

Original Article**STAT5 in Human Basophils: IL-3 Is Required for Its FcεRI-Mediated Phosphorylation**

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Basophils are key effector cells in allergic inflammatory reactions. However, the mechanisms of FcεRI-induced degranulation are complex and remain to be disentangled. Signal transducer and activator of transcription (STAT) molecules modulate various cell functions. STAT5 appears to be essential for IgE-mediated mast cell function, but its role in human basophils after cross-linking FcεRI is unknown. In this study, STAT5 phosphorylation was investigated by flow cytometry, and combined with analyses of the degranulation marker CD63 at single cell level. Kinetics of STAT5 phosphorylation were studied in basophils of birch pollen allergic patients and showed a fast phosphorylation induced by interleukin (IL)-3, but not with antigen alone. Stimulating basophils with a mixture of allergen and IL-3 resulted in a two to three fold higher phosphorylation of STAT5 than induced by IL-3 alone. In the presence of IL-3, antigen elicited a dose-dependent STAT5 response. In conclusion, this study demonstrates that STAT5 in human basophils is activated through both the IL-3 and the FcεRI signaling pathway. © 2011 International Clinical Cytometry Society

Key terms: basophils; STAT5; IL-3; FcεRI; flow cytometry; CD63; allergen; human; Bet v 1

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Basophils respond to various IgE-dependent and -independent stimuli and exert more than a simple bystander role in initiating and maintaining allergic reactions (1). Basophils are involved in the communication between immunocompetent cells such as macrophages, dendritic cells, T or B lymphocytes and exert major immunoregulatory functions, both in innate and adaptive immunity. First, basophils are critical orchestrators of the Th2-type immune responses due to their capacity to generate Th2 polarizing cytokines (1–3). Second, they contribute to immunoglobulin synthesis and class switching, angiogenesis, autoimmunity, tumor immunity, and hematopoiesis (4,5). Finally, there is evidence that (murine) basophils might act as antigen presenting cells (6–8). However, the mechanisms that govern basophil activation upon encounter of specific allergens that cross-link high-affinity IgE receptors (FcεRI) are complex and remain incompletely elucidated. It is likely that the final response that leads to degranulation with release of mediators is controlled by the cross talk of activatory and inhibitory signals.

Signal transducer and activator of transcription (STAT) molecules modulate several cell functions by activating specific genes in various cell types (9,10). It has been disclosed that STAT5 is essential for development and expansion of both murine mast cells and basophils (11–13). An important activator of STAT5 in these cells is interleukin (IL)-3, which plays a fundamental role in mast cell and basophil biology (14,15). Murine models have revealed that activation of STAT5 is also essential for IgE-mediated mast cell activation that includes

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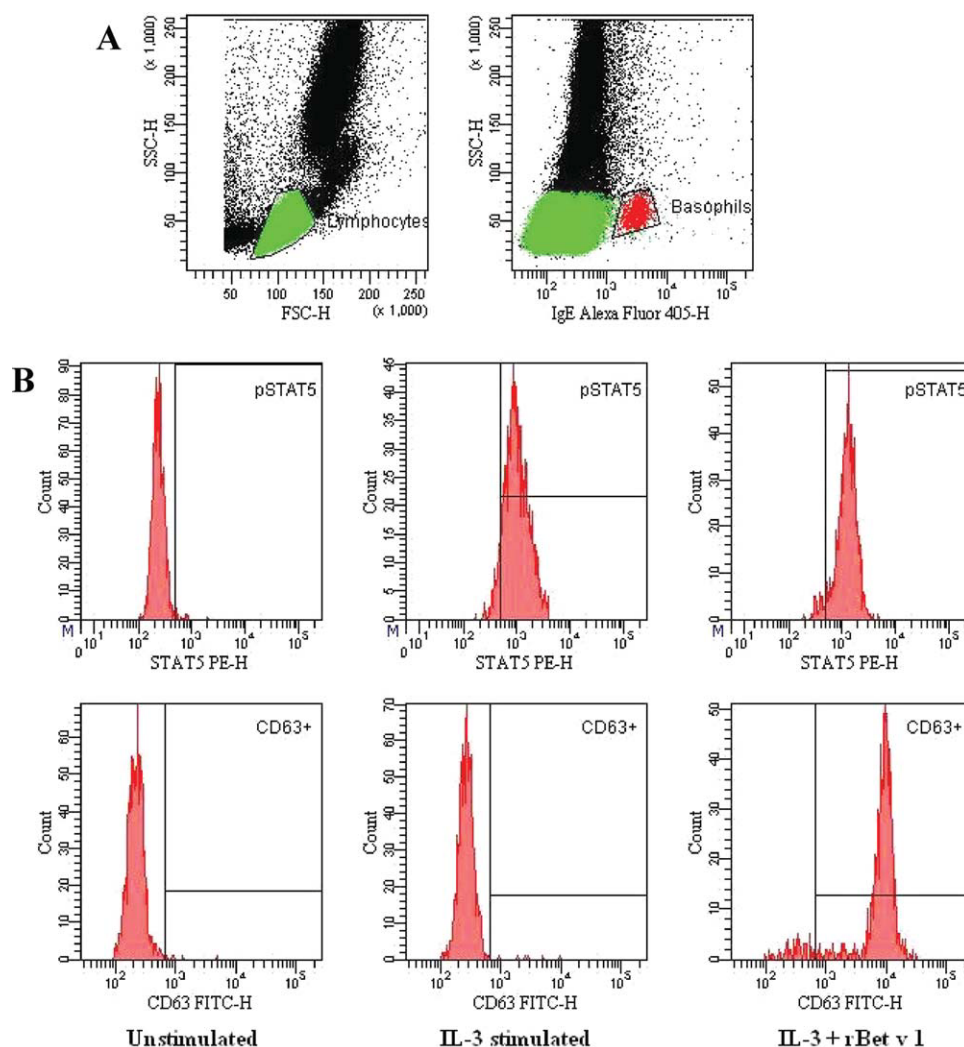


FIG. 1. Flow cytometric analysis of pSTAT5 and CD63 in basophils in a representative sample. **A:** Lymphocytes are selected based on forward and side scatter. From these lymphocytes basophils are gated out based on their IgE expression. **B:** Histograms for pSTAT5 and CD63 expression in basophils after different stimuli. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

degranulation and cytokine production (16,17). Alternatively, IL-10 was shown to inhibit IgE induced STAT5 phosphorylation (17).

In contrast to murine mast cells, antigen was not able to induce STAT5 phosphorylation in human mast cells (18). This discrepancy probably reflects different signaling pathways in human and murine mast cells. However, to the best of our knowledge, the effect of antigen on STAT5 phosphorylation has currently not been studied in human basophils. Therefore, we sought to investigate the effect of IL-3, IL-10, and Fc ϵ RI cross-linking on the possible phosphorylation of STAT5 in human basophils by flow cytometry.

METHODS

Subjects

Blood samples from 15 birch pollen allergic patients, 11 male and 4 female, median age 23 years (range

7–49), were included to study phosphorylation of STAT5 (pSTAT5) kinetics by flow cytometry. All patients reported rhinoconjunctivitis and/or asthma during the birch pollen season and their diagnosis was confirmed by positive specific IgE (sIgE > 0.35 kUA/L) to birch pollen, *Betula verrucosa*, (Immuno-CAP FEIA, Phadia AB, Brussels, Belgium) and/or positive skin prick test to birch pollen (HAL, Haarlem, the Netherlands) (19).

Basophil Activation by Cytokines

Endotoxin-free heparinized whole blood samples were obtained from patients and aliquots of 200 μ L whole blood challenged with the cytokines IL-3 (PeproTech, Paris, France) and/or IL-10 (PeproTech, Paris, France) at 37°C. Reactions were stopped on ice. Different cytokine concentrations (1–100 ng/mL) and incubation times (0–60 min) were used to assess the effect of these cytokines on phosphorylation of STAT5 in basophils.

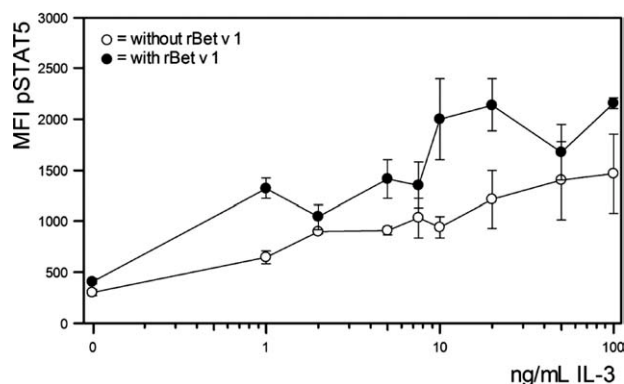


Fig. 2. Dose-dependent upregulation of pSTAT5 induced by IL-3. Cells were incubated with different concentrations of IL-3 during 30 min (open symbols). Next to IL-3 stimulation alone, cells were incubated with a mixture of IL-3 and rBet v 1 (5 min; 0.1 μ g/mL; closed symbols). ($N = 3$).

Basophil Activation by Cross-Linking Fc ϵ R1

Cross-linking of the Fc ϵ R1 was induced with recombinant major birch pollen allergen rBet v 1 (0.001–10 μ g/mL), kindly provided by Stallergenes (Antony, France), or anti-IgE (10 μ g/mL clone G7-18, Pharmingen, BD Biosciences, Erembodegem, Belgium). Various time points were investigated (0–15 min). Stimulation of the cells with allergens was done in the presence or absence of cytokines. Negative control implied stimulation with activation buffer (Hank's balanced salt solution with 20 μ M HEPES and 7.5% NaHCO₃, pH = 7.4). Reactions were stopped on ice.

Immunophenotyping and Intracellular Staining

Basophils were stained for their surface markers with 30 μ L of monoclonal anti-human IgE (clone GE-1, Sigma-Aldrich Chemic GmbH, Steinheim, Germany) labeled with Alexa Fluor 405 (Molecular probes, Invitrogen, Eugene, OR) and 5 μ L of monoclonal anti-human CD63-FITC (clone H5C6, Pharmingen, BD Biosciences) for 20 min at 4°C. Cells were lysed and fixed with Lyse/Fix (Phosflow, BD Biosciences) for 15 min at 37°C. Cells were washed in phosphate-buffered saline (PBS, pH 7.4, Invitrogen) and then permeabilized with PermIII (Phosflow, BD Biosciences) during 30 min at 4°C. Cells were washed again in PBS, stained with 20 μ L anti-pSTAT5 (pY694, Phosflow, BD Biosciences) conjugated with phycoerythrin (PE) and resuspended in 100 μ L PBS during 30 min at room temperature. Afterwards cells were washed again and resuspended in PBS with 0.1% NaN₃.

Flow Cytometric Analysis

Basophils were analyzed with BD FACSCanto II Flow Cytometer (BD Biosciences). A population selected on forward scatter and side scatter and containing mostly lymphocytes and basophils was selected. From this population basophils were gated out based on side scatter and positive IgE staining (Fig. 1). At least 500 basophils were counted. The median fluorescence intensity of

pSTAT5-PE for each sample was measured and standardized using Ultra Rainbow Calibration beads (URCP-38-2K, Spherothec, Lake Forest, IL) for calibration. The membrane expression of CD63 on basophils was expressed as net percentage of positive cells.

Inhibition Experiments

As no specific STAT5 inhibitor was available, the Janus kinase 2 (JAK2) inhibitor WP1066 (Sigma-Aldrich) (20) was used, since the IL-3 induced phosphorylation of STAT5 is performed by JAK2 (21). Next to WP1066 also the effect of Syk-inhibitor Piceatannol (Sigma-Aldrich) (22) was analyzed, as it has been shown that STAT5 is not only phosphorylated by JAK2, but could be phosphorylated by Syk as well (23,24). WP1066 (stock solution 10 mg/mL in DMSO) was further diluted in activation buffer and used in the final concentrations of 100 and 10 ng/mL. Piceatannol (stock solution 10 mg/mL in DMSO) was diluted in activation buffer and used in the final concentrations 40, 4, and 0.5 ng/mL. Inhibitors were added to the cells together with the cytokines. Vehicle (DMSO) experiments were done to exclude toxic effect on basophil activation.

Statistics

All results are expressed as median (range). Nonparametric tests were applied where appropriate. P -values <0.05 were considered as significant. All statistics were performed using SPSS version 16.0 (IBM Corporation, Somers, NY).

RESULTS

Effect of Cytokines Alone on STAT5 Phosphorylation

Stimulation of cells with different concentrations (1–100 ng/mL) of IL-3 for 30 min induced a clear dose-dependent phosphorylation of STAT5 in basophils (Fig. 2: open symbols), but did not elicit an up-regulation of CD63 (data not shown). The submaximal concentration of 5 ng/mL was chosen for further experiments as this leaves space for a possible increase of STAT5 phosphorylation in the different settings.

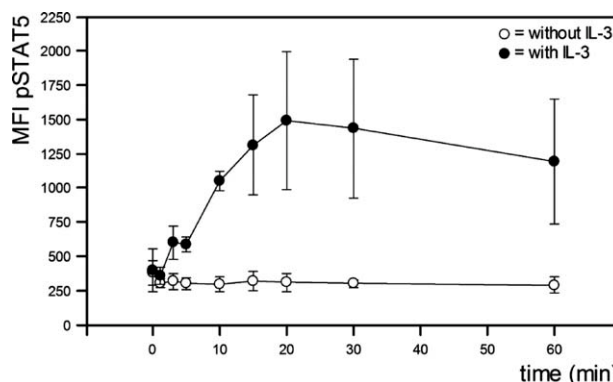


Fig. 3. Kinetics of pSTAT5 after IL-3 stimulation. Cells were stimulated with 5 ng/mL IL-3 (closed symbols) or activation buffer as negative control (open symbols) during different time points. ($N = 3$).

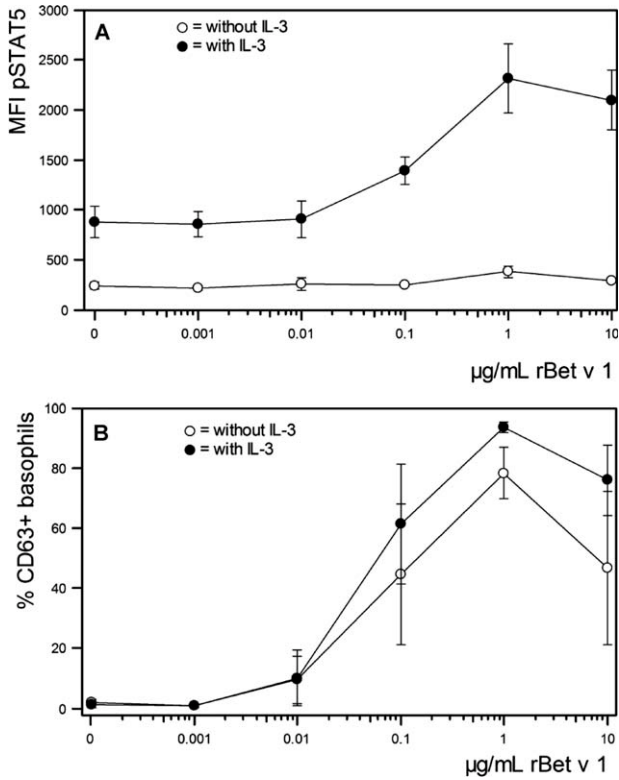


Fig. 4. STAT5 phosphorylation and CD63 expression in basophils after antigen stimulation. Cells were challenged with different concentrations of rBet v 1 for 3 min, with (closed symbols) or without (open symbols) IL-3–5 ng/mL for 20 min. **A:** STAT5 phosphorylation in basophils. **B:** CD63 expression in the same cells. ($N = 3$).

Stimulating the cells with a concentration of 5 ng/mL showed that IL-3 induced STAT5 phosphorylation reaches its maximum after 10–20 min and starts to decrease after 30 min (Fig. 3: closed symbols). Without IL-3, no STAT5 phosphorylation occurred (Fig. 3: open symbols).

In a separate experiment, IL-10 alone or in the presence of IL-3 was added to the cells. However, this cytokine did not affect STAT5 phosphorylation or CD63 upregulation nor inhibit the IL-3 induced pSTAT5 (data not shown).

Effect of Cross-Linking Fc ϵ RI on STAT5 Phosphorylation

To investigate if allergen could induce phosphorylation of STAT5 in human basophils, cells of birch pollen allergic patients were stimulated with different concentrations of rBet v 1 (Fig. 4a: open symbols) and during different time points (data not shown). However, rBet v 1 alone did not elicit pSTAT5, although basophils were clearly activated, as CD63 was upregulated in the same cells (Fig. 4b: open symbols).

Effect of Cross-Linking Fc ϵ RI in the Presence of Cytokines on STAT5 Phosphorylation

Since allergen alone failed to induce pSTAT5, additional analysis were performed in which cells were challenged by a combination of IL-3 and rBet v 1. From these experiments, it appeared that this mixture induced a pSTAT5 response, which was 2 to 3-fold higher than after IL-3 incubation alone (Fig. 4a: closed symbols). Stimulating the cells with different concentrations of rBet v 1, in the presence of IL-3–5 ng/mL, elicited a response which was dose dependent on rBet v 1. The maximal STAT5 response was reached after stimulating with 1 $\mu\text{g/mL}$ rBet v 1 (Fig. 4a: closed symbols). This was based on the maximal basophil activation as also CD63 expression was maximal for 1 $\mu\text{g/mL}$ rBet v 1 (Fig. 4b). The mixture of IL-3 and rBet v 1 enhanced not only pSTAT5, but also the CD63 expression was raised on basophils activated by the mixture compared to rBet v 1 stimulation alone (Fig. 4b: closed symbols).

To investigate if this effect on STAT5 phosphorylation was antigen specific or could be elicited by a specific stimulus as well, stimulation with aEgE was used to

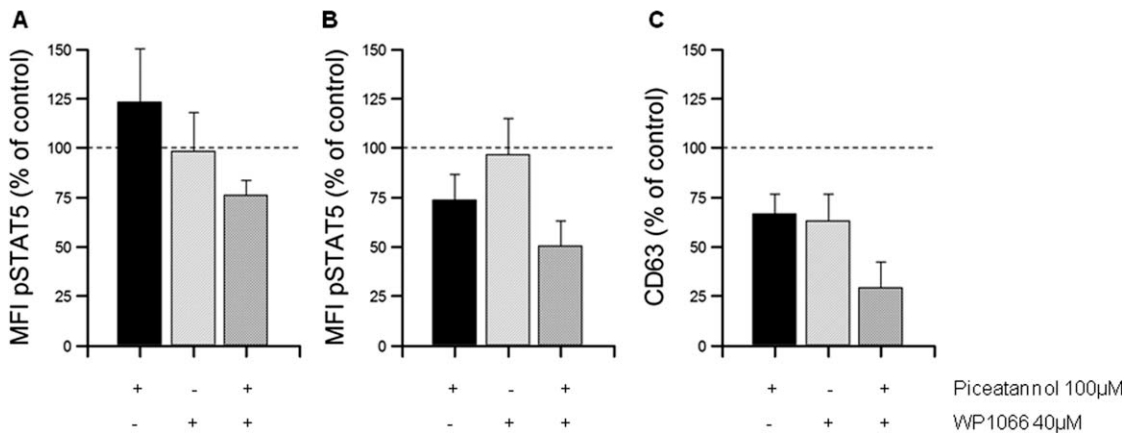


Fig. 5. The effect of Syk inhibitor Piceatannol and JAK2 inhibitor WP1066 on the phosphorylation of STAT5 and CD63 expression in basophils. Cells were incubated with 1 $\mu\text{g/mL}$ rBet v 1 for 3 min and/or IL-3–5 ng/mL for 20 min. Inhibitors were added as indicated. Results are expressed as percentage of STAT5 phosphorylation or CD63 expression in basophils stimulated with Bet v 1 and/or IL-3 without inhibitor. **A:** The effect of inhibitors on STAT5 phosphorylation on IL-3 stimulated cells. **B:** The effect of inhibitors on STAT5 phosphorylation on rBet v 1 and IL-3 stimulated cells. **C:** Inhibition of CD63 expression on basophils after stimulating cells with rBet v 1 and IL-3. ($N = 3$).

cross-link Fc ϵ RI. Both stimuli induced a comparable STAT5 phosphorylation in the presence of IL-3 (data not shown).

Inhibition of pSTAT5

To elucidate possible pathways of STAT5 phosphorylation, inhibition experiments were performed. Neither WP1066 nor Piceatannol reduced STAT5 phosphorylation. In contrast, incubation of basophils with the mixture of WP1066 and Piceatannol resulted in a decrease in STAT5 phosphorylation (Figs. 5A and 5B). Nevertheless, both inhibitors separately induced a dose-dependent inhibition of the CD63 expression (data not shown) and using mixture of both inhibitors resulted in a more pronounced inhibition of CD63 (Fig. 5C).

DISCUSSION

This study demonstrates a significant phosphorylation of STAT5 by IL-3 in human basophils. Besides, although cross-linking of the high-affinity Fc ϵ RI receptor by relevant allergen or anti-IgE alone did not elicit phosphorylation of STAT5, together with IL-3 a quantitatively increased pSTAT5 response was observed.

Traditionally, analysis of signaling in human basophils and mast cells has been performed using Western blot techniques (18,25). Recently, it has been demonstrated that flow cytometry can generate qualitative results concerning dose responses and kinetics of signaling molecules similar and complementary to the data obtained with blotting techniques (19,26–28). Flow cytometry does not require a purification of the basophils. It ensures reliable measurements without interference of additional manipulations and negligible contribution of contaminant cells. In addition, flow cytometry enables characterization of cells with heterogenic responsiveness rather than providing results that represent merely a mean value for the total isolated cell population.

Stimulation of the basophils with IL-3 was confirmed to induce a rapid, dose-dependent and sustained phosphorylation of STAT5 that peaks after 10–20 min and remains on plateau for over 1 h (25). However, as expected, this was not associated with degranulation of the cells (as assessed by the upregulation of CD63) (29,30).

Next to IL-3, the effect of IL-10 was examined as this cytokine has been shown to inhibit histamine release from basophils (31) and temper IL-3 and antigen induced pSTAT5 in murine cells (17). In our settings, IL-10 failed to affect STAT5, in contrast with the data obtained by Kennedy et al. (17). This discrepancy might relate to differences in materials, for example, the use of fresh human basophils in their natural environment versus IL-3 cultured murine mast cells and/or study protocols, for example, stimulation with IL-10 of 20 min compared to 4 days.

In contrast to IL-3 stimulation, cross-linking of Fc ϵ RI by allergen or anti-IgE antibodies rapidly triggered basophil activation without demonstrable phosphorylation of STAT5. Although these findings contrast to studies

performed in murine models (16,17), our results parallels the data by Hundley et al. (18) who observed that Kit ligand but not antigen stimulated the phosphorylation of STAT5 in human mast cells.

Although IL-3 was confirmed not to upregulate CD63 (29,30) and allergen did not trigger STAT5 phosphorylation, the observation that a mixture of IL-3 and allergen significantly augmented both phosphorylation of STAT5 and CD63 expression endorses the idea of a cross-talk between these two distinct signaling pathways. This is further illustrated by the inhibition experiments as only a mixture of WP1066 and Piceatannol was able to reduce STAT5 phosphorylation. Furthermore, since CD63 upregulation was inhibited by the syk inhibitor and JAK2 inhibitor, it seems that although STAT5 phosphorylation may not cause basophil degranulation, blocking the JAK2/STAT5 pathway resulted in a reduced degranulation as assessed by CD63.

The exact function of STAT5 phosphorylation in IL-3-activated basophils remains elusive. Since IL-3 has been demonstrated to increase synthesis and expression of the Fc ϵ RI β (25,32), probably in a STAT5 dependent manner (25), it can be hypothesized that IL-3 induced STAT5 phosphorylation might affect basophil responsiveness as the β -chain amplifies Fc ϵ RI signaling (33). Interestingly, basophils regulate their own priming with IL-3 by releasing autocrine IL-3 after activation of the basophils with anti-IgE (34), which might affect basophil sensitivity even more.

In conclusion, in this study, we investigated the role of STAT5 phosphorylation in human basophils and showed that not only IL-3 is able to induce STAT5 phosphorylation but that also the Fc ϵ RI signaling pathway affects this molecule.

LITERATURE CITED

1. Gibbs BF. Human basophils as effectors and immunomodulators of allergic inflammation and innate immunity. *Clin Exp Med* 2005;5:43–49.
2. Sokol CL, Medzhitov R. Role of basophils in the initiation of Th2 responses. *Curr Opin Immunol* 2010;22:73–77.
3. Siracusa MC, Perrigoue JG, Comeau MR, Artis D. New paradigms in basophil development, regulation and function. *Immunol Cell Biol* 2010;88:275–284.
4. Crivellato E, Travan L, Ribatti D. Mast cells and basophils: a potential link in promoting angiogenesis during allergic inflammation. *Int Arch Allergy Immunol* 2010;151:89–97.
5. Jensen-Jarolim E, Achatz G, Turner MC, Karagiannis S, Legrand E, Capron M, Penichet ML, Rodríguez JA, Siccardi AG, Vangelista L, Riemer AB, Gould H. AllergoOncology: The role of IgE-mediated allergy in cancer. *Allergy* 2008;63:1255–1266.
6. Yoshimoto T, Yasuda K, Tanaka H, Nakahira M, Imai Y, Fujimori Y, et al. Basophils contribute to T(H)2-IgE responses in vivo via IL-4 production and presentation of peptide-MHC class II complexes to CD4⁺ T cells. *Nat Immunol* 2009;10:706–712.
7. Sokol CL, Chu NQ, Yu S, Nish SA, Laufer TM, Medzhitov R. Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. *Nat Immunol* 2009;10:713–720.
8. Perrigoue JG, Saenz SA, Siracusa MC, Allenspach EJ, Taylor BC, Giacomini PR, et al. MHC class II-dependent basophil-CD4⁺ T cell interactions promote T(H)2 cytokine-dependent immunity. *Nat Immunol* 2009;10:697–705.
9. Chen W, Khurana Hershey GK. Signal transducer and activator of transcription signals in allergic disease. *J Allergy Clin Immunol* 2007;119:529–541.
10. Paukku K, Silvennoinen O. STATs as critical mediators of signal transduction and transcription: Lessons learned from STAT5. *Cytokine Growth Factor Rev* 2004;15:435–455.

11. Shelburne CP, McCoy ME, Piekorz R, Sexl V, Roh KH, Jacobs-Helber SM, et al. Stat5 expression is critical for mast cell development and survival. *Blood* 2003;102:1290-1297.
12. Ikeda K, Nakajima H, Suzuki K, Watanabe N, Kagami S, Iwamoto I. Stat5a is essential for the proliferation and survival of murine mast cells. *Int Arch Allergy Immunol* 2005;137 Suppl1:45-50.
13. Ohmori K, Luo Y, Jia Y, Nishida J, Wang Z, Bunting KD, et al. IL-3 induces basophil expansion in vivo by directing granulocyte-monocyte progenitors to differentiate into basophil lineage-restricted progenitors in the bone marrow and by increasing the number of basophil/mast cell progenitors in the spleen. *J Immunol* 2009;182:2835-2841.
14. Valent P, Dahinden CA. Role of interleukins in the regulation of basophil development and secretion. *Curr Opin Hematol* 2010;17:60-66.
15. Lantz CS, Boesiger J, Song CH, Mach N, Kobayashi T, Mulligan RC, et al. Role for interleukin-3 in mast-cell and basophil development and in immunity to parasites. *Nature* 1998;392:90-93.
16. Barnstein BO, Li G, Wang Z, Kennedy S, Chalfant C, Nakajima H, et al. Stat5 expression is required for IgE-mediated mast cell function. *J Immunol* 2006;177:3421-3426.
17. Kennedy NS, Barnstein B, Brenzovich J, Bailey DP, Kashyap M, Speiran K, et al. IL-10 suppresses mast cell IgE receptor expression and signaling in vitro and in vivo. *J Immunol* 2008;180:2848-2854.
18. Hundley TR, Gilfillan AM, Tkaczuk C, Andrade MV, Metcalfe DD, Beaven MA. Kit and FcεpsilonRI mediate unique and convergent signals for release of inflammatory mediators from human mast cells. *Blood* 2004;104:2410-2417.
19. Aerts NE, Dombrecht EJ, Bridts CH, Hagendorens MM, De Clerck LS, Stevens WJ, et al. Simultaneous flow cytometric detection of basophil activation marker CD63 and intracellular phosphorylated p38 mitogen-activated protein kinase in birch pollen allergy. *Cytometry Part B* 2008;76B:8-17.
20. Ferrajoli A, Faderl S, Van Q, Koch P, Harris D, Liu Z, et al. WP1066 disrupts Janus kinase-2 and induces caspase-dependent apoptosis in acute myelogenous leukemia cells. *Cancer Res* 2007;67:11291-11299.
21. Guthridge MA, Stomski FC, Thomas D, Woodcock JM, Bagley CJ, Berndt MC, et al. Mechanism of activation of the GM-CSF, IL-3, and IL-5 family of receptors. *Stem Cells* 1998;16:301-313.
22. Tkaczuk C, Villa I, Peronet R, David B, Mecheri S. FcεpsilonRI-mediated antigen endocytosis turns interferon-gamma-treated mouse mast cells from inefficient into potent antigen-presenting cells. *Immunology* 1999;97:333-340.
23. Ng SW, Nelson C, Parekh AB. Coupling of Ca(2+) microdomains to spatially and temporally distinct cellular responses by the tyrosine kinase Syk. *J Biol Chem* 2009;284:24767-24772.
24. Kanie T, Abe A, Matsuda T, Kuno Y, Towatari M, Yamamoto T, et al. TEL-Syk fusion constitutively activates PI3-K/Akt, MAPK and JAK2-independent STAT5 signal pathways. *Leukemia* 2004;18:548-555.
25. Miura K, Saini SS, Gauvreau G, MacGlashan DW Jr. Differences in functional consequences and signal transduction induced by IL-3, IL-5, and nerve growth factor in human basophils. *J Immunol* 2001;167:2282-2291.
26. Haas A, Weckbecker G, Welzenbach K. Intracellular Phospho-Flow cytometry reveals novel insights into TCR proximal signaling events. A comparison with Western blot. *Cytometry Part A* 2008;73A:799-807.
27. Lin CC, Huang WL, Su WP, Chen HH, Lai WW, Yan JJ, et al. Single cell phospho-specific flow cytometry can detect dynamic changes of phospho-Stat1 level in lung cancer cells. *Cytometry Part A* 2010;77A:1008-1019.
28. Verweij MM, De Knop KJ, Bridts CH, De Clerck LS, Stevens WJ, Ebo DG. P38 mitogen-activated protein kinase signal transduction in the diagnosis and follow up of immunotherapy of wasp venom allergy. *Cytometry Part B* 2010;78B:302-307.
29. Ebo DG, Dombrecht EJ, Bridts CH, Aerts NE, De Clerck LS, Stevens WJ. Combined analysis of intracellular signalling and immunophenotype of human peripheral blood basophils by flow cytometry: A proof of concept. *Clin Exp Allergy* 2007;37:1668-1675.
30. Hauswirth AW, Sonneck K, Florian S, Krauth MT, Bohm A, Sperr WR, et al. Interleukin-3 promotes the expression of E-NPP3/CD203C on human blood basophils in healthy subjects and in patients with birch pollen allergy. *Int J Immunopathol Pharmacol* 2007;20:267-278.
31. Pierkes M, Bellinghausen I, Hultsch T, Metz G, Knop J, Saloga J. Decreased release of histamine and sulfidoleukotrienes by human peripheral blood leukocytes after wasp venom immunotherapy is partially due to induction of IL-10 and IFN-gamma production of T cells. *J Allergy Clin Immunol* 1999;103:326-332.
32. Saini SS, Richardson JJ, Wofsy C, Lavens-Phillips S, Bochner BS, MacGlashan DW Jr. Expression and modulation of FcεpsilonRIα and FcεpsilonRIβ in human blood basophils. *J Allergy Clin Immunol* 2001;107:832-841.
33. Kraft S, Rana S, Jouvin MH, Kinet JP. The role of the FcεpsilonRI beta-chain in allergic diseases. *Int Arch Allergy Immunol* 2004;135:62-72.
34. Schroeder JT, Chichester KL, Bieneman AP. Human basophils secrete IL-3: Evidence of autocrine priming for phenotypic and functional responses in allergic disease. *J Immunol* 2009;182:2432-2438.