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Lyn but Not Fyn Kinase Controls IgG-Mediated Systemic Anaphylaxis

Yves T. Falanga,* Natalia S. Chaimowitz,† Nicolas Charles,‡ Fred D. Finkelman,¶,* Nicholas A. Pullen,* Suzanne Barbour,‡ Kevin Dholaria,* Travis Faber,* Motunrayo Kolawole,* Bernice Huang,† Sandra Odom,§ Juan Rivera,§ Jason Carlyon,‡ Daniel H. Conrad,† Sarah Spiegel,** Carole A. Oskeritzian,** and John J. Ryan*  

Anaphylaxis is a rapid, life-threatening hypersensitivity reaction. Until recently, it was mainly attributed to histamine released by mast cells activated by allergen crosslinking (XL) of FcεRI-bound allergen-specific IgE. However, recent reports established that anaphylaxis could also be triggered by basophil, macrophage, and neutrophil secretion of platelet-activating factor subsequent to FcyR stimulation by IgG/Ag complexes. We have investigated the contribution of Fyn and Lyn tyrosine kinases to FcyRIIB and FcγRIII signaling in the context of IgG-mediated passive systemic anaphylaxis (PSA). We found that mast cell IgG XL induced Lyn, Lyn, Akt, Erk, p38, and JNK phosphorylation. Additionally, IgG XL of mast cells, basophils, and macrophages resulted in Fyn- and Lyn-regulated mediator release in vitro. FcγR-mediated activation was enhanced in Lyn-deficient (knockout [KO]) cells, but decreased in Fyn KO cells, compared with wild-type cells. More importantly, Lyn KO mice displayed significantly exacerbated PSA features whereas no change was observed for Fyn KO mice, compared with wild-type littermates. Intriguingly, we establish that mast cells account for most serum histamine in IgG-induced PSA. Taken together, our findings establish pivotal roles for Fyn and Lyn in the regulation of PSA and highlight their unsuspected functions in IgG-mediated pathologies. The Journal of Immunology, 2012, 188: 000–000.

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Address correspondence and reprint requests to Prof. John J. Ryan, Department of Biology, Virginia Commonwealth University, 1000 W. Cary Street, Richmond, VA 23284. E-mail address: jjryan@vcu.edu

Abbreviations used in this article: BMMMC, bone marrow–derived mast cell; KO, knockout; LTC4, leukotriene C4; PAF, platelet-activating factor; PAFAH, platelet-activating factor acetylhydrolase; PSA, passive systemic anaphylaxis; pY, tyrosine-phosphorylated; SLE, systemic lupus erythematosus; WT, wild-type; XL, crosslinking.

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kinase in IgG signaling has been demonstrated (29, 30), little is known about Src family kinase functions. In this study we investigated the importance of the Lyn and Fyn kinases in FcγR signaling using mast cells, basophils, and macrophages. Our findings show that Fyn, Lyn, Akt, Erk, p38, and JNK are activated upon FcγR stimulation, and that Fyn and Lyn regulate FcγR-mediated degranulation and cytokine and chemokine release not only in mast cells, but also in basophils and macrophages. Furthermore, we demonstrate that mast cells account for the total amount of circulating histamine during FcγR-induced passive systemic anaphylaxis (PSA), which is regulated by Fyn and Lyn. Moreover, we show that Lyn kinase, but not Fyn kinase, is a major regulator of IgG-mediated PSA. These results bring new insights to the function of Fyn and Lyn kinases downstream of FcγR stimulation.

Materials and Methods

Animals

C57BL/6 × 129sv wild-type (WT), C57BL/6 × 129sv Fyn-deficient (KO), 129sv WT, and 129sv Lyn KO inbred strains were described previously (21, 26). Mast cell-deficient Wsh−/− and their C57BL/6 control mice were purchased from The Jackson Laboratory. All mice were used at a minimum of 9 wk age, and all experiments received approval from the Virginia Commonwealth University Institutional Animal Care and Use Committee.

Cytokines and reagents

Cytokines and ELISA assay kits were purchased from PeproTech (Rocky Hill, NJ). The Bio-Plex Pro cytokine assay kits were purchased from Bio-Rad (Hercules, CA). Histamine and leukotriene C4 (LTC4) ELISA kits were purchased from Cayman Chemicals (Ann Arbor, MI). Cytokine measurements were performed as per the manufacturer’s directions. Abs specific for tyrosine-phosphorylated (pY) Lyn and Lyn total were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Abs specific for the tyrosine-phosphorylated form of the Src kinase activation loop (pY416) and for Fyn were purchased from Cell Signaling Technologies (Danvers, MA). Anti-mouse FcεRIα was purchased from eBioscience (San Diego, CA). Rat anti-mouse FcγRIIIA (2.4G2), purified mouse IgE, rat anti-mouse IgE, rat IgG isotype control, and rat IgG anti-c-Kit (CD117) were purchased from BD Pharmingen (San Diego, CA).

Cells

Mouse bone marrow-derived mast cells (BMMCs) were derived by culture in complete RPMI 1640 medium (Invitrogen Life Technologies, Carlsbad, CA) containing 10% heat-inactivated FBS, 2 mM l-glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin, 1 mM sodium pyruvate, and 1 mM HEPES (all from Biofluids, Rockville, MD), supplemented with IL-3-containing supernatant from WEHI-3 cells and stem cell factor-containing supernatant from BHK-MKL cells. The final concentration of IL-3 and stem cell factor was adjusted to 1 and 10 ng/ml, respectively, as measured by ELISA. Mouse bone marrow-derived basophils were derived by culturing bone marrow cells for 6 d in complete RPMI 1640 medium supplemented with 5 ng/ml H-FCS (PeproTech, Rocky Hill, NJ). At day 6, they were analyzed and sorted by flow cytometry on the basis of FcεRI-positive and Kit-negative characteristics. Mouse bone marrow-derived macrophages were derived by culturing bone marrow cells for 8 d in complete RPMI supplemented with 50 ng/ml macrophage CSF (PeproTech).

Flow cytometry

Mice were bled via tail vein nick. Cells were labeled following RBC lysis and filtration through 40-μm cell strainers. Abs included unlabeled 2.4G2, FITC-conjugated anti-mouse c-Kit (2B8) and Gr-1 (RB6-8C5); Alexa 647-conjugated anti-mouse CD49b (DX5) and B220 (RA3-6B2); PE-Cy7–conjugated anti-mouse FcεRI (MAR-1) and Gr-1 (RB6-8C5); Alex 488-conjugated anti-mouse CD49b (DX5) and B220 (RA3-6B2); PE-Cy7–conjugated anti-mouse CD3 (145-2C11) and CD11b (M1/70) from BioLegend; and Alexa 647–conjugated anti-FcγRII (2.4G2), purified mouse IgE, rat anti-mouse IgE, rat IgG isotype control, and rat IgG anti–c-Kit (CD117) were purchased from BD Pharmingen (San Diego, CA).

Western blot

Cells were dissociated in immunomunoprecipitation assay buffer and Western blotting was performed using 50 μg total cell lysate per sample. Proteins were loaded and separated over an 8–16 or 4–20% gradient SDS-polyacrylamide gel (Bio-Rad). Proteins were transferred to nitrocellulose membranes ( Pall, Ann Arbor, MI) and blocked for 60 min in Blotto B buffer (Rockland Immunochemicals, Gilbertsville, PA) plus 0.1% Tween-20. Blots were incubated in a solution of TBS supplemented with 0.1% Tween-20 and 5% BSA (TBST), with the indicated Abs overnight at 4°C with gentle rocking. Blots were washed six times for 10 min each in TBST, followed by incubation in Blotto B containing a 1:5000 dilution of HRP-linked anti-IgG matched to the relevant species, from Cell Signaling Technology (Danvers, MA). Size estimates for proteins were obtained using molecular mass standards from Bio-Rad.

Passive systemic anaphylaxis

Mice were injected i.v. with 200 μl PBS containing 5 mg histamine, 700 ng platelet-activating factor (PAF), or 500 μg rat anti-mouse CD16/CD32, clone 2.4G2 (13, 31). The body temperature of each animal was measured using a rectal microprobe (Physitemp Instruments). Mice were then euthanized, and blood was collected by cardiac puncture to prepare serum.

PAF acetylhydrolase activity assays

Substrate (50 μM 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine with 0.05 μCi hexadecyl-2-acetyl-sn-glycero-3-phosphocholine, 1-O-[acyetyl-(N)-] [NEP/PerkinElmer; 13.5 Ci/mmol added as a tracer] was combined with serum and incubated for 30 min at 37°C to determine PAF acetylhydrolase (PAFAH) enzymatic activity. PAFAH activity was expressed as release of [14C]acetate, using a method that we described previously (32). The 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine (PAF) was purchased from Avanti Polar Lipids (Alabaster, AL).

Statistical analysis

Data are presented as the means ± SEM of at least three independent experiments. Comparisons were made by the two-tailed Student t test for independent samples. A p value of <0.05 was considered statistically significant. Analysis was performed with GraphPad Prism software.

Results

FcγR stimulation induces rapid Fyn, Lyn, Akt, Erk, p38, and JNK activation in BMMCs

To compare the effect of the IgE versus IgG receptor stimulation on the phosphorylation state of Fyn kinase, BMMCs were stimulated in vitro with IgE plus Ag or 2.4G2 plus anti-IgG (referred to as IgE or 2.4G2 crosslinking [XL], respectively). Fyn kinase was then immunoprecipitated and proteins were separated by SDS-PAGE. The phosphorylated form of Fyn was detected by immunoblotting for the activated loop of the Src kinase family on residue 416 (pY416) (Fig. 1A). Our results show that Fyn kinase (59 kDa) is rapidly activated downstream of both the IgE and the IgG receptors. Quantification of Western blot signal intensity by densitometry after adjusting for loading showed that FcγRI and FcεRI activate Fyn to a similar extent.

Similar to Fyn, we found that both Lyn kinase isoforms (53 and 56 kDa) (17) were also activated following 2.4G2 XL (Fig. 1B). FcγR-mediated Lyn activation was nearly as strong as IgE-mediated effects as attested by densitometry analysis of band intensity.

Intracellular signaling events occurring in BMMCs after FcεRI activation of mast cells has been intensively studied and is well established. Because our results showed that comparable activation levels of Fyn and Lyn can be achieved through FcγRI or FcεRI stimulation, we next decided to investigate the kinetics of phosphorylation events subsequent to Fyn and Lyn activation. We found that Fyn activation after 2.4G2 XL occurred rapidly, with maximal phosphorylation observed within 3 min (Fig. 1C). Additionally, FcγR activation of mast cells triggered the activation of Akt, Erk, p38, and JNK (Fig. 1D). A representative densitometry analysis of band intensity shows the phosphorylation kinetics of these signaling molecules (Fig. 1E), with ERK phosphorylation being the strongest, reaching ∼70-fold increase in less than a minute after the initiation of 2.4G2 XL. Our results showed parallel kinetics for Fyn, Akt, and Erk during the early time points...
of FcγR activation of mast cells, with only Lyn displaying sustained phosphorylation during ~30 min. Collectively, our results show that comparable Fyn and Lyn activation can be achieved through FcγR-mediated signals activated similar downstream targets to those previously shown for FcεRI signaling (26, 33–39), especially the robust and early Akt and ERK signaling.

**Effects of Fyn or Lyn deficiency downstream of mast cell IgG receptor signaling**

Fyn and Lyn kinases act as opposite regulators of IgE-induced mast cell activation (21), (26, 27). We therefore investigated their roles in IgG-mediated BMMC activation. To fairly compare how downstream signaling molecules are affected by Fyn or Lyn deficiency, we depleted these kinases with specific small interfering RNA on C57BL/6 mast cells, given that Fyn KO and Lyn KO were generated on different backgrounds (B6.129sv and 129sv, respectively). Fyn KO BMMCs displayed increased phospho-JNK whereas no change was observed for phospho-Akt and phospho-p38 subsequent to IgG XL (Fig. 2). Alternatively, Lyn-deficient BMMCs displayed significantly high phospho-Akt whereas there was no change in JNK or p38 phosphorylation status in comparison with WT BMMCs. Complementing the data in Fig. 1, these data demonstrate that Fyn and Lyn regulate particular signaling pathways upon IgG XL in mast cells by targeting different downstream signaling molecules.

_Fyn kinase deficiency diminishes FcγR-mediated activation of mast cells, basophils, and macrophages_

To determine the functional importance of Fyn kinase downstream of FcγR signaling, BMMCs derived from WT or Fyn KO bone marrow progenitors were stimulated by 2.4G2 XL, and cell culture supernatants were collected to measure degranulation and cytokine release. Fyn deficiency significantly decreased histamine release (Fig. 3A) and profoundly reduced IL-6 (Fig. 3B) and IL-13 (Fig. 3C) levels. There were no striking differences in LTC4, MIP-1α, or TNF-α secretion (data not shown). We used the calcium ionophore ionomycin as a positive control for all of our cytokine and mediator release measurements in this study and observed that the deficiency in either Fyn or Lyn did not significantly impact the amount of mediators secreted (data not shown). These data mirror previous reports of the importance for Fyn kinase in IgE-mediated stimulation (21, 26), expanding its significance to FcγR stimulation.

Basophils and macrophages share FcγRIIb/III expression with mast cells and are also involved in innate immunity. In macro-
phages, FcγRs regulate a variety of functions, such as macrophage activation or inhibition as well as opsonization of Ab-neutralized pathogens (40–44). Recent studies have identified the pivotal importance of basophils and macrophages in IgG-related allergic and anaphylactic reactions (13, 45–51). To determine the functional relevance of Fyn kinase downstream of FcγR signaling in basophils and macrophages, these cells were derived from WT and Fyn KO bone marrow as described in Materials and Methods. Interestingly, after 2.4G2 XL, we observed that Fyn KO basophils and macrophages displayed a significant decrease in secreted cytokines and chemokines in comparison with the WT cultures (Figs. 3D–H). These data demonstrate that Fyn kinase is required for optimal FcγR-mediated activation of basophils and macrophages in addition to mast cells.

Lyn kinase deficiency enhances FcγR-mediated activation of mast cells, basophils, and macrophages

To determine the role of Lyn in FcγR signaling, WT and Lyn KO mast cells were stimulated by 2.4G2 XL. We found that Lyn KO BMMCs displayed a significant increase in the secretion of early phase mediators such as LTC4 (Fig. 4A). Additionally, the amount of IL-13 and MIP-1α released was significantly increased in Lyn KO BMMCs compared with the control cells (Fig. 4B, 4C). However, we did not observe a significant increase in histamine and other cytokines, including TNF-α (data not shown). These results support the idea that Lyn kinase selectively regulates the amount of mediators released upon FcγR stimulation in BMMCs. Similar results were observed in macrophages (Fig. 4D, 4E) and basophils (Fig. 4F–H), where Lyn deficiency also selectively enhanced FcγR-mediated cytokine/chemokine release. Taken together, our findings support that Fyn and Lyn kinases are key opposing regulators of FcγR-dependent mast cell, basophil, and macrophage activation.

Regulatory functions of Fyn and Lyn kinases during FcγR-induced passive systemic anaphylaxis

The importance of Fyn and Lyn in FcγR signaling prompted us to investigate their regulatory roles in vivo, in the context of PSA. We performed an IgG-mediated PSA assay using i.v. injected 2.4G2 (31). Recent studies have reported that mast cells are not required for IgG-mediated PSA, and identified basophils, macrophages, and neutrophils as the key players (11, 13, 14). Both Fyn KO age- and gender-matched littermate mice developed anaphylaxis as assessed by decreased core body temperature, followed by a recovery period of ∼2 h. Unlike in vitro FcγR stimulation (Fig. 3), Fyn deficiency did not convey protection from anaphylaxis severity (Fig. 5A).

In contrast, 2.4G2-induced PSA was exacerbated in Lyn KO mice compared with their WT littermates (Fig. 5B), correlating with our in vitro observations with mast cells, basophils, and macrophages (observed in Fig. 4). Despite a dramatic (11˚C) drop
in body temperature, none of the Lyn KO mice died in this experiment. Also, we did not observe any changes in circulating cytokines (Bio-Plex for GM-CSF, IL-1β, IL-4, IL-5, IL-6, IL-10, IL-13, IL-17a, MCP-1, MIP-1α, MIP-1β, and TNF) in the sera of mice 2 h after 2.4G2-induced PSA (data not shown). In general, 129Sv mice (Lyn WT and KO) displayed a lower nadir than did their Lyn WT and KO counterparts. This could be due to increased blood basophils and neutrophils on this genetic background (Fig. 5C–E). We found that Lyn or Lyn deficiency did not affect circulating B cell, T cell, or eosinophil numbers (data not shown).

Histamine and PAF are very potent mediators causing bronchoconstriction, vascular leak, and vasodilation, three features observed during human and mouse systemic anaphylaxis (13, 49, 52, 53). Due to a paucity of sensitive assays for measuring PAF, and given that both PAF and histamine cause anaphylaxis, we next investigated the effects of 2.4G2-induced PSA on circulating histamine levels in the context of Lyn or Lyn deficiency. Our results showed that the serum of Lyn KO mice contained significantly lower histamine levels than did their WT counterparts (Fig. 6A). In contrast, Lyn KO mice displayed elevated serum histamine compared with their littermates, in agreement with their exacerbated PSA phenotype (Fig. 6B).

To assess the impact of Lyn and Lyn kinase deficiency in the vascular sensitivity to histamine, we i.v. injected 5 mg histamine into Lyn KO, Lyn KO, and their WT counterparts (Fig. 6C). Whereas Lyn KO mice exhibited anaphylactic responses mirroring their littermates, Lyn KO mice were more responsive than controls when given the same amount of histamine, suggesting that Lyn deficiency might also affect the vasculature in addition to regulating the amount of released histamine.

**Mast cells account for the amount of serum histamine released in IgG-induced PSA**

Mast cells and basophils degranulate and release histamine once activated through their FcεR. Our results show that the amount of histamine released in the serum during IgG-induced PSA is regulated in a Fyn- and Lyn-dependent manner (Fig. 6A, 6B). However, the cell population accounting for the amount of released histamine remained unclear. To address this question, we induced PSA by 2.4G2 i.v. injection into WT or mast cell-deficient mice (Wsh−/−) mice and monitored the drop in core body temperature. As seen in Fig. 7A, Wsh−/− mice developed PSA to a similar extent as did their WT controls (C57BL/6). Not surprisingly, mast cell-deficient mice had little serum histamine 2 h after the induction of PSA in comparison with their controls (Fig. 7B). Our data demonstrate that mast cells account for most histamine released during IgG-induced PSA.

**PAF injection recapitulates IgG-mediated PSA in Lyn KO mice while the deficiency in Lyn or Fyn does not affect PAFAH**

PAF is known to be the main mediator regulating IgG-induced PSA (11–14). To assess its importance in our model, we i.v. injected PAF (700 ng) into Lyn KO, Lyn KO, and matched WT mice (Fig. 8A). PAF administration recapitulated IgG-mediated PSA kinetics in Lyn and Lyn KO (Fig. 8A, 4B). Furthermore, we observed that Lyn deficiency not only worsened anaphylaxis severity (Figs. 5B, 6C, 8A) but also lengthened the recovery time to ∼5 h, instead of the 2 h observed with littermate controls (data not shown). These findings could be explained by increased leakage from endothelial cells, or altered decay rates for these anaphylactic mediators. Because PAF is the key factor in IgG-mediated anaphylaxis, we assessed serum activity levels of its inactivating enzyme, PAFAH. As shown in Fig. 8B, steady-state PAFAH serum enzymatic activity was unaffected by Lyn or Fyn deficiency. Collectively, these data suggest that Lyn deficiency exacerbates responses to both histamine and PAF. Paired with enhanced macrophage and basophil responsiveness to IgG, the absence of Lyn kinase appears to exaggerate the severity and recovery time of anaphylaxis.

**Discussion**

Recently, the Src family kinases Lyn and Fyn have been found to exert opposing effects on IgE-mediated mast cell activation (21, 26, 54), but little is known about the role of these enzymes in IgG signals. Our findings show that FcεR triggers Fyn and Lyn kinase activation similar to FceRI crosslinkage in mast cells (Fig. 1), which might be explained by the fact that these two receptor families share the common γ-chain (55). Furthermore, Lyn and Lyn appear important for the regulation of IgG-induced degranulation and cytokine production, suggesting an important role in
mast cell functions. In this study, we show that Lyn deficiency significantly increases Akt phosphorylation subsequent to mast cell IgG receptor stimulation. These data mirror previous observations of FcεRI-mediated mast cell activation from Kitaura et al. (33). In that report, IgE XL of Lyn KO mast cells induced increased Akt phosphorylation, correlating with enhanced NF-κB, NF-AT, and AP-1 transcriptional activation and subsequently elevated IL-2 and TNF production. Taken together, these data demonstrate that Lyn and Fyn kinase play important and similar roles in FcεRI and FcγRIII activation of mast cells. The fact that Fyn and Lyn deficiency similarly altered FcγR signaling in basophils and macrophages (Figs. 3, 4) supports the importance of these antagonistically paired Src family kinases in IgG-mediated immune responses.

The onset and regulation of anaphylaxis have been exclusively attributed to mast cells, delineating the generally known classical pathway of anaphylaxis involving IgE-mediated FcεRI mast cell activation and subsequent histamine release (1). In addition to this well-characterized pathway, recent reports in the literature demonstrate that in a murine model, the anaphylactic reaction can be triggered by an IgG/FcγR pathway. Basophils, macrophages, and recently neutrophils (14), but not mast cells, have been identified as crucial players in this “alternative pathway of anaphylaxis,” because mast cell-deficient mice demonstrated IgG-induced anaphylaxis. In contrast, in vivo depletion of basophils with the mAb Ba103 (12, 13) or inhibiting macrophage function via gadolinium injection (11) markedly decreased the severity of FcγRIII-

![FIGURE 5.](image1.png) Lyn but not Fyn kinase deficiency is pivotal to IgG-induced passive systemic anaphylaxis. (A and B) PSA was induced with 500 μg 2.4G2 injected i.v. Changes in the core body temperature were measured by rectal probe (Fyn WT [PBS, n = 4; 2.4G2, n = 6], Fyn KO [PBS, n = 3; 2.4G2, n = 5], Lyn WT [PBS, n = 3; 2.4G2, n = 3], Lyn KO mice [PBS, n = 3; 2.4G2, n = 4]). Data shown represent the means ± SE. (C) Blood basophils (IgE+CD49b+), (D) monocytes (CD11b+Gr1−), and (E) neutrophils (CD11b+Gr1−) counts for Fyn (WT, n = 8; KO, n = 8) and Lyn (WT, n = 8; KO, n = 8) mice. Data shown represent the means ± SEM. *p < 0.05 based on Student t test WT to Lyn KO cells.

![FIGURE 6.](image2.png) Fyn and Lyn kinases regulate the amount of serum histamine during IgG-induced PSA. (A and B) Circulating histamine levels 2 h after PSA induction (Fyn WT [PBS, n = 4; 2.4G2, n = 6], Fyn KO [PBS, n = 3; 2.4G2, n = 5], Lyn WT [PBS, n = 3; 2.4G2, n = 3], Lyn KO mice [PBS, n = 3; 2.4G2, n = 4]). Data shown represent the means ± SE. (C) Fyn KO (n = 4) and Lyn KO (n = 4) mice or control littermates (Fyn, n = 4; Lyn, n = 4) were injected i.v. with 5 mg histamine and core body temperature was measured by rectal microprobe. Data shown represent the means ± SEM. ***p < 0.0001 based on Student t test WT to Lyn KO cells.

![FIGURE 7.](image3.png) Mast cells account for most serum histamine during IgG-induced PSA. (A) PSA was induced in mast cell-deficient mice (Wsh−/−, n = 4) as well as their WT controls (C57BL6, n = 5) by i.v. injection of 500 μg 2.4G2. The core body temperature was monitored by rectal microprobe. Data shown are the means ± SEM. Two hours after the induction of PSA in (A), blood was collected from mice, serum was prepared, and the amount of circulating histamine was assessed by ELISA (B). Data shown are the means ± SE. ***p < 0.0001 based on Student t test WT to Lyn KO cells.
mediated anaphylaxis. A recent report further showed that FcγRIV-neutrophil–induced anaphylaxis also occurs, as mice deficient in FcγRI/FcγRIIB/FcγRIIA/FcεRI/FcεRII \( ^{-/-} \) (5KO mice) still developed IgG-mediated anaphylaxis (14). In this alternative form of anaphylaxis, PAF, rather than histamine, has been indicated as a pivotal mediator. Although PAF serum levels are quite challenging to measure, the in vivo administration of a PAF antagonist protected mice from developing IgG- but not IgE-mediated anaphylaxis (13). Thus, it is thought that IgG-induced anaphylaxis operates through FcγR-mediated activation of basophils, neutrophils, and macrophages, with subsequent PAF release triggering vasodilation, vascular fluid leak, and loss of core body temperature (11, 13, 14, 48).

Our data support and extend the understanding of IgG-mediated anaphylaxis at the molecular signaling level. First, we found that Fyn or Lyn deletion has opposing effects on histamine release and cytokine secretion during IgG activation of mast cells, basophils, and macrophages in vitro. Interestingly, although the effects on histamine release were consistent in vivo, they did not predict the severity of FcγR-induced PSA. Whereas Lyn KO mice showed increased histamine and worsened hypothermia, Fyn KO mice had diminished histamine but no change in hypothermia versus respective WT mice. Additionally, although histamine and PAF are responsible for anaphylaxis, we found that mast cells were mainly responsible for the amount of circulating histamine present in the serum during the course of FcγR-induced PSA (Fig. 7), extending the involvement of mast cells in IgG-related pathologies. Interestingly, a recent study found histamine produced by non-mast cell sources in a model of contact hypersensitivity (56), suggesting that in vivo histamine production varies with the eliciting stimulus. Based on previous reports (11–14), the drop in body temperature observed in mast cell-deficient animals over the course of anaphylaxis should be attributed to PAF released by basophils, macrophages, and neutrophils after FcγRIII activation. Furthermore, we did not observe any significant difference in perivascular cell infiltration and pulmonary edema between Fyn KO mice, Lyn KO mice, and their controls subsequent to 2.4G2-induced PSA (data not shown).

The exacerbated response of Lyn KO mice could be ascribed to many factors: increased PAF secretion, decreased PAF metabolism, or enhanced PAF signaling in vascular endothelium. We found no changes in PAFAH activity, suggesting that reduced PAF catabolism is not an explanation. Furthermore, we found no overt changes in circulating basophils, monocytes, or neutrophils in Lyn KO mice. It is also possible that Lyn KO endothelial cells, just like mast cells, basophils, and macrophages, are hyperresponsive to any stimuli involving Lyn kinase. In fact, Kuruvilla et al. (57) have demonstrated that Fyn and Lyn are phosphorylated upon PAF receptor activation, leading to phosphorylation of the p85 regulatory subunit of PI3K. Additionally, Yu et al. (58) showed that phosphotyrosine activated Lyn kinase colocalized with PI3K in the lipid body fraction of PMN leukocytes subsequent to PAF receptor stimulation. Taken together, and in line with our findings, we therefore speculate that Lyn KO endothelial cells are hyperresponsive to PAF stimulation, triggering a longer and more severe vasodilation episode in comparison with Lyn-sufficient cells.

Based on our data, we can hypothesize that patients with altered Lyn function or expression could be hyperresponsive to IgE- and IgG-mediated pathology. There is precedence for this, as most systemic lupus erythematosus (SLE) patients have been shown to have reduced Lyn expression in two clinical studies (59, 60). Although SLE is regarded as an IgG immune complex-mediated disease, Charles et al. (61) recently showed that basophils activated by IgE may participate in the onset or the exacerbation of lupus-like symptoms in a mouse model. In the same report, this group demonstrated that aged Lyn KO mice displayed increased basophil numbers in the lymph nodes, blood, and spleen. Furthermore, Lyn KO mice display significantly elevated circulating autoantibodies, which, in conjunction with many other factors, lead to the development of SLE-like symptoms and contribute to the elevated mortality in this population (62–64). Combined with our data, Lyn deficiency is postulated to worsen either IgG- or IgE-mediated inflammation, which could contribute to the development of SLE in aged mice.

Several studies have reported the presence of allergen-specific IgG in allergic individuals. The involvement of this Ig isotype in the onset and development of the allergic reaction remains poorly understood (65, 66). However, Bandukwala et al. (67) used a murine model to demonstrate the importance of FcγRI and FcγRIII in airway inflammation and hyperresponsiveness. This study showed that C57BL/6 mice that were sensitized with non-infectious parasitic *Schistosoma mansoni* eggs and challenged with soluble egg Ag displayed airway inflammation, including eosinophil infiltration and severe peribronchial and perivascular inflammation. This response was greatly decreased in FcγRII \( ^{−/−} \) or FcγRIII \( ^{−/−} \) mice. This group also demonstrated that deficiency of FcγRIII, but not FcγRI, reduced lung resistance upon methacholine challenge, compared with littermate controls. Thus, in addition to the well-established role of IgE/FcεRI in the onset and the development of allergy and hypersensitivity, this study provides evidence that FcγRIII activation can participate in airway disease pathogenesis in a murine model. Combined with our data, these findings support the theory that the exacerbated 2.4G2-mediated PSA we noted in Lyn KO mice is elicited by FcγRIII, as previously supported by Daeron and colleagues (68) and by Ravetch and colleagues (69–71).

The high-affinity (FcγRI) and low-affinity (FcγRIII) IgG receptors are known to be important in the activation of numerous cell types of the immune system and in the phagocytosis of opsonized microbes. In contrast, one of the members of the FcγR family, FcγRIIb, has emerged as an inhibitory receptor (31, 72, 73). Using a mouse model of allergic asthma, Dharajiya et al. (74) demonstrated that in addition to upregulating the expression of FcγRIIb in CD14–MHC class II–mononuclear as well as in CD11b+ cells in the lungs, ragweed extract challenge also led to signs of severe allergic asthma-like symptoms in FcγRIIb-deficient mice, delineated by increased airway eosinophilia, mu-
cin production, and allergen-specific IgE. Furthermore, a tremendous increase in macrophage, eosinophil, and lymphocyte recruitment was observed in the bronchoalveolar lavage fluid of ragweed extract-challenged FcγRIIb KO mice in comparison with WT controls. These data, as well as many others, consolidate the idea that IgG and Fcγ receptors play important roles in the regulation of immune system homeostasis. Our data support the hypothesis that Fyn and Lyn kinases are pivotal antagonistic regulators of this paired IgG receptor system.

The data in this study demonstrate that Fyn and Lyn kinases are activated during IgG-mediated signaling and have opposing regulatory functions in mast cells, basophils, and macrophages. In addition to Fyn and Lyn activation, we found that FcεRII stimulation also led to the phosphorylation of Akt, Erk, p38, and JNK. Furthermore, we also uncover the unsuspected contribution of mast cells as major producers of serum histamine during IgG-induced PSA, regulated in a Fyn- and Lyn-dependent manner. More importantly, we show that overall Lyn but not Fyn kinase regulates the severity of IgG-induced passive systemic anaphylaxis, by enhancing the amount of vasoactive mediators secreted and exacerbating endothelial cell responsiveness to PAF and histamine.

In previous studies with previous findings, we extend the understanding of IgG-related pathologies and demonstrate the pivotal role of Lyn kinase as the key regulator of IgG-mediated inflammation.

Disclosures

The authors have no financial conflicts of interest.

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