

Monotherapy with an inhibitor of IL-1 is the treatment of choice. This report provides *ex vivo* evidence to support clinical observations.

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Syk-deficient basophils from donors with chronic idiopathic urticaria exhibit a spectrum of releasability

To the Editor:

Population studies of human basophils stimulated with anti-IgE have revealed a wide range of histamine release (HR) for reasons that are just beginning to be explored.¹ After FcεRI aggregation, Lyn transphosphorylates the FcεRI β and γ subunits, allowing Syk to be recruited to the phosphorylated γ chain, and propagate the signaling resulting in secretion. A poorly secreting subpopulation of human basophils, termed nonresponder basophils from a healthy donor (NOR-NR basophils), release less than 10% of their total histamine content on stimulation with an optimal dose of anti-IgE.^{2,3} Protein levels of the tyrosine kinase Syk but not its message were found to be 10-fold lower in NOR-NR basophils than Syk levels in basophils averaging 65% HR. In one study both Lyn and Syk kinases were deficient in NOR-NR basophils.² The initial studies identified NOR-NR

basophils as coming from donors who did not have allergic disease, suggesting this downregulation of Syk kinase might have a protective effect. A deficiency of Syk kinase, as studied in the RBL-2H3 cell line, resulted in truncated FcεRI signaling and the complete absence of both preformed and newly synthesized mediator secretion.⁴

Kern and Lichtenstein⁵ reported minimal HR from basophils isolated from donors with chronic idiopathic urticaria (CIU) to an anti-IgE stimulus and that the responsiveness of their basophils normalized as their symptoms improved. A subpopulation of patients with CIU (40%) has serum autoantibodies directed to either the FcεRI α subunit or IgE.⁶ Furthermore, a recent study demonstrated that basophils downregulate expression of Syk protein after chronic (18 hours) anti-IgE or suboptimal antigen stimulation.⁷ Therefore in our study we tested the hypothesis that a deficiency of Syk kinase was responsible for the reduced secretion in basophils from patients with CIU.

Healthy adult volunteers and those with either CIU or cold urticaria were recruited from the Johns Hopkins Asthma and Allergy Center. After informed consent through a protocol approved by the Western Institutional Review Board, subjects underwent venipuncture. For HR assays, venous blood was subjected to dextran sedimentation to obtain mixed leukocytes with a basophil purity of less than 1%. Cells were stimulated with anti-IgE (FcεRI) or N-formyl-methionyl-leucyl-phenylalanine (a G protein-coupled receptor). HR was quantified by using an automated fluorometric assay, and the results are expressed as the percentage of total cellular histamine content minus spontaneous release. Basophils were purified by using double Percoll gradients and negative selection to greater than 85% purity. Detergent extracts were prepared and subjected to quantitative Western blotting with an anti-Syk mAb (clone 4D10; Santa Cruz Biotechnology, Santa Cruz, Calif; Fig 1, A, upper panel). A fixed amount of human T-lymphocyte lysate was loaded on every gel and used to normalize the scanned values for Syk obtained by using a digital camera and densitometry. Prior quantification of Syk in 2-fold dilutions of an RBL standard lysate by means of Western blotting established the linear range of staining for the anti-Syk antibody. All film exposures included in the analysis fell within this linear range. Our previous study of basophils isolated from donors with CIU identified a population that also was poorly secreting (<10%) to a 0.1 μg/mL polyclonal anti-IgE dose, which we termed anti-IgE nonresponders (CIU-NRs).⁸ A second population, termed CIU anti-IgE responder basophils (CIU-R basophils), release greater than 10% histamine on 0.1 μg/mL anti-IgE stimulation. All donors tested released histamine to N-formyl-methionyl-leucyl-phenylalanine, with the CIU-NR group having the lowest release. However, no correlation was seen between the presence of a serum autoantibody and CIU-NR status.⁸ As shown in Fig 1, B, 2 of the 7 donors classified as CIU-NR have basophils that are indeed deficient in Syk (NR-1 and NR-2, black bars, normalized based on cell equivalents). However, 8 healthy donors (Fig 1, B) and 5 additional CIU-NR donors (not shown) had basophils with a normal Syk protein level.⁸ In addition, we identified 4 donors classified as CIU-R, CIU-inactive, cold-induced urticaria, or healthy (no urticaria) as Syk deficient of a total of 25 donors tested (Fig 1, B). Stimulation with anti-IgE of the basophils from the CIU-inactive and cold-induced urticaria donors provoked moderate HR. On retesting 4 years later, NR-2 basophils converted to a CIU-R phenotype (R-2), resulting in 19% HR, which is consistent with an improvement of symptoms (L. Gober and S. Saini,

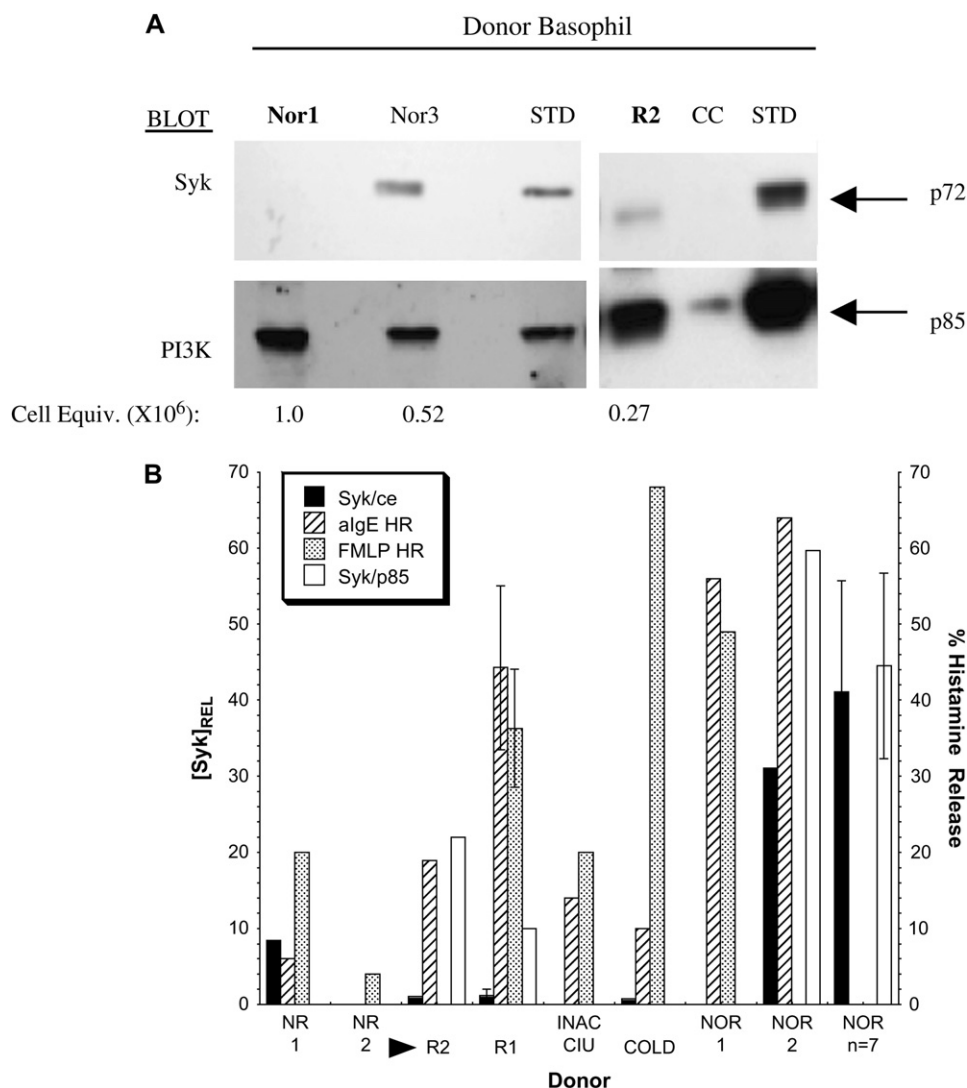


FIG 1. HR in the context of Syk deficiency. **A**, Representative anti-Syk Western blots (Syk) and PI3K reprobes of basophils. *Nor*, Healthy; *STD*, T lymphocyte standard; *R2*, CIU anti-IgE responder 2; *CC*, lysate of column contaminants to correct for basophil purity. **B**, Relative Syk protein expression ($[Syk]_{REL}$) normalized through cell equivalents Syk/ce or PI3K Syk/p85 (Y1 axis). HR (0.1 μ g/mL anti-IgE (algE) or 1 μ mol/L N-formyl-methionyl-leucyl-phenylalanine (FMLP; Y2 axis). *NR*, CIU anti-IgE nonresponder; *R*, CIU anti-IgE responder; *INAC*, inactive CIU; *COLD*, cold-induced urticaria; *NOR*, healthy.

unpublished data, 2007). However, to our surprise, the CIU-R1 donor's basophils, tested on 3 separate occasions, produced $44.3\% \pm 10.8\%$ HR, whereas 1 healthy donor (NOR1) had 56% HR. Therefore Syk deficiency does not always prevent HR. Additional mechanisms must exist to bypass the requirement for active Syk kinase in these Syk-deficient but normally secreting basophils.

Syk deficiency was found at a frequency of 42.8% (3/7 donors) in the CIU-NR population versus $\leq 20\%$ in the CIU-R, cold CIU, atopic, or healthy donor basophil populations (Table I, $n = 5-9$).^{8,9} χ^2 Analysis indicates that Syk deficiency is overrepresented in the CIU-NR group compared with the CIU-R, Cold CIU, atopic (human recombinant histamine releasing factor responders), or healthy donors ($P < .05$). All 7 Syk-deficient basophil lysates contained additional signaling proteins, as determined by means of

Western blotting (p85 phosphoinositide-3 kinase [PI3K] [Upstate Biotechnology, Lake Placid, NY]; Fig 1, A, lower panel; 6/7 donors), and Src homology domain 2-containing inositol phosphatase 1 and 2 (data not shown, 7/7 donors).⁸ Fig 1, B (open bars), depicts the Syk expression data normalized to p85 PI3K expression for a subset of the donors. The T-cell standard was not detected on the 4 oldest blots (from January 2002 to August 2002), likely because of the age of the blots. By means of cell equivalent normalization, our Syk-deficient donors ($n = 6$) range from 0 to 8.5 relative units, whereas healthy donors were 41 ± 14.7 units (mean $\pm$ SEM, $n = 7$). By means of p85 PI3K normalization, our 2 Syk-deficient CIU-R donors were 10 and 22 relative units versus 44.5 ± 12.2 units for healthy donor basophils. As such, both normalization methods gave similar results.

TABLE I. Syk protein expression and HR in basophils stratified by disease category

Basophil donor	Syk, + or –	No. HR positive to anti-IgE (%)*	No. HR negative to anti-IgE (%)*
CIU-R	+	5	0
CIU-R	–	1 (16.6%)	0
CIU-NR	+	0	4
CIU-NR	–	0	3 (42.8%)
Normal	+	8	0
Normal	–	1 (11.1%)	0
HrHRF-R	+	5	0
HrHRF-R	–	0	0
Cold CIU	+	4	0
Cold CIU	–	1 (20%)	0
Inactive CIU	+	0	0
Inactive CIU	–	1	0

HrHRF-R, Human recombinant histamine releasing factor responders (atopic subjects with $\geq 10\%$ HR to HrHRF-R).

*Percentage of total subjects.

Our Syk-deficient CIU donors contain sufficient IgE to allow a normal Fc ϵ RI expression on their basophils, eliminating a lack of Fc ϵ RI as the cause of their hypodegranulation.* CIU-NR basophil HR averages were less than 12% at all anti-IgE doses tested (0.01–3 μ g/mL).⁸ In conclusion, we provide preliminary evidence that anti-IgE-mediated degranulation is intact in a subset of donor basophils, despite Syk deficiency. Additionally, the NOR-NR phenotype, previously reported only in healthy donors, exists in diseases such as CIU.

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*The serum IgE levels were quantified by means of FEIA and were found to be 15 IU for NR-1, 38 IU for NR-2, 47 IU for NR-3 (R-2 on reassay), and 526 IU for R-1 (an atopic subject).