

Syk deficiency in non-releaser basophils

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Abstract:

Background: Peripheral blood basophils from 10% to 20% of donors fail to degranulate in response to cross-linking the high-affinity IgE receptor FcεRI. The molecular mechanisms underlying the nonreleaser phenotype have not been established. **Objectives:** Our aim was to compare the expression of FcεRI-associated protein tyrosine kinases between nonreleaser and releaser basophils. **Methods:** With use of Western blotting we investigated Syk and Lyn protein levels in highly purified basophils from 3 anti-IgE nonreleasers and 2 releasers. **Results:** We identified 3 healthy nonatopic donors whose nonreleaser basophils express FcεRI normally but fail to express protein for the tyrosine kinase Syk, which is implicated in the initiation of FcεRI-mediated secretion. Protein levels for the tyrosine kinase Lyn are somewhat reduced but not absent in nonreleaser basophils. Levels of Lyn and Syk protein are similar in B cells, eosinophils, and neutrophils from releaser and nonreleaser donors. During these studies one nonreleaser “converted” into a releaser with concomitant basophil Syk expression. **Conclusion:** The absence of detectable Syk could explain the nonreleaser phenotype of basophils from some donors.

Keywords: Basophils | signal transduction | nonreleaser | tyrosine kinase | Lyn | Syk | human

Article:

Abbreviations

FcεRI: High-affinity receptor for IgE

fMet peptide: N-formyl-methionyl-leucyl-phenylalanine

PMA: Phorbol myristate acetate

Cross-linking the high-affinity IgE receptor (FcεRI) on mast cells and basophils initiates the release of mediators involved in allergic inflammation.¹ Details of the signaling pathway have been established primarily in the RBL-2H3 mast cell model and in mouse bone marrow-derived

mast cells. In these cells FcεRI cross-linking sequentially activates 2 protein tyrosine kinases, Lyn, which principally phosphorylates the immunoreceptor tyrosine-based activation motif—containing β and γ subunits of the FcεRI, and Syk, which binds the phosphorylated receptor and in turn recruits and phosphorylates additional kinases, adaptor proteins, and enzymes, including phospholipase Cγ isoforms. These early events in turn stimulate Ca⁺⁺ mobilization, guanosine triphosphatase activation, and the activation of multiple downstream enzymes, including protein kinase C isoforms and Erk and Jnk mitogen-activated protein kinase family members, resulting in secretion.²

We showed recently that cross-linking the FcεRI on human basophils activates the protein tyrosine kinases Lyn, Syk, and Zap70, and the resulting tyrosine phosphorylation of multiple cellular substrates is essential for FcεRI-mediated basophil degranulation.³ Others have reported the wide variability in the secretory response of human basophils to anti-IgE antibody and, in particular, have established that basophils from 10% to 20% of donors do not release histamine in response to FcεRI cross-linking.^{4,5}

Here we identify 3 donors with nonreleaser basophils. These basophils fail to express Syk and have normal to lower levels of Lyn. B cells, eosinophils, and neutrophils from these donors have normal Syk and Lyn levels. Understanding the basophil-specific regulation of Lyn and Syk expression could yield new therapies for allergic inflammation.

MATERIAL AND METHODS

Reagents

Antibodies to Lyn and Syk were from Santa Cruz Biotechnology, Santa Cruz, Calif. Affinity-purified goat antihuman IgE antibody was from Biosource, Camarillo, Calif. Antibody 29C6 to the FcεRI-α chain was a generous gift from Dr J. Kochan, Hoffman LaRoche. Calcium ionophore A23187, N-formyl-methionyl-leucyl-phenylalanine (fMet peptide), phorbol myristate acetate (PMA), Iscove's medium, antibodies specific for leukocyte subsets and erythrocytes (CD antibodies and antiglycophorin), FITC-CD16, and antiactin were from Sigma, St Louis, Mo.

Isolation of peripheral blood cells

Basophil-enriched cell populations were isolated by Percoll gradient centrifugation and either used directly (at 25% to 60% purity for degranulation assays) or further purified to >99% basophils by sequential negative and positive selection and flow sorting, as described elsewhere.^{3,6}

Isolation of other leukocytes

To isolate B cells, the monocyte-lymphocyte fraction obtained after Percoll-gradient centrifugation was washed twice with PBS–bovine serum albumin followed by sequential incubation for 30 minutes at 4°C with mouse antibodies to CD19 and CD22 and with FITC-goat antimouse IgG antibody. FITC-positive B cells were isolated with use of an EPICS Elite cell sorter. Eosinophils and neutrophils in the granulocyte fraction from these gradients were

separated by incubation with FITC-conjugated CD16 mAb and flow sorting into the CD16-positive neutrophils and the CD16-negative eosinophils. Wright's stain analysis confirmed that these preparations were >98% neutrophils or eosinophils.

Histamine release and measurement

Percoll-enriched basophils (1×10^5 cells per assay) were activated as described previously³ with the addition of anti-IgE (0.01-10 $\mu\text{g}/\text{mL}$), A23187 (500 ng/mL), fMet peptide (10^{-7} mol/L), or PMA (50 ng/mL). Total histamine content and fractional histamine release were measured with an RIA (ALPCO, Windham, NH).

Western blotting for Lyn and Syk protein

Highly purified basophils, B cells, eosinophils, and neutrophils were solubilized, and equal amounts of protein (10-25 $\mu\text{g}/\text{lane}$) or equal numbers of cell equivalents ($0.6\text{-}2.5 \times 10^5$ cells/lane) were separated by SDS-PAGE and transferred to nitrocellulose. Western blotting with 1 $\mu\text{g}/\text{mL}$ of antibodies to Lyn, Syk, and actin or with normal rabbit serum as a negative control (1:500 dilution) was performed as described previously.³ Membranes were developed with use of the ultrasensitive enhanced chemiluminescence detection reagents (ECL, Amersham).

RESULTS

In multiple assays of Fc ϵ RI-induced secretion basophils from 3 of 37 (8%) healthy blood donors consistently failed to degranulate in response to anti-IgE antibody. These donors, made up of a 46-year-old man, his 16-year-old son, and a 31-year-old male, were taking no medications and had no history of symptomatic allergy or asthma. The first two patients had positive skin tests to one or more of a panel of common allergens during the time of these studies, indicating that their mast cell IgE-mediated pathways were probably intact. The third patient had a negative skin test result.

Nonreleaser and releaser basophils contain similar amounts of histamine (Fig 1, *A*).

As previously reported,⁵ releaser basophils secrete optimally in response to between 0.1 and 1 $\mu\text{g}/\text{mL}$ concentrations of anti-IgE (Fig 1, *A*). IgE-primed nonreleaser basophils showed no degranulation over a range of cross-linker concentrations from 0.01 to 10 $\mu\text{g}/\text{mL}$ anti-IgE (Fig 1, *A*).

Although Fc ϵ RI-induced secretion was absent, nonreleaser basophils secreted normally when challenged with fMet peptide, which activates the G protein-coupled chemoattractant receptor, and with Ca^{++} ionophore and phorbol ester, which are thought to induce secretion in part by direct effects on cytoplasmic Ca^{++} levels and protein kinase C activity. These and similar results published previously^{4,5} indicate that the nonreleaser phenotype results from the failure of early events in the Fc ϵ RI signaling cascade. Consistent with the earlier reports, the expression of cell surface Fc ϵ RI, measured by flow cytometry in cells labeled with mouse anti-Fc ϵ RI mAb followed by FITC-anti-mouse IgG, was in the normal range (data not shown).

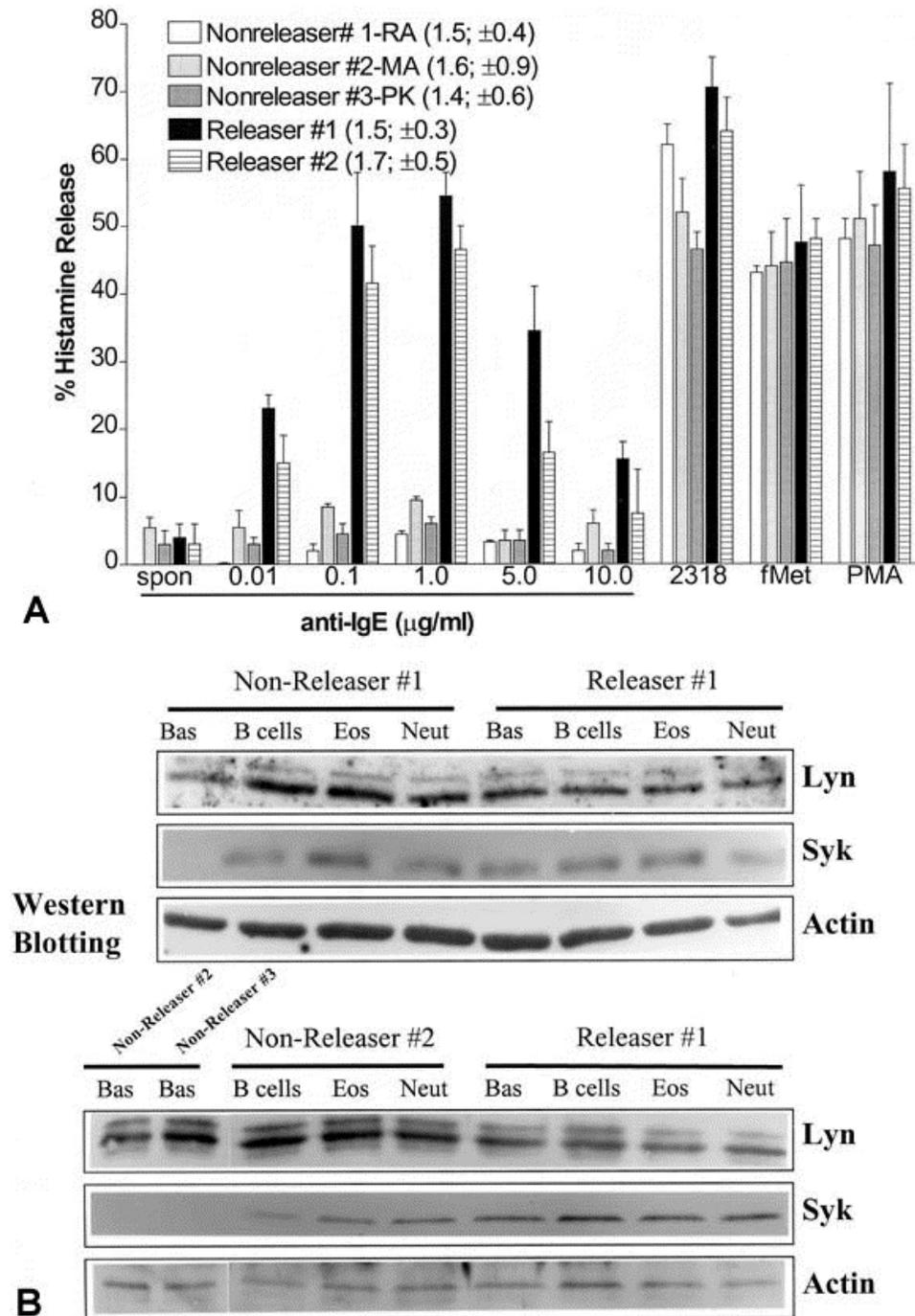


Fig. 1. A, Specific impairment of FcεRI-dependent degranulation in nonreleaser basophils. Percoll-enriched nonreleaser and releaser basophils were challenged with different concentrations of anti-IgE, other non-IgE-mediated stimuli (A23187, fMet, or PMA), or with no stimulus (*spon*) for 30 minutes and assayed for degranulation. Data are results of 3 separate experiments for each donor, each done in duplicate (\pm SEM, $n = 3$). Numbers in parentheses indicate total histamine per 10^6 basophils for each donor from 2 separate experiments (\pm SEM; $n = 2$) **B**, Nonreleaser basophils lack protein tyrosine kinase Syk. Lysates of highly purified (>99% pure) releaser and nonreleaser basophils (*Bas*), as well as B cells, eosinophils (*eos*), and neutrophils (*neut*) from same donors, were analyzed by Western blotting for Lyn, Syk, and actin protein expression. *Upper panel*, Equal amounts of protein (13 μ g/lane) were added to gel; *lower panel* (nonreleasers 2 and 3), equal numbers of cell equivalents were added to gel (2×10^5 cell equivalents/lane). Results are representative of 3 separate experiments for each donor.

FcεRI-mediated degranulation in human basophils involves the early activation of protein tyrosine kinases, particularly Lyn and Syk.³ We therefore compared levels of these 2 kinases between releaser and nonreleaser basophils. Kinase protein and messenger RNA levels were also analyzed in B cells, eosinophils, and neutrophils from the same donors.

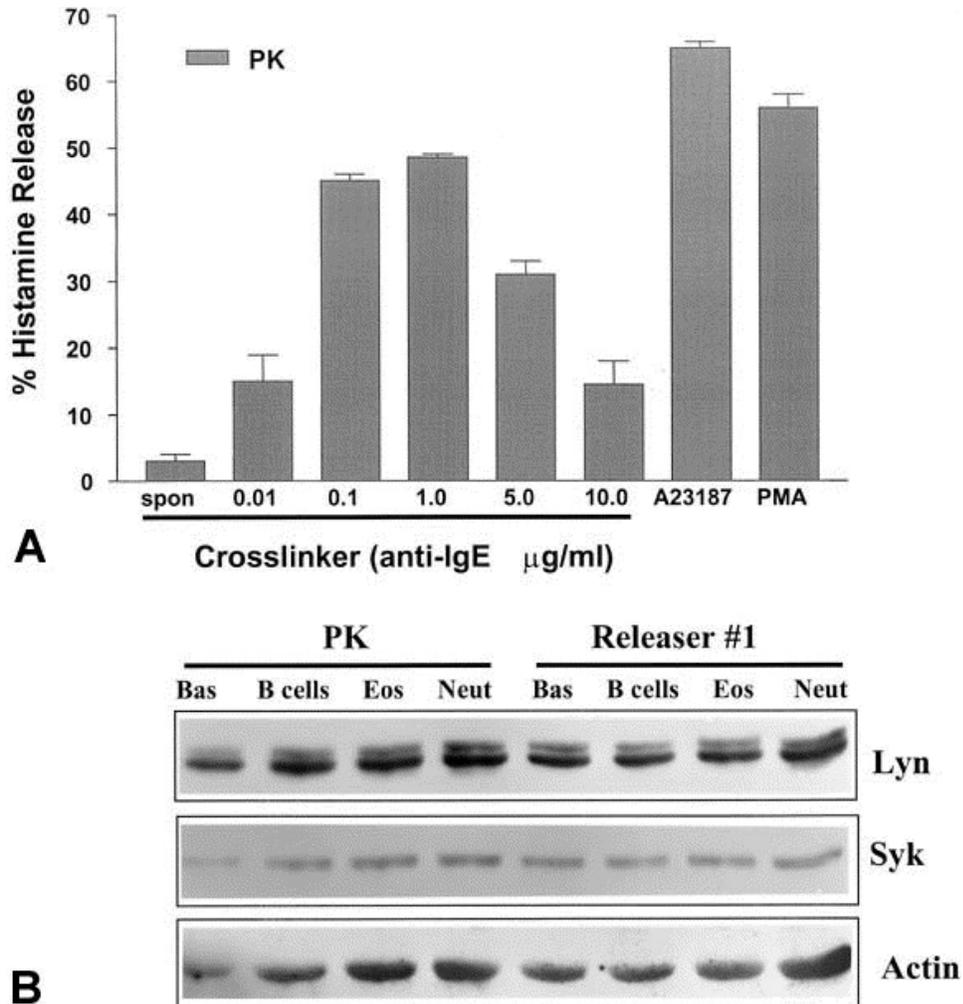


Fig. 2. A, Conversion of nonreleasing basophils to releasing basophils with concomitant Syk protein expression. Percoll-enriched basophils from nonreleaser 3 (tested approximately 1 month from the third of 3 experiments reported in Fig 1, *A*) were challenged with different concentrations of anti-IgE, other non-IgE-mediated stimuli (A23187 or PMA), or no stimulus (*spcn*) for 30 minutes and assayed for histamine release. Data are results of 2 separate experiments, each performed in duplicate (\pm SEM, $n = 2$). **B**, Lysates of highly purified (>99% pure) nonreleaser basophils (*Bas*) from preparation used for fifth secretion analysis, as well as B cells, eosinophils (*Eos*), and neutrophils (*Neut*) from the same donor, were analyzed by Western blotting for Lyn, Syk, and actin protein expression. Equal numbers of cell equivalents were added to gel (2.6×10^5 cell equivalents/lane). Results are representative of 2 separate experiments.

In the experiments illustrated in Fig 1, *B*, solubilized cell proteins were separated by SDS-PAGE and Western blots probed with antibodies to Lyn or Syk. Highly purified nonreleaser basophils contain the Src-related tyrosine kinase Lyn at lower levels than releaser basophils. Lyn levels were similar among B cells, eosinophils, and neutrophils from all donors regardless of the releaser or nonreleaser status of their basophils. Importantly, Syk protein was consistently

undetectable in nonreleaser basophils. In contrast, Syk levels were similar among B cells, eosinophils, and neutrophils from donors with releaser and nonreleaser basophils. In contrast with Syk, Zap-70, previously detected at low levels in releaser basophils,³ was detectable in highly purified preparations of nonreleaser basophils (data not shown).

Assays for kinase messenger RNA expression showed that both releaser and nonreleaser basophils contained messenger RNA for Lyn. Syk and Lyn were consistently detected in the monocyte-lymphocyte population from both releaser and nonreleaser donors. We were able to detect Syk message in 2 of 4 experiments for nonreleaser 1, 0 of 2 experiments for nonreleaser 2, and 3 of 3 experiments for nonreleaser 3 (data not shown).

Remarkably, we detected anti-IgE-induced histamine release from basophils obtained from nonreleaser 3 in the last 2 of 5 experiments (Fig 2, *B*).

Western blotting performed at the time of the nonreleaser “converting” into a releaser revealed the presence of Lyn and Syk protein in the basophils at levels similar to those found in releaser basophils (Fig 2, *B*). There were no obvious changes in levels of Lyn and Syk in B cells, neutrophils, and eosinophils before or after conversion of the basophils to the responder phenotype.

DISCUSSION

It has been known since 1973 that basophils from as many as 10% to 20% of nonatopic donors fail to secrete in response to FcεRI cross-linking.⁷ This phenotype was found with somewhat lower frequency (3/37 or approximately 8%) among our donors (approximately 50% men and 50% women, age range 16-50 years, approximately 25% Hispanic and 75% non-Hispanic white, all nonallergic by self-report). Mechanistic studies have so far not explained this phenotype. Nonreleaser basophils express the FcεRI α subunit in the normal range, and donors do not show altered serum IgE levels.^{5,8} In addition, studies of receptor composition demonstrated normal expression of the β subunit in nonreleaser basophils and showed additionally that the α, β, and γ subunits of nonreleaser FcεRI have no primary structural changes.^{8,9} Other explanations for the nonreleaser phenotype have been explored. Antibodies to the cell membrane phosphatase CD45 can down-regulate Ca⁺⁺ mobilization and histamine release in human basophils.¹⁰ However, flow cytometric experiments demonstrated no difference in CD45 expression between releaser and nonreleaser basophils.¹¹

We have established that nonreleaser basophils from 3 healthy donors lack the tyrosine kinase Syk and express normal-to-reduced levels of the protein tyrosine kinase Lyn. We hypothesize that Syk deficiencies account, at least in part, for the lack of FcεRI-induced secretion. In contrast, small amounts of Zap70 were detectable in both releaser and nonreleaser basophils. These results demonstrate that Zap70 cannot substitute for Syk in the FcεRI signaling pathway leading to degranulation. Its function in human basophil signaling remains to be determined.

Remarkably, Syk and Lyn appear to be expressed normally in B cells, eosinophils, and neutrophils from nonreleaser donors, suggesting that kinase expression may be regulated separately in basophils in comparison with other leukocytes. Because 2 of the 3 nonreleasers had

positive skin test results to common antigens, we suppose that mast cell Syk is probably also expressed in adequate levels.

The mechanism(s) underlying the absence of Syk from nonreleaser basophils is not yet known. Reduced Syk gene transcription, perhaps related to a mutation in the Syk promoter region or to fluctuation levels of basophil-specific transcriptional activators (or repressors) and reduced Syk messenger RNA stability are both possible. Syk protein is particularly susceptible to cleavage from the full-length 72-kd form to a 40-kd form¹² and it is possible that mutations or modifications that selectively affect Syk itself or a Syk binding partner such as Cbl¹³ or tubulin¹⁴ may also contribute to the consistent absence of Syk protein from nonreleaser basophils. The recent discovery of an acridone-related Syk inhibitor that blocks FcεRI-coupled Syk activation without affecting B-cell Syk activation¹⁵ supports the occurrence of cell type-specific Syk-protein interactions that might affect Syk stability. The demonstration by Yamaguchi et al⁸ that 4 days in culture with IL-3 causes a partial recovery of secretion in nonreleaser basophils, and our observation that this recovery is accompanied by increased Lyn and Syk expression (data not shown) provides a system to explore events regulating the transcription or translation of basophil kinases.

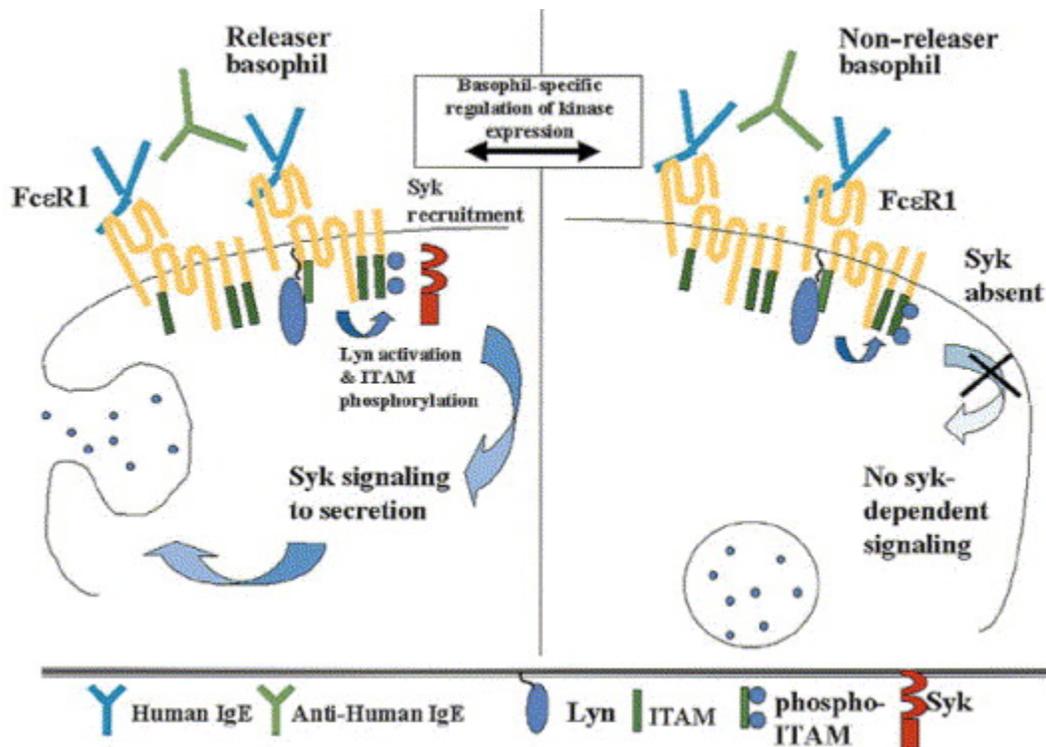


Fig. 3. Model for FcεRI-mediated activation of releaser basophils (*left*) and for absence of FcεRI-mediated activation in nonreleaser basophils (*right*). This schematic links basophil-specific absence of Syk to nonreleaser phenotype and indicates that fluctuation between Syk and Syk⁺ phenotype is possible in vivo. Future treatments for allergic inflammation may include drugs that target basophil-specific kinase expression.

In the course of the current studies (approximately 1 year), we were never able to detect Syk protein in basophils from nonreleasers 1 and 2 and were never able to demonstrate FcεRI-mediated secretion in these cells. In the fourth and fifth experiments examining nonreleaser 3 we did detect anti-IgE-induced histamine release and concomitant basophil Syk expression. These

data support anecdotal evidence that basophils from individual donors can cycle in and out of responsiveness over time. They provide further evidence that the expression of Syk protein is the critical component missing from nonreleaser basophils.

The scheme in Fig 3 summarizes and interprets our results.

In releaser basophils (left panel), previous work^{2, 3} indicates that FcεRI cross-linking activates Lyn, leading to the phosphorylation of immunoreceptor tyrosine-based activation motifs in the FcεRI β and γ subunit cytoplasmic tails. Syk in turn is recruited via its tandem SH2 domains to the γ subunit phosphoimmunoreceptor tyrosine based activation motifs and activated. The subsequent Syk-mediated phosphorylation of target proteins is the critical event leading to signal propagation and physiologic responses, including secretion. In nonreleaser basophils (right panel), the sequence of events leading to signal propagation is blocked by the absence of Syk and no secretion occurs. We indicate that Syk expression is controlled by basophil-specific factors (because Syk-deficient basophils can coexist with Syk-containing B cells, eosinophils, and neutrophils) and that basophils are capable of fluctuating between the 2 phenotypes (because one donor changed from the nonreleaser to the releaser phenotype, with concomitant basophil Syk expression, during our studies). The evidence for basophil-specific kinase regulation raises the exciting possibility of developing new treatments for allergic inflammation based on drugs that target basophil-specific kinase expression.

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References

1. Schwartz LB, Huff TF. Biology of mast cells and basophils. In: Middleton E Jr, Reed CE, Ellis EF, Adkinson NF Jr, Yunginger JW, Busse WW, editors. Allergy: principles and practice. St Louis: Mosby-Year Book; 1993. p 135-68.
2. Beaven MA, Baumgartner RA. Downstream signals initiated in mast cells by FcεRI and other receptors. *Curr Opin Immunol* 1996;8:766-72.
3. Kepley CL, Wilson BW, Oliver JM. Identification of the FcεRI-activated tyrosine kinases Lyn, Syk, and Zap-70 in human basophils. *J Allergy Clin Immunol* 1998;102:304-15.
4. Nguyen KL, Gillis S, MacGlashan DW Jr. A comparative study of releasing and nonreleasing human basophils: nonreleasing basophils lack an early component of the signal transduction pathway that follows IgE cross-linking. *J Allergy Clin Immunol* 1990;85:1020-9.
5. Knol EF, Mul FPJ, Kuijpers TW, Verhoeven AJ, Roos D. Intracellular events in anti-IgE nonreleasing human basophils. *J Allergy Clin Immunol* 1992;90:92-103.

6. Kepley C, Craig S, Schwartz L. Purification of human basophils by density and size alone. *J Immunol Methods* 1994;175:1-9.
7. Ishizaka T, Soto CS, Ishizaka K. Mechanisms of passive sensitization, III: number of IgE molecules and their receptor sites on human basophil granulocytes. *J Immunol* 1973;111:500-11.
8. Yamaguchi M, Hirai K, Ohta K, Suzuki K, Kitani S, Takaishi T, et al. Nonreleasing basophils convert to releasing basophils by culturing with IL-3. *J Allergy Clin Immunol* 1996;97:1279-87.
9. Kuster H, Zhang L, Brini AT, MacGlashan DW, Kinet JP. The gene and cDNA for the human high affinity immunoglobulin E receptor beta chain and expression of the complete human receptor. *J Biol Chem* 1992;267:12782-7.
10. Hook WA, Berenstein EH, Zinsser FU, Fischler C, Siraganian RP. Monoclonal antibodies to the leukocyte common antigen (CD45) inhibit IgE-mediated histamine release from human basophils. *J Immunol* 1991;147:2670-6.
11. MacGlashan DW Jr. Signal transduction and cytokine production by human basophils. *Chem Immunol* 1995;61:88-113.
12. Zioncheck TF, Harrison ML, Isaacson CC, Geahlen RL. Generation of an active protein-tyrosine kinase from lymphocytes by proteolysis. *J Biol Chem* 1988;263:19195-202.
13. Ota Y, Scharenberg AM, Donovan JA, Kinet JP, Samuelson LE. Characterization of Cbl tyrosine phosphorylation and a Cbl-Syk complex in RBL-2H3 cells. *J Exp Med* 1996;84:1713-23.
14. Peters JD, Furlong MT, Asai DJ, Harrison ML, Geahlen RL. Syk, activated by cross-linking the B-cell antigen receptor, localized to the cytosol where it interacts with and phosphorylates alpha-tubulin on tyrosine. *J Biol Chem* 1996;271:4755-62.
15. Moriya K, Rivera J, Odom S, Sakuma Y, Muramoto K, Yoshiuchi T, et al. ER-27319, an acridone-related compound, inhibits release of antigen-induced allergic mediators from mast cells by selective inhibition of Fce receptor I-mediated activation of Syk. *Proc Natl Acad Sci U S A* 1997;94:12539-44.