

Original article

Relationship between spleen tyrosine kinase and phosphatidylinositol 5' phosphatase expression and secretion from human basophils in the general population

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Background: Previous studies have suggested that expression levels of spleen tyrosine kinase (syk) or phosphatidylinositol 5' phosphatase (SHIP) may explain certain extreme human basophil phenotypes.

Objective: This study is designed to explore whether variability in syk and SHIP expression levels in the general population, alone or in concert, can account for the variability in basophil function.

Methods: A survey of maximum responsiveness to IgE-mediated stimulation, sensitivity, and expression levels of 6 early signaling elements was performed on 36 subjects' basophils.

Results: Of the 6 signaling elements, only syk and SHIP showed a correlation with maximum histamine release or cellular sensitivity. In a multiple regression, syk and SHIP together could account for 67% of population variance, although most of the variance was explained by syk expression. The pattern of expression variance $\text{syk} > \text{SHIP1} > \text{SHIP2} \approx \text{lyn} \approx \text{p85} \approx \text{cbl}$ suggested a process that primarily modulated syk levels. IL-3 is known to modulate syk levels, but we found that a 3-day incubation with IL-3 resulted in increased expression of other signaling elements to a greater degree: $\text{cbl} > \text{SHIP1} > \text{SHIP2} \approx \text{lyn} \approx \text{p85} \approx \text{syk}$, opposite the pattern in the population survey. In contrast, 18-hour stimulation with anti-IgE antibody led to marked downregulation of syk expression, modest downregulation of FcεRI expression, weak downregulation of lyn expression, and no effect on 23 other signaling elements.

Conclusion: Unlike studies in mice, we conclude that expression of syk is a good preconditioning predictor of basophil function in the general population.

Clinical implications: The finding that expression of syk levels may strongly influence functional responses of basophils suggests a mechanism underlying the severity of atopic diseases. (*J Allergy Clin Immunol* ■■■■;■■■;■■■-■■■.)

Key words: Human, basophil, allergy, Fc receptors

Abbreviations used

ED50: Effective density for 50% of the maximal response

HRF: Histamine-releasing factor (p23)

PI3: Phosphatidylinositol 3'

PIP3: Phosphatidylinositol 3,4,5 trisphosphate

SHIP: Phosphatidylinositol 5' phosphatase

Syk: Spleen tyrosine kinase

Secretion of mediators from basophils and mast cells is considered an important component of an immediate hypersensitivity reaction. Human basophils have provided a convenient model of IgE-mediated secretion from a human IgE-bearing cell type for several decades. It has been known since the earliest studies of this cell type that there is a broad range of natural variation in the ability of basophils to respond to antigenic stimulation. There are some obvious reasons for this variation, such as the possession of adequate amounts of antigen-specific IgE to initiate a response to a given antigen. However, when anti-IgE antibodies became available and it became possible to stimulate basophils through IgE and its receptor without regard to the inherent antigenic specificity, it became apparent that there remained considerable variation in responsiveness. Even when it could be demonstrated that basophils from 2 different subjects expressed equal densities of IgE, there remained extreme differences in response to anti-IgE antibody.^{1,2} In the intervening years, 2 parameters of IgE-mediated basophil histamine release were characterized. The maximum amount of histamine release that can be obtained by aggregation of FcεRI ranges from 0 to 100%, and this parameter of basophil activation is the one most often studied because it is the most accessible assay of function. However, it became apparent that the maximum achievable histamine release could be obtained with relatively low densities of cell surface IgE and that there was a second critical parameter that was needed to characterize the response fully.^{3,4} The number of cell surface antigen-specific IgE molecules required to reach half-maximal histamine release is not as easily determined, but several studies have demonstrated considerable variation in sensitivity among subject basophils.^{3,4}

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In the first population survey of this parameter, it was not found to be correlated to maximum histamine release, suggesting that there were 2 points of regulation in the control of cellular responsiveness⁴ (maximum release and sensitivity).

Using animal models or cell lines, a fair amount of detail concerning the early signal transduction cascade has been elucidated. Some of this detail has been validated with human basophils or mast cells. A testable hypothesis is that the 2 intrinsic parameters of basophil responsiveness, maximum response and sensitivity, may result from differences in the expression of a select set of early signaling elements. The possibility that expression levels control responsiveness is strengthened by studies of 2 different basophil phenotypes. It had been recognized since the earliest studies of basophil histamine release that some subjects' basophils do not secrete in response to anti-IgE Ab stimulation. We showed that this was not the trivial result of an absence of cell surface IgE.⁵ Indeed, basophils of this phenotype, which we called *nonreleasers*, do sense the anti-IgE antibody in a limited way⁵; they simply do not release mediators. More recent studies by our group and others demonstrated that expression of spleen tyrosine kinase (syk) was low in these nonreleasers.⁶⁻⁸ This low expression of syk was confined to the basophil. Other circulating leukocytes that express syk kinase did so at levels similar to levels found in subjects having releasing basophils.⁶

A second basophil phenotype was identified in studies of the so-called *histamine-releasing factor* (HRF), which has been identified as a 23-kd protein normally found to be intracellular.⁹ A small subset of subjects, often those that are categorized as subjects with allergic asthma, have basophils that can respond to this protein.¹⁰ Although the reason for responsiveness to HRF remains unclear, Vonakis et al¹¹ found that basophils with this phenotype also express lower levels of phosphatidylinositol 5' phosphatase (SHIP)-1, the SHIP that has been identified as being important in downregulating B-cell lymphocyte activation.¹² SHIP1 has been studied in murine mast cells or rat basophilic leukemia cells and is found to participate in the activation cascade, although its precise role is not entirely defined.¹³⁻¹⁵ It is possible that it acts a negative regulator of secretion, because the generation of phosphatidylinositol 3,4,5 trisphosphate (PIP3) is considered to be an important component of the activation cascade. Notably, expression of SHIP1 has been proposed to be an important element in controlling secretion in mouse mast cells.¹³ In human basophils, we have found that the enzyme considered critical in the formation of PIP3, phosphatidylinositol 3' (PI3) kinase, is absolutely required for propagating the early signaling steps.¹⁶ Inhibition of all 3 classes of mediator secretion follows inhibition of PI3 kinase with relatively selective agents, such as wortmannin or LY294002; therefore, the negative influence of a phosphatase such as SHIP on the presence of PIP3 could be an important regulator of secretion in these cells.

It is not yet known whether these 2 basophil phenotypes represent special cases of signal element expression or

whether differences in syk and SHIP expression are associated with the functional differences found in the larger population. Therefore, we explored the relationship of these 2 signaling elements to the 2 described parameters of IgE-mediated activation, maximum histamine release and basophil sensitivity, in the general population. The primary hypothesis was that together, variation in expression levels of these 2 signaling elements could account for the majority of response variation in the general population.

METHODS

Materials and buffers

The Online Repository (www.jacionline.org) provides the description of standard materials, buffers, and antibodies used for these studies. Briefly, various piperazine-N,N'-bis(2-ethanesulfonic acid) buffers, as described previously, were used for short periods of stimulation, and supplemented RPMI-1640 was used for overnight incubations.¹⁷ RPMI-1640 for overnight cultures contained 8 μ g/mL gentamycin and 300 μ g/mL human serum albumin, supplemented to 1 mmol/L final Ca^{++} concentration. The syk inhibitor, NVP-QAB205, was a gift from GlaxoSmithKline (Stevenage, England).

Basophil purification

Two sources of basophils were used for these experiments. To develop the population profile of signal element expression, basophils were obtained by venipuncture for functional analysis and signal element expression. For this study, blood was first enriched in basophils by a 2-step Percoll (Pharmacia, Piscataway, NJ) gradient (53%/62% Percoll).¹⁸ Approximately $\frac{1}{3}$ of the enriched cells were set aside for functional analysis, and the remaining cells were purified to near homogeneity by negative selection using the basophil purification kit (Stem Cell Technologies, Vancouver, British Columbia, Canada) and columns from Miltenyi Biotec (Auburn, Calif).⁷ After the 2-step enrichment step, the basophils were maintained at 4°C throughout the steps necessary for final purification. The average purity of these basophils by Alcian blue staining¹⁹ was 99%. For many of the remaining studies, residual cells of normal donors undergoing leukapheresis were enriched in basophils using Percoll density gradients and countercurrent-flow elutriation, as previously described.¹⁸ Basophils were further purified by negative selection as described. Basophil purity exceeded 99%.

Subjects

Recruitment was quasi-random; there were no specific exclusion criteria other than those specified by good practice for blood drawing. The mean age was 35 (22-58) years, and 61% were female. With respect to atopy, subjects were simply asked if they had any known allergies after the study was complete; 21% reported having allergies to common antigens (animal danders, aeroallergens, insects). A medication history was not obtained.

Functional analysis

A portion of the 2-step Percoll enriched cells were divided for sensitization under 5 conditions; no IgE Ab, 5, 2.5, 1.25, and 0.625 μ g/mL anti-gp120peptide-specific biotinylated IgE (SE44B, a gift from Tanox Corp, Houston, Tex). Sensitization was performed at 4°C for 1 hour in PIPES-albumin-glucose (see this article's Online Repository at www.jacionline.org for details) buffer containing 10 μ g/mL heparin. After washing, an aliquot of cells from each sensitization condition were set aside for flow cytometry, and the remaining cells were stimulated with either 0.2 μ g/mL goat polyclonal anti-IgE

Ab or 0.6 $\mu\text{g/mL}$ gp120-peptide-ovalbumin conjugate (both concentrations were previously determined to be optimal for histamine release (HR) at all IgE densities^{4,20}). For the cells not sensitized with SE44B, the antigen challenge condition was replaced with a monoclonal mouse IgM anti-IgE Ab, 6061 (Hybridoma Reagent Laboratory, Baltimore, Md), so that for comparative purposes, non-sensitized cells were tested for response to either goat anti-IgE Ab or IgM anti-IgE Ab. Supernatants were harvested after 45 minutes of stimulation in PAGCM and analyzed for histamine (see this article's Online Repository at www.jacionline.org for details). The maximum histamine release value (max HR) was obtained from the best response to either antigen or goat anti-IgE antibody from any condition. The effective density for 50% of the maximal response (ED50) was extrapolated from the antigen responses plotted against the SE44B density obtained from flow cytometry (see this article's Online Repository at www.jacionline.org), as described previously.²⁰

Western analysis

Purified basophils were lysed and run in gels in groups of 8 with a 3 concentration standard basophil lysate used for internal calibration so that each blot could be compared with other Western blots. The details of the procedure and its calibration are provided in this article's Fig E1 in the Online Repository at www.jacionline.org.

Statistical analysis

In pilot studies, errors in signal element measurements were found to be proportional to the level of expression, and therefore, these measurements were log-transformed for analysis. Multiple regression, forward stepwise with $F > 4.5$ for inclusion of a parameter, was performed for syk and SHIP against maximum histamine release (and ED50, with the exception noted in the Results). The loss or gain of signaling components was analyzed by t test of stimulated/control ratios (against the expected value of 1.0).

RESULTS

To assess the variation in interassay sample error, whole cell lysates from 9 different subjects were run in multiple Western blots. From these measurements, we found approximately $\pm 24\%$ (error relative to measured amount) variation for the syk kinase determination and $\pm 11\%$ variation for SHIP1. We also examined expression levels in basophils of 9 subjects with a second purification and Western analysis; expression levels showed a relative variation of 21% and 35% for syk and SHIP1, respectively. Fig 1 shows the population distributions for 4 additional signaling elements as well as the population distribution for maximal histamine release and ED50. Although the study was focused on syk and SHIP1 measurements, we also examined several other potentially interesting signaling elements demonstrated to operate early during IgE-mediated signaling. There was little variation in expression of these elements, and there was no correlation between these 4 signaling elements and HR or ED50 (see this article's Table E1 in the Online Repository at www.jacionline.org). There was a broader expression of syk kinase; Fig 2 shows that there was a strong correlation between syk kinase expression and maximal histamine release ($r = 0.766$; $P < .0001$). Removing nonreleasers from the correlation retained the statistically significant positive correlation between syk and HR ($r = 0.585$). There was also a

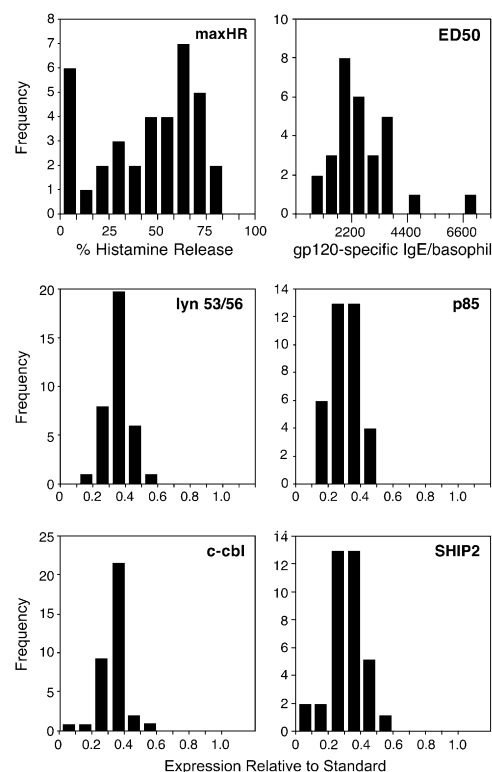


FIG 1. Population distributions of 6 of the 8 obtained measures. For the signaling elements, the x coordinate is the band intensity relative to the standard included in each blot (see Methods).

weak, although statistically significant, negative correlation between expression of SHIP1 and maximal histamine release ($r = -0.343$; $P = .0403$). A stepwise multiple regression using expression of syk and SHIP1 as 2 independent variables resulted in the regression equation (improving the overall correlation coefficient to 0.83): $\text{maxHR} = 0.015 + 0.753 \cdot \text{syk} - 0.314 \cdot \text{SHIP1}$ (F to enter SHIP into the regression was 10.32; F to enter syk was 72).

To obtain a quantitative sense for the expression levels of syk (and lyn), we compared our basophil standard to a known quantity of recombinant syk and lyn. Based on this calibration, we found that the median syk and lyn expression levels for this population of basophils were equivalent to 20,000 and 100,000 molecules of syk and lyn per cell, respectively. The plot in Fig 2 includes this information.

In our previous study examining the relationship between maximum histamine release and the ED50, there was not a statistically significant correlation between these 2 parameters.⁴ This remained true for the current study but only when using the response to goat anti-IgE antibody to determine the maximum histamine release ($r = -0.269$; $P = .151$). However, for the current study, we noticed that in some cases, the highest level of antigen stimulation would induce somewhat greater histamine release than goat anti-IgE Ab. If the value for maximum histamine release was derived from the best histamine release obtained—whether induced by goat anti-IgE Ab or antigen,

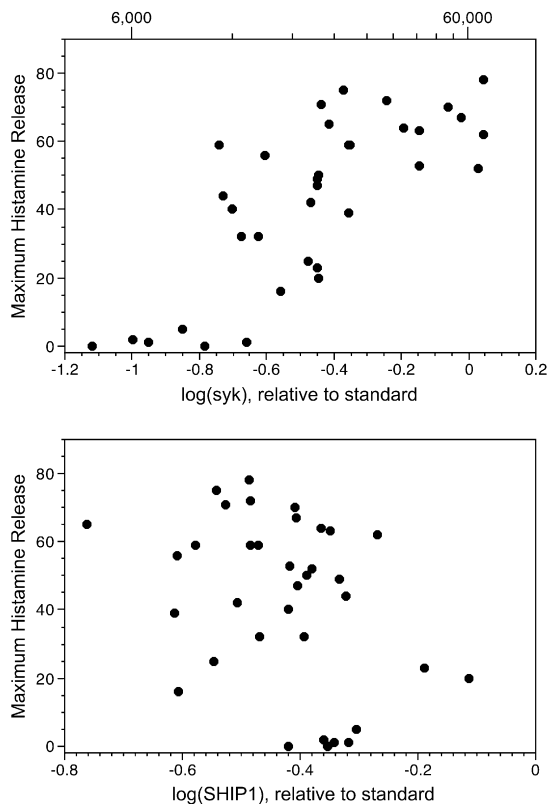


FIG 2. Correlation between syk and SHIP1 expression and maximum histamine release. Each point represents the measurement from each of the survey subjects. At the top of the syk correlation plot is the absolute number of molecules of syk per basophil as estimated from the calibration performed with recombinant syk kinase.

as used in these correlations—and this value is used to generate the correlation between ED50 and maximum release, a weak inverse correlation is observed ($r = -0.486$; $P = .044$). The possible correlation between these 2 parameters was expected in our earlier study (but not found) and would be expected from the heuristic model to be discussed, but their weak correlation does make it more difficult to interpret the correlation between ED50 and syk or SHIP expression. The correlation between ED50 and syk expression was -0.367 ($P = .046$, $n = 30$, which excludes the nonreleasing cells for which an ED50 cannot be determined). However, if the relationship between maximum HR and ED50 is factored out, the correlation between syk and ED50 becomes nonsignificant ($r = -0.137$). There was also no correlation between SHIP1 and ED50, with or without factoring out the HR/ED50 correlation.

These results suggest that expression of syk kinase can largely determine the final outcome of maximal histamine release. Because previous studies of nonreleasing basophils found that expression of syk mRNA levels was not statistically different between releasers and nonreleasers,^{7,21} it is possible that these levels of syk expression are determined by a posttranslational event. We are currently aware of only 2 possible regulators of syk expression. It has been noted that incubation of basophils with high

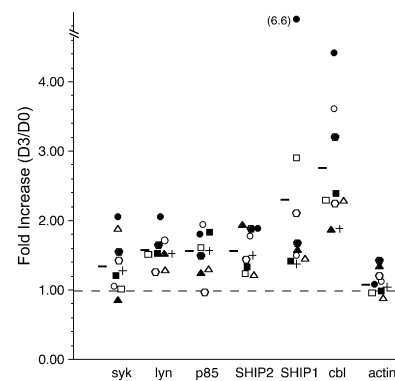


FIG 3. Upregulation of signaling elements after a 3-day incubation with IL-3. Purified basophils were lysed on day 0 (300,000 per 20 μ L electrophoresis sample buffer), or cultured in RPMI-1640 supplemented with 2% FCS, nonessential amino acids, glutamine, IL-3 at 10 ng/mL, and gentamycin at 8 μ g/mL. Cells were harvested on the third day, counted with erythrocin B for viability (which was greater than 97% for all preparations), and cells lysed at a density of 300,000 per 20 μ L electrophoresis sample buffer. After Western blotting the lysates, the ratio of day 3/day 0 expression was plotted for each of the 9 subjects examined. Each symbol in the plot represents 1 of the 9 subjects; the horizontal bar represents the mean for the groups.

concentrations of IL-3 for several days will upregulate syk levels in nonreleasing basophils, and it is not yet clear whether IL-3 is inducing posttranslational regulators or regulators of gene expression.^{21,22} We examined the effects of IL-3 on signal element expression in 9 subjects selected from the survey for having a range of syk expression. Purified basophils were cultured for 3 days in the presence of 10 ng/mL IL-3. A comparison was made between expression levels on day 0 and day 3. The normalizing parameter was a cell count. Fig 3 summarizes the observations. Notably, all signaling elements were upregulated to some extent, although surprisingly, syk expression was the least upregulated, and only basophils expressing < 0.1 units of syk showed meaningful upregulation. The order of upregulation was $cbl > SHIP1 > SHIP2 \approx p85 \approx lyn \geq syk$ (day 3/day 0 > 1.00 ; $P < .05$ for all elements). Note that although we used the cell count to determine the lysis conditions, the actin measurements also indicated equal levels between day 0 and day 3 ($P > .05$). The matrix of correlations among elements showed a range of moderate positive correlations (0.31-0.81) between lyn, SHIP, SHIP2, p85, and c-cbl, but syk upregulation did not correlate with lyn, SHIP1, or p85 upregulation (changes in actin did not correlate with other elements).

Recently, we showed that activation of basophils through Fc ϵ RI would strongly modulate syk kinase levels,¹⁷ and similar results have also been found for tissue-derived human mast cells.²³ It is possible, however, that this form of regulation also involves downregulation or upregulation of other signaling species. With the possible exception of a modest SHIP1 distribution, only the syk distribution was broad in this population. Therefore, we performed a survey of which signaling elements

TABLE I. Loss of signaling elements after 18 hours of stimulation with an optimal concentration of anti-IgE Ab (0.2 µg/mL)

Signal element	Fraction of control	syk*	n	Significance
syk	0.33 ± 0.04	—	29	<.0001
FcεRIα	0.70 ± 0.05	0.36	18	<.0001
lyn	0.84 ± 0.03	0.31	9	.0017
SHIP1	0.90 ± 0.09	0.32	9	NS
SHIP2	0.97 ± 0.05	0.32	9	NS
c-cbl	1.01 ± 0.06	0.52	4	NS
p85	1.05 ± 0.03	0.37	20	NS
p110δ	0.90 ± 0.05	0.18	5	NS
fyn	1.02 ± 0.10	0.50	4	NS
PLCγ1	1.13 ± 0.09	0.36	4	NS
PLCγ2	0.99 ± 0.06	0.17	5	NS
btk	1.10 ± 0.10	0.35	6	NS
PLD1/2	0.94 ± 0.08	0.17	5	NS
SLP-76	1.25 ± 0.19	0.59	3	NS
LAT	1.14 ± 0.04	0.47	4	NS
shc	1.04 ± 0.05	0.59	3	NS
Grb2	1.01 ± 0.10	0.47	4	NS
SOS2	1.07 ± 0.05	0.41	4	NS
p56dok	1.24 ± 0.37	0.47	4	NS
VAV	0.98 ± 0.07	0.35	6	NS
GAB2	1.01 ± 0.12	0.59	3	NS
SHP-1	0.99 ± 0.13	0.16	5	NS
PTEN	1.09 ± 0.05	0.16	5	NS
Akt	0.89 ± 0.06	0.16	5	NS
Erk	0.86 ± 0.06	0.16	5	NS

p110δ, Catalytic subunit of phosphatidylinositol 3' kinase; *PLC*, phospholipase C; *btk*, bruton's tyrosine kinase; *PLD*, phospholipase D; *SLP-76*, SH2 domain-containing leukocyte-specific phosphoprotein; *LAT*, linker for activation of T cells; *shc*, SH2 domain containing transforming protein C; *Grb2*, growth factor receptor bound protein 2; *SOS2*, son-of-sevenless 2; *p56dok*, docking protein; *GAB2*, Grb2-associated binder-2; *SHP-1*, SH2-containing tyrosine phosphatase-1; *PTEN*, phosphatase and tensin homolog deleted on chromosome Ten; *Erk*, extracellular signal regulated kinase; *NS*, not significant.

*Syk loss for the same experiments in which the specified element was measured.

are altered by previous stimulation through FcεRI (see this article's Fig E2 in the Online Repository at www.jacionline.org for a schematic of the elements examined). Because of limitations in the cell numbers, we used Western blotting to make a determination of changes in 25 elements. Most of the signaling elements in Table I have been demonstrated by either translocation or phosphorylation to participate in an IgE-mediated reaction in human basophils. There are a couple of exceptions that were examined as well. It is notable that only 3 elements were observed to change: syk kinase, FcεRIα, and lyn kinase. The change in lyn kinase is marginal although statistically significant. As we will show in an upcoming publication, the change in receptor expression is very slow and likely results from a process of downregulation different from the loss of syk kinase. The changes, however, are also modest despite all surface IgE being aggregated by the stimulus, anti-IgE Ab.

To explore what kind of signaling was required to observe changes in syk expression after aggregation of FcεRI, we examined the effect of several agents on the loss of syk kinase induced by stimulation with anti-IgE antibody. Table E2 and Figs E3 and E4 in the Online Repository at www.jacionline.org show that the PI3 kinase inhibitor, LY294002, the actin polymerization inhibitor, latrunculin A, the protein kinase C (PKC) inhibitor, Bis II, and IL-3 did not alter the loss of syk kinase in these 18-hour experiments. In contrast, PP1 inhibited the loss of

syk, and there was partial inhibition with a new syk inhibitor, NVP-QAB205. However, the concentration-dependence of inhibition with NVP-QAB205 was not consistent with its activity as a syk inhibitor; inhibition of mediator release required ≈300-fold lower concentrations than inhibition of the loss of syk kinase.

DISCUSSION

Previous studies on the relationship between signal element expression and basophil function examined extreme differences in function. In addition, the codependent relationship between signal element expression and function has not been examined. The current studies provide a broader context for signal elements expression. There are certainly many signaling elements absolutely required for mediator release, but it appears that syk expression is a focus of natural variation. Indeed, the distribution of expression of all but SHIP1 appears very tightly regulated, being nearly identically expressed in all samples. There is a slightly larger variance in SHIP1 expression, and a multiple regression allows entry of SHIP1 into the regression equation with a consequent improvement in the overall correlation. This result suggests that SHIP1 introduces a counterregulation, although one that is relatively modest: there is a ≈6-fold difference in the

contribution to variance by syk versus SHIP1. The previous studies of the relationship between SHIP1 expression and the ability of basophils to respond to p23 histamine releasing factor¹¹ demonstrate a somewhat larger range of SHIP1 expression than found here. Because the normal population shows a relatively narrow distribution of SHIP1 expression, the HRF results suggest that there is a unique process in these HRF-responsive subjects, further influencing the expression of SHIP1. The range of syk expression, on the basis of the calibration of our basophil standard, is interesting for its relationship to receptor expression. A typical basophil expresses $\approx 100,000$ Fc ϵ RI. Syk expression ranged between 5000 and 60,000 molecules per cell. These studies suggest that it may be syk kinase that is the limiting species in this reaction rather than lyn kinase or SHIP as suggested by studies of rat or mouse mast cells or cell lines.^{13,24}

Histamine release is a far downstream measure of function and an integrated response as well. An outcome that is demonstrably dependent on syk activity but is more proximal to syk's place in the signaling cascade is the cytosolic calcium response. We have shown that basophils that poorly express syk (so-called *nonreleasers*) show weak calcium responses relative to the rest of the population.⁸ We have also shown that there is a tight correlation, among subjects, between the magnitude of the cytosolic calcium response and histamine release.²⁵ We infer from these relationships that variation in syk expression accounts not only for variability in histamine release but also for variability in the calcium response, a far more proximal signaling event. This article's Fig E5 in the Online Repository at www.jacionline.org also demonstrates, in a limited study, a similar dependence of 2 other early signals, phosphorylation of Src homology 2 domain-containing transforming protein C (shc) and c-cbl, on the level of syk expression.

The abundance of syk is but 1 parameter that defines the enzymatic activity of this critical enzyme. Without directly assessing the activity of syk, it remains possible that variation in syk expression could be offset by changes in other parameters that determine syk function. Direct measurements of enzymatic rates are not technically feasible at this time. Even with a known *in situ* substrate, one would have to eliminate the back-reactions to assess an actual rate. Therefore, there is an implicit assumption in an interpretation of these observations that variations in abundance translate to variations in enzymatic rate. In a similar context, the lack of correlations between expression of other signaling elements, such as lyn, and histamine release does not remove the possibility that other determinants of their activity (such as single-nucleotide polymorphism variations and so forth) could also induce variability in the population response. Mitigating this possibility is that the combined correlation between the syk-SHIP pair and histamine release is sufficiently close to a statistically perfect expectation that there is little room for other factors to contribute.

Because we know that syk kinase is a necessary signaling element in human basophils (from transfection

studies²⁶ and pharmacological studies, demonstrated here) and tissue mast cells,²³ and we now know that natural variation in syk kinase expression correlates with the natural variation in maximal histamine release, we suggest that the next step will be to understand the origins of the natural variation in syk kinase expression. Notably, the control of syk expression is not confined to a special basophil phenotype but applies to the whole population. The survey provided an expression signature that could be compared to 2 known methods of modulating expression. First, IL-3 induces upregulation of multiple basophil functions in response to multiple classes of stimuli. In this context, it is not surprising that there is upregulation of all signaling elements. It was more surprising that syk kinase expression was the least affected signaling element, and only meaningfully upregulated in those subjects whose basophils expressed the lowest levels of syk. A second surprise was that SHIP expression was markedly upregulated. If SHIP were acting as a negative regulatory element, we might have expected no change or downregulation. However, the cells are exposed to IL-3 at a high concentration for a long duration, and because IL-3-induced signaling also results in activation of PI3 kinase and PIP3 formation (as determined by phosphorylation of Akt²⁷), perhaps the upregulation of SHIP represents a feedback control on strong IL-3 receptor activation (in addition to negative signaling elements like suppressors of cytokine signaling, suppressor of cytokine signaling [SOCS], proteins). In addition, the pan-upregulation of the non-syk elements suggests that the correlation among these components (see this article's Table E1 in the Online Repository at www.jacionline.org) may not be a result of a technical issue but may be a general reflection of the state of the circulating basophil and its historical exposure to a cytokine like IL-3.

If circulating basophils experience some level of Fc ϵ RI-mediated activation *in vivo*, one would expect downmodulation of syk kinase. Indeed, the cells do not even need strong activation to see some regulation of syk by this mechanism.^{17,23} Activation of syk itself does not even appear necessary to result in syk loss (see this article's Fig E4 in the Online Repository at www.jacionline.org). As shown in Table I, of the signaling elements examined, only syk is markedly downregulated by stimulation. If this were the only change, the results would be consistent with the limited variation in the population for elements other than syk. However, Table I also shows that there is some loss of lyn and Fc ϵ RI α . There is little context for understanding the significance of the changes in lyn; we sometimes observed 90% loss of syk and little loss of lyn. However, if our computation of syk and lyn expression for the population is relatively correct (20,000 vs 100,000 molecules/basophil for syk and lyn, respectively) then a similar numerical loss of syk and lyn would lead to just this result (eg, losing 18,000 molecules of each would result in 90% loss of syk and 20% loss of lyn). If lyn serves other purposes in the basophil (eg, mediating IL-3 signaling in a manner observed for IL-5 receptor in eosinophils²⁸), then the higher expression acts as a buffer

against FcεRI-mediated loss mechanisms. In our previous study of nonreleaser basophils,⁷ there was altered expression of syk, but we also observed altered expression of lyn kinase. These results were not replicated in the current study; in fact, there was not a statistical difference between the nonreleasers and releasers in this study. The observation that some lyn downregulation occurs with long-term FcεRI aggregation demonstrates the possibility of finding different expression of lyn among subjects by at least 1 mechanism, but the reason for a discrepancy between the 2 studies is not yet known.

The loss of FcεRIα, although it appears to be a distinct process requiring much longer times than the loss of syk (as described in a forthcoming publication), shows a more significant range of loss than lyn kinase. From previous studies, we know that expression of FcεRIα and maximal release show a correlation coefficient that is near 0.⁴ However, we also know that FcεRIα is strongly controlled by IgE titer²⁹; indeed, the range of possible regulation induced by IgE is 100-fold, considerably greater than the 1.5-fold to 2-fold range of regulation by strong activation of FcεRI. The IgE effect could easily mask any influence of subliminal aggregation on FcεRI expression. In addition, basophils require relatively low densities of cell surface IgE for a maximal response. Coupled with the masking effect of monomeric IgE mediated upregulation, the absence of a correlation between maximum release and FcεRIα expression would not be surprising. Therefore, the data in Table I are not entirely incompatible with a hypothesis that natural variation in syk expression arises from subliminal aggregation of FcεRI *in vivo*. Alternatively, if syk kinase is used by another receptor-mediated process, perhaps selective loss of syk could occur by a non-FcεRI-dependent process. In this context, it is notable that SHIP1 or SHIP2 expression is not regulated by aggregation of FcεRI, but may be following activation of another receptor.

Although it is apparent that syk kinase expression is one of the natural points of control, SHIP1 expression as a point of control is not as clear. However, it is useful to explore expectations for these correlations. If one had a completely deterministic model of histamine release and scattered the data from such a model according to known measurement errors for the parameters of syk, SHIP1, maxHR, and ED50, expectations for these correlations could be assessed. We have performed this simulation. The model we used was heuristic, a minimal control systems description of events that mimics early signal transduction as described in a previous study.³⁰ We coupled 2 heuristic models, using this control systems model as a front end for generating a signal that drives the heuristic model of histamine release described by Goldstein et al.³¹ Because syk and SHIP both contribute to function in this model, a simple correlation between syk expression and maximum histamine release for a hypothetical randomly chosen population is already somewhat scattered because SHIP expression is hidden in a 1-independent-variable correlation. The median value of *r* in this simulation is 0.88. If the data are subjected to Monte Carlo

scattering, using known measurement errors for these parameters, the median value of *r* is 0.82. A similar 1-independent-variable analysis can be generated for SHIP expression and maximum release, and here the median *r* values, plus or minus Monte Carlo scattering, are −0.49 and −0.50, respectively. In the survey, the values for syk and SHIP1 were +0.77 and −0.34, respectively. Because the model has only been shown to mimic some aspects of the response, the significance of this analysis is unknown, but it does indicate that for a fully deterministic model—where both syk and SHIP1 are explicitly regulating secretion—the correlational data generated from a hypothetical population are not always high for both parameters.

In summary, with syk being demonstrated to be critical in early IgE-mediated signaling,^{23,26} the results of this survey also indicate that syk expression is a strong predictor of subsequent basophil secretion. SHIP1 expression appears to influence maximal release, although the contribution is either weak because there is only modest natural variation, or, as a signal element, SHIP itself has only modest effects on secretion. These data suggest that the nonreleaser phenotype is simply part of a continuum but that the HRF-responsive phenotype (with low levels of SHIP) may result from a distinct process that generates this condition in selected subjects. Two methods of altering syk expression, IL-3 treatment or FcεRI aggregation, generate patterns of change in signal element expression that are not strictly compatible with the signature gleaned from the survey. Nevertheless, FcεRI aggregation most strongly downmodulates syk, and a similar process *in vivo*, with certain caveats, may generate the profile of signaling element expression observed in the survey.

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