splenomegaly, as well as the appearance of monocytic tumors on some of the mice (42). Mice in which the Fyn gene has been deleted to ablate both alternatively spliced isoforms of Fyn (FynB and the hematopoietic specific FynT) show a pronounced neurological defect in learning and memory (55). This is a likely consequence of the role of Fyn kinase in N-methyl-D-aspartate (NMDA) receptor function and in oligodendroglial development and myelin-sheath formation (56–58). In the immune system, the loss of the FynT isoform results in the selective loss of NK1.1+ T cells

(59), impaired but not completely defective TCR-induced caspase activation and apoptosis (60), and in subtle changes in thymocyte development that affect their sensitivity to low-affinity ligands (61). No obvious defects are observed in B-cell signaling and proliferation (62). Thus in the immune system FynT-deficiency does not result in an overt phenotype, and because its loss results in a few selected impairments, Fyn may function more narrowly than Lyn or Lck in promoting immune cell signals.

The contributory role of Lyn and Fyn in the allergic response was investigated *in vivo* by a passive systemic anaphylaxis challenge. Young mice (4 wk) were sensitized with DNP-specific IgE and subsequently challenged with the antigen (DNP-HSA). Lyn-deficient mice showed a hyperresponsive phenotype when compared to wild-type mice, as determined by measurement of circulating histamine (32). In contrast, Fyn-deficient mice were resistant to anaphylaxis because the circulating histamine, upon challenge, was reduced by an average of 82% when compared to wild-type mice. This *in vivo* challenge of mast cells strongly supports the aforementioned cell studies describing a negative or positive role for Lyn and Fyn, respectively, in mast-cell degranulation. Interestingly, our finding of an anaphylactic response in Lyn-deficient mice contradicted a previously published report where these mice were found to be resistant to a passive cutaneous anaphylactic challenge (14). This led us to investigate the anaphylactic response of these mice at various ages and we determined that increasing age caused increased resistance to anaphylaxis (32). Because increased sensitivity to IL-4-mediated immunoglobulin class switching had been described, we tested the IgE levels of young (<4 wk) and older mice (>7 wk). Young mice showed normal

levels of IgE but older mice showed as much as a 10-fold increase in circulating IgE. Peritoneal mast cells from older *Lyn*^{-/-} mice had higher levels of FcεRI expression and their receptors were occupied with IgE (32). These findings may explain the apparent discrepancy in the anaphylaxis data on older mice. Because the mast cells of older mice are completely saturated with IgE, passive sensitization with a DNP-specific IgE could not occur and thus these cells would not respond to the DNP challenge. In contrast to *Lyn*^{-/-} mice, *Fyn*^{-/-} mice had normal serum IgE levels regardless of age and, although degranulation was impaired, the binding of DNP-specific IgE to their mast cells was identical to wild-type mice.

Additional evidence of a role for Lyn in negative regulation of the allergic response in vivo was provided by finding that the levels of circulating histamine in unchallenged *Lyn*^{-/-} mice was at least twofold higher than wild-type mice (32). Moreover, analysis of mast-cell numbers in the skin and peritoneum revealed a twofold increase in mast-cell numbers, and in the peritoneum a striking eosinophilia was observed (32). In contrast, *Fyn*^{-/-} mice have normal mast-cell numbers in both the skin and peritoneum, and prior to challenge circulating histamine levels are identical to unchallenged wild-type mice. Nonetheless, once challenged, *Fyn*^{-/-} mice have much lower levels of circulating histamine than wild-type mice. This would argue that *Fyn*^{-/-} mice may be more resistant to allergic disease. However, mice carrying the selective deletion of FynT were described to have an exacerbated pulmonary inflammation in an allergic mouse model (63). Whether this enhanced inflammatory response is mast cell-dependent is not clear. However, the apparent dichotomy of positive regulation of mast-cell degranulation, and negative regulation of pul-

monary inflammation, by Fyn might be explained by opposite regulatory roles for Fyn in degranulation vs cytokine/ eicosanoid production. This intriguing possibility merits further investigation.

An obvious puzzle in the observation of an allergic-like phenotype in *Lyn*^{-/-} mice is the underlying cause. Although it is possible that mice housed in a clean facility may develop allergies to environmental allergens, the likelihood that most (if not all) *Lyn*^{-/-} mice would become allergic is rather small. Nonetheless, we observed the allergic-like phenotype in all of the mice tested. This suggests that the underlying cause is more likely a defect in common to all *Lyn*^{-/-} mice because of the deletion of this gene. Our study supports two distinct defects as central to the allergic phenotype. First, as mentioned previously, mast cells from these mice are hyperresponsive. Thus, the required stimulus (antigen) for full activation is about 10-fold less than that required for wild-type mast cells (16,32). The second key change is the dramatic increase in circulating IgE levels in *Lyn*^{-/-} mice. This is important because mast cells from these mice have increased FcεRI expression, which increases the sensitivity of a mast cell to an allergen challenge (64,65). Moreover, our data showed a tight link between Lyn and control of IgE production as restoration of both Lyn alleles reverses the high IgE phenotype (32). This brings into play the role of monomeric IgE in mast-cell responses (66). Of particular relevance is the observation that very high levels of monomeric IgE can induce mast-cell degranulation through aggregation of FcεRI by a mechanism that is undefined. Interestingly, the concentration of circulating IgE (mg/mL) in *Lyn*^{-/-} mice easily surpasses the concentration of monomeric IgE (μg/mL) required for in vitro induction of

mast-cell degranulation by certain monoclonal IgEs (66). It is notable that like *Lyn*^{-/-} mice, *SHIP-1*^{-/-} mice also show a hyperresponsive phenotype that makes them highly susceptible to mast-cell degranulation by monomeric IgE (67). This is notable because our preliminary data showed that *Lyn*-deficient mast cells have an apparent defect in SHIP-1 activation (YF and JR, unpublished observation). Therefore, it is possible that the high circulating IgE levels combined with the hyperresponsive phenotype are the critical driving force in the allergic-like phenotype of *Lyn*^{-/-} mice. This possibility is currently favored as the most plausible explanation for the high (all mice) occurrence of this phenotype in *Lyn*^{-/-} mice. Moreover, it begs the question of whether atopy and allergy might be linked in some populations through *Lyn* polymorphisms, which might increase circulating IgE levels and reduce the threshold for mast-cell degranulation by reducing Lyn enzymatic activity.

Closing Remarks

The summarized work provides a new and still evolving paradigm on the functional coupling of FcεRI to mast-cell responses and the role of Src PTKs in promoting and controlling the allergic response. Principally, we have established the requirement of two proximal Src PTKs (*Lyn* and *Fyn*) for functional coupling of FcεRI. It is still unclear as to how *Fyn* kinase is activated by FcεRI, but evidence for its role in promoting mast-cell degranulation is increasingly convincing. On the other hand, the cumulative evidence on *Lyn* kinase shows a principal role in controlling the extent of B-cell IgE production and mast-cell degranulation. Importantly, the evidence suggests that the negative regulatory role of *Lyn* is essential, whereas its role in promoting FcεRI phosphorylation and mast-cell activation is

dispensable, because both mast-cell degranulation and cytokine production can occur in the absence of *Lyn*. This implies molecular redundancy in early steps likely provided by other Src PTKs, because treatment of *Lyn*-deficient mast cells with Src-selective inhibitors ablates mast-cell degranulation (SO and JR, unpublished observation). Although *Fyn* kinase is the positive modulator of mast-cell degranulation (16), we know less of the role for *Fyn* in lymphokine production. Preliminary evidence suggests that *Fyn* may also be a positive regulator of selected lymphokine gene expression (GG and JR, unpublished observation). Given the aforementioned negative regulatory control by *Lyn* on *Fyn*, it is important to note that the loss of *Lyn*'s ability to downregulate *Fyn* activity, in *Lyn*^{-/-} mice, is not the cause of increased B-cell IgE production because *Lyn/Fyn*^{-/-} double-deficient mice still have high IgE levels. Thus, how *Lyn* functions to regulate IgE production remains to be determined.

The new findings force us to rethink the strategy of targeting *Lyn* kinase to therapeutically intervene in IgE-dependent allergies (11). As we explore in more detail the IgE-dependent activation of *Fyn* kinase in mast cells, its interactions with FcεRI and other proteins, and its cellular substrates, we are likely to appreciate whether suitable therapeutic targets exist in this signaling pathway. Of current importance, however, is that the findings described herein will hopefully provoke new thought and promote new areas of investigation into the allergic response.

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