Published in final edited form as:

Curr Opin Allergy Clin Immunol. 2010 February ; 10(1): 42–47. doi:10.1097/ACI.0b013e328334f642.

Signaling Pathways Critical for Allergic Airway Inflammation

John D. Colgan and Isaiah L. Hankel

Roy J. and Lucille A. Carver, College of Medicine, Department of Internal Medicine, University of Iowa, Iowa City, IA, USA

Abstract

Purpose of review—Activated mast cells, basophils, and CD4⁺ helper T cells have critical roles in allergic inflammation. Therefore, devising ways to specifically inhibit these cells will likely be useful for controlling allergic inflammation. We summarize recent findings regarding the role of mast cells and basophils in allergic responses and the regulation of signaling pathways downstream the IgE receptor, the chief inducer of mast cell and basophil activation. We also highlight studies addressing the roles of the protein tyrosine kinases Zap-70 and Itk in immune system development and in the regulation of CD4⁺ helper T cell responses.

Recent findings—Recent work has demonstrated that mast cell function is unexpectedly diverse and that basophils have a more prominent role in Th2-type immune responses than previously appreciated. Biochemical analysis of the IgE receptor signaling pathway has led to insights regarding the roles of phosphatases and other enzymes in this process. Studies of Zap-70 and Itk have helped to define the potential outcomes and complications of inhibiting these enzymes in order to suppress allergic inflammation.

Summary—Analysis of genetically engineered mice and biochemical studies continue to help unravel the molecular pathways that drive allergic inflammatory reactions. The knowledge acquired may lead to novel approaches for suppressing allergic inflammation.

Keywords

Intracellular signaling; immunoglobulin E receptor; T cell receptor; protein and inositol phosphatases; protein tyrosine kinases

Introduction

Allergic inflammatory reactions can be either acute (Type I) or delayed (Type IV). Acute reactions are initiated by contact between allergen and IgE bound to receptor complexes expressed on basophils and mast cells, resulting in physiologic responses within seconds. In the respiratory tract, acute allergic reactions cause coughing, sneezing and congestion and, in rare cases, can result in anaphylaxis. Delayed allergic reactions are promoted by cells within the innate and adaptive immune systems, with symptoms appearing several hours after allergen is encountered. In the airway, delayed reactions drive asthma development as manifested by chronic airway inflammation that leads to airway obstruction, airway hyper-responsiveness and lung tissue remodeling. These responses compromise lung function and, in severe cases, can cause death. Helper T cells that secrete IL-4, -5 and -13, together with mast cells and basophils, are the dominant players in chronic allergic inflammation. Substantial progress has been made in defining the intracellular signaling pathways that regulate these cell types, with

the goal being to define molecular targets for compounds that can be used as therapeutics for allergic inflammation. Here, we summarize recent findings regarding how mast cells and basophils contribute to allergic inflammation and discuss recent progress in characterizing signaling pathways that regulate these cells. We also highlight findings from studies that address how protein tyrosine kinases regulate T cell development and function and discuss the prospect of inhibiting these enzymes as a means to suppress allergic inflammation.

Mast cells and basophils in allergic inflammation

Recent studies have examined how mast cells contribute to host defense and revealed links between these cells and diseases with inflammatory components [1]. Evidence that mast cells suppress immune responses has also emerged [2]. As hypothesized by Kalesnikoff and Galli [1], the diversity of functions attributed to mast cells suggests that this compartment is comprised of specialized subsets rather than a homogeneous population.

Mast cells are derived bone marrow progenitors and enter the bloodstream in an immature form. These cells migrate through blood vessel endothelium and localize to vascularized connective tissue beneath epithelial layers, including those that form the skin and line the digestive and respiratory tracts. Progenitors then differentiate into cells capable of effector function; these express the high affinity FcεR1, which constitutively binds IgE. Interaction between receptor-bound IgE and antigen results in mast cell activation and the release of multiple inflammatory mediators. Via this mechanism, mast cells initiate and amplify acute allergic inflammation and also contribute to chronic allergic responses. The latter role was further defined by recent studies showing that mast cells indirectly supply antigen for presentation to CD4⁺ T cells and can provide costimulatory signals to these cells [3,4].

Basophils are derived from bone marrow progenitors and enter the blood as mature cells. Circulating basophils enter peripheral tissues in response to inflammation and promote the late phase of acute allergic reactions. Like mast cells, basophils express the FcεR1 and are activated by antigen-induced aggregation of the receptor. Basophils can also be stimulated by various cytokines [5]. For example, recent reports showed that IL-33 produced by epithelial cells and other cell types activates basophils as well as mast cells [6–12]. Sokol *et al.* demonstrated that the protease allergen papain elicits basophil activation, inducing their migration to draining lymph nodes and thus causing enhanced Th2 differentiation by CD4⁺ T cells [13]. These results suggest that basophils participate in immune responses to protease allergens, which have a significant role in human allergic disease.

Emerging data suggest that basophils have greater influence on adaptive Th2 responses than previously thought [5,14]. Denzel *et al.* showed that, upon secondary exposure to antigen, basophils activated via FcεR1 engagement augment immunoglobulin production by interacting with B cells and providing IL-4 and -6 [15]. These data indicate that basophils have a role in memory humoral immune responses. Three publications collectively demonstrated that basophils present peptide-MHC II complexes to CD4⁺ T cells while secreting IL-4, thus initiating Th2 cell differentiation [16–18]. These data suggest that antigen presentation by basophils has a key role in the generation of allergen-specific Th2 cells in humans. It will be important to determine whether basophils present antigen to memory Th2 cells, which likely drive chronic allergic responses. Charles *et al.* demonstrated that the absence of Lyn, which has a key role in suppression of FcεR1 signaling, causes basophilia and strong skewing toward Th2-type responses [19]. These studies support the hypothesis that targeted suppression of FcεR1 signaling could be effective for controlling chronic, as well as acute, allergic inflammation. Overall, these findings indicate that further characterization of basophil function will reveal novel strategies for suppressing allergic disease.

New insights regarding the IgE receptor signaling pathway

Interaction between FcεR1-bound IgE and antigen induces an elaborate network of signaling pathways that elicits cell activation (Figure 1). The FcεR1 consists of an IgE-binding α chain, a β chain and a disulfide-linked γ chain homodimer. The cytoplasmic tails of the β and γ subunits each contain ITAMs, which are targets for Src and Syk family protein tyrosine kinases. FcεR1 engagement activates the SFKs Fyn and Lyn by an undefined mechanism, leading to ITAM phosphorylation and thus the generation of high-affinity docking sites for proteins that contain SH2 domains. These include Fyn, Lyn and the protein tyrosine kinase Syk. All three likely bind the phosphorylated ITAMs, promoting trans- and auto-phosphorylation events that increase kinase activity and result in the tyrosine phosphorylation of multiple downstream substrates. Chief among these are the proteins LAT and LAT2 [LAB/NTAL], which are phosphorylated on several tyrosines and thus associate with multiple SH2 domain-containing proteins. These include the adapter proteins SLP-76, Grb2, Gab2 and Gads and the GTP exchange factors SOS and Vav [2,20]. The latter two proteins activate Ras, which induces MAPK signaling cascades. SOS, Vav and Gab2 also promote activation of PI3-kinase, which generates membrane-docking sites for proteins containing pleckstrin homology domains. These include the key enzyme PLCγ, which associates with the membrane and LAT/LAT2-containing complexes and becomes activated via tyrosine phosphorylation, generating second messenger molecules that induce calcium flux and protein kinase C activity. These events, combined with signals provided by MAPK cascades, induce the multiple events associated with mast cell and basophil activation.

Given their critical roles in FcεR1 signaling, the protein tyrosine kinases Fyn, Lyn and Syk continue to be the focus of intense investigation and are discussed in recently published reviews [21,22]. The central role of Syk in promoting FcεR1 signaling has made this molecule a predominant target for molecular inhibitors. However, recent reports have implicated Syk in neutrophil function and phagocytosis [23,24] while others have demonstrated that Syk is important for mechanisms that detect fungal infections [25,26]. These findings emphasize the need to assess how targeted inhibition of Syk affects immune responses to microorganisms. Such studies will help determine whether molecules that inhibit Syk function can suppress allergic inflammatory responses without impairing host defense.

Recent reports have illustrated how protein tyrosine phosphatases regulate FcεR1 signaling. Grochoway *et al.* re-examined the role of the phosphatase CD45 using mouse BMMCs [27]. Lack of CD45 dramatically reduced degranulation, calcium flux and IL-6 production, which correlated with increased inhibitory phosphorylation of Lyn. Reciprocally, a hyperactive variant of CD45 increased FcεR1 signaling, but, surprisingly, failed to rescue Lyn from increased inhibition. Instead, greater PI3-kinase activity was observed, likely due increased Fyn activation. These results support the proposal by Weiss and colleagues that CD45 inhibition could be effective for controlling allergic disease [28]. Akimoto *et al.* [29] showed that protein tyrosine phosphatase epsilon (PTPε) negatively regulates FcεR1 signaling by suppressing Syk activation. Consistent with this conclusion, the responses of PTPε-deficient mice to anaphylactic challenge were exaggerated. *Motheaten* mice lack the tyrosine phosphatase SHP-1 and spontaneously develop immunodeficiency and autoimmunity. Nakata *et al.* [30] demonstrated that the absence SHP-1 increased phosphorylation of LAT and SLP-76 and MAPK activation but did not affect the upstream regulator Syk. Paradoxically, PLCγ activation and calcium flux were impaired, suggesting that the adaptor function by SHP-1 is important or that SHP-1 functions to counteract inhibitory pathways. McPherson *et al.* assessed the role of the tyrosine phosphatase SHP-2 in FcεR1 signaling [31]. Although degranulation by SHP-2-deficient cells was normal, the level of inhibitory Fyn phosphorylation was increased and correlated with reduced activation of the downstream Fyn targets Gab2 and PI3-kinase. In contrast, the levels of inhibitory and activating phosphorylation of Lyn were normal and

increased, respectively. These results suggest that SHP-2 has a role in maintaining the balance between Fyn and Lyn activity. Such regulation is likely important given the dual activating and inhibitory roles of Lyn [21].

FcεR1 signaling activates PI3-kinase, which generates phosphatidylinositol 1,4,5- triphosphate [PIP3] and thus membrane docking sites for other signaling proteins [32]. PIP3 is converted to PIP2 by the inositol phosphatases SHIP-1, SHIP-2 and PTEN, resulting in downregulation of signaling. To assess the role of these proteins in FcεR1 signaling, RNA interference was employed by different groups to knockdown expression of SHIP-1, SHIP-2 or PTEN. It was previously shown that the absence of SHIP-1 increases FcεR1 signaling and cell activation [33]. Langdon *et al.* extended these results by showing that knocking down SHIP-1 in bone marrow-derived human basophils caused greater release of inflammatory mediators upon FcεR1 engagement [34]. Leung and Bolland showed that knockdown of SHIP-2 in mouse BMMCs augments degranulation and microtubule formation while increasing transcription of IL-4 and -13 RNA [35]. These effects correlated with increased activation of the PIP3-dependent kinase Akt and the small GTPase Rac-1, which both regulate microtubule assembly. Saini *et al.* reported that SHIP-2 expression is significantly lower in peripheral blood mast cells from chronic idiopathic urticaria [CIU] patients [36], which correlated with higher levels of Syk and increased spontaneous histamine release. Furumoto *et al.* showed that PTEN knockdown in human mast cells increases calcium flux and Akt activation and elicits spontaneous IL-8 and GM-CSF secretion [37]. Taken together, these reports suggest that strategies for augmenting inositol phosphatase activity could be effective for treating allergic inflammatory reactions.

CD4⁺ T cells and chronic allergic inflammation

Responses by CD4⁺ T cells have a central role in chronic allergic inflammatory responses. Upon engagement of the TCR by peptide-MHCII complexes, naive CD4⁺ T cells proliferate and differentiate into effector cells that can be classified into 4 groups: Th1 cells that produce interferon-γ [IFNγ]; Th2 cells, which generate IL-4, IL-5, IL-13, and IL-25; Th17 cells that secrete IL-17A, IL-17F, IL-21 and IL-22; and T regulatory [Treg] cells, which produce suppressive cytokines. Additional effector CD4⁺ T cell subsets, such as Th9 and T follicular helper cells, have recently been described [38,39], suggesting that the biology of CD4⁺ T cells is more complex than previously thought. Nevertheless, there is abundant evidence that Th2 cells have a central role in chronic allergic inflammatory disease [40]. For example, assessment of asthma patients has shown that airway infiltration by Th2-type CD4⁺ T cells is a prominent feature of allergic airway inflammation [41]. Yet, it is becoming increasingly apparent that Th17 and Treg cells among others influence immune responses in the lung [38,42,43]. Thus, manipulation of these cells may prove useful for controlling asthma and other diseases.

The T cell receptor signaling pathway

The signaling pathways downstream of the TCR [44] and the FcεR1 are similar in many respects. The TCR consists of antigen-specific α and β chains associated with CD4 and the CD3 complex (Figure 2). Receptor engagement induces phosphorylation of ITAMs in the cytoplasmic tails of CD3ζ subunits by the SFK Lck, resulting in recruitment of the Syk family kinase Zap-70. Cross- and auto-phosphorylation upregulates Zap-70 kinase activity, which, together with Lck and the Tec kinase Itk, mediates phosphorylation of LAT and SLP-76. As in the FcεR1 signaling pathway, LAT and SLP-76 support the formation of a complex containing PLCγ1. The resulting increases in calcium flux and MAPK activity induced by PLCγ1 and other pathways lead to the induction of multiple transcription factors and changes in cytoskeletal structure associated with T cell activation. Notably, the Tec family kinase Itk functions to phosphorylate PLCγ1 and thus amplifies calcium flux and ERK MAPK activation.

T cell protein tyrosine kinases and allergic inflammation

The critical roles of protein tyrosine kinases in the TCR signaling pathway makes these enzymes attractive targets for inhibitors that could be used to suppress chronic allergic inflammatory responses. In this regard, Zap-70 and Itk have received the most attention with recent studies underscoring the complex role that each kinase plays in immune system development and in CD4⁺ T cell responses.

Zap-70 is required for normal T cell development in both mice and humans [45]. Structural analysis by Deindl *et al.* showed that Zap-70 is auto-inhibited by a mechanism involving intramolecular interactions [46]. Stabilizing this arrangement with a small molecule could be an effective way to suppress Zap-70 activity. To date, no small molecule inhibitors of Zap-70 have been reported. Zap-70 contains tyrosine residues that have been implicated in both negative regulation and interactions with other proteins that promote TCR signaling. Hsu *et al.* created mice expressing a form of Zap-70 containing alanine in place of the relevant tyrosines and found that the function of Zap-70 was compromised [47]. These mice developed rheumatoid factor [i.e. antibodies for IgG] but not overt autoimmune disease. Siggs *et al.* characterized mice expressing two different Zap-70 variants, each functionally impaired due to an amino acid substitution [48]. Zap-70 mutant mice developed hyperglobulinemia and autoantibodies and responded poorly to foreign antigen. The data reported by Hsu *et al.* and Siggs *et al.* suggest that inhibiting Zap-70 beyond a certain threshold could lead to autoimmune responses and compromised host defense. However, both studies found that decreased Zap-70 function affected thymic T cell development, suggesting that the autoimmune responses observed could be due to defects in negative selection or Treg development. In contrast to these data, Picard *et al.* reported that signs of autoimmunity were absent in an immunodeficient patient expressing a form of Zap-70 with reduced function [49]. Guided by structural data, Levin *et al.* generated Zap-70 variants that retain normal kinase activity but are sensitive to a small molecule inhibitor [50]. Mice expressing these Zap-70 variants instead of the normal protein would be predicted to undergo normal T cell development. If so, such mice will be a useful tool for determining how pharmacological inhibition of Zap-70 affects the function of the mature immune system.

Itk represents an intriguing target for inhibitors that could be used to treat chronic allergic inflammation. Indeed, several studies have provided evidence that Itk is crucial for the responses of effector Th2 cells to secondary stimulation [51]. In contrast, the responses of Th1 effector cells to such secondary challenge are less affected, likely due to expression of the functionally redundant Tec kinase Rlk in Th1 cells. Gomez-Rodriguez *et al.* recently reported that the absence of Itk expression decreased the ability of Th17-differentiated cells to produce IL-17A, but had no effect on the synthesis of other Th17 cytokines [52]. These data suggest that the impact of inhibiting Itk on CD4⁺ T cell function may extend beyond the Th2 subset.

As demonstrated by analysis of gene-deficient mice, Itk is not absolutely required for the development of T cells or other immune cell types [53]. Interestingly, Itk-deficient mice have reduced responses when tested using a model of allergic airway inflammation [54]. Ferrara *et al.* recently showed that this phenotype correlates with reduced airway hyper-responsiveness [55]. Yet, interpretation of data obtained from such studies is complicated by the fact that multiple aspects of immune system development are perturbed in Itk-deficient mice [53]. Two recent reports demonstrated that the $\gamma\delta$ T cell compartment in Itk-deficient mice is expanded and that these cells are required for the increased serum IgE levels seen in Itk-deficient mice [56,57]. This latter effect may have a negative impact on mast cell function, since the responses of Itk-deficient mice to allergen/IgE challenge are decreased [58]. These studies suggest that mice in which Itk expression can be ablated in a lineage-specific manner would be very helpful for clarifying what defects in Itk-deficient mice are responsible for the reduced responses to

experimental asthma challenge. In addition the approach taken with Zap-70, i.e. creation of an Itk variant that is sensitive to a specific inhibitor, would likely be useful for defining the impact of Itk inhibition the function of the mature immune system in the absence of pre-existing developmental defects.

Conclusion

It is well established that mast cells and basophils have important roles in allergic inflammatory responses and that the FcεR1 signaling pathway is a critical inducer of these cells. Further, it there is strong evidence that Th2-type CD4⁺ T cells are the driving force behind chronic allergic inflammatory reactions. More detailed knowledge of the molecular pathways that regulate the functions of these cells will facilitate the development of novel and more specific inhibitors of allergic inflammation, for which there is a critical need. The studies described here help illustrate the complexity of this task but also provide hope that such inhibitors can be developed.

Acknowledgments

We thank our colleagues for providing comments and suggestions regarding this article. The authors declare the absence of conflicts of interest, financial or otherwise. Work in our laboratory is supported by NIH/NIAID grant R01AI067489 (awarded to J.D.C.).

Abbreviations

FcεR1	Immunoglobulin E receptor
TCR	T cell receptor
ITAM	immunoreceptor tyrosine-based activation motif
SHK	Src family protein tyrosine kinase
SH2	Src Homology 2
LAT	linker for activation of T cells
PI3-kinase	Phosphoinositide-3 kinase
PLCγ	phospholipase Cγ

References and recommended reading

1. Kalesnikoff J, Galli SJ. New developments in mast cell biology. *Nat Immunol* 2008;9:1215–1223. [PubMed: 18936782]
2. Galli SJ, Grimbaldston M, Tsai M. Immunomodulatory mast cells: negative, as well as positive, regulators of immunity. *Nat Rev Immunol* 2008;8:478–486. [PubMed: 18483499]
3. Nakae S, Suto H, Iikura M, Kakurai M, Sedgwick JD, Tsai M, Galli SJ. Mast cells enhance T cell activation: importance of mast cell costimulatory molecules and secreted TNF. *J Immunol* 2006;176:2238–2248. [PubMed: 16455980]
- 4*. Kambayashi T, Baranski JD, Baker RG, Zou T, Allenspach EJ, Shoag JE, Jones PL, Koretzky GA. Indirect involvement of allergen-captured mast cells in antigen presentation. *Blood* 2008;111:1489–1496. Shows that mast cells indirectly provide antigen for presentation to CD4⁺ T cells. [PubMed: 18032707]
5. Schroeder JT. Basophils beyond effector cells of allergic inflammation. *Adv Immunol* 2009;101:123–161. [PubMed: 19231594]
6. Kroeger KM, Sullivan BM, Locksley RM. IL-18 and IL-33 elicit Th2 cytokines from basophils via a MyD88- and p38alpha-dependent pathway. *J Leukoc Biol* 2009;86:769–778. [PubMed: 19451398]

7. Pecaric-Petkovic T, Didichenko SA, Kaempfer S, Spiegl N, Dahinden CA. Human basophils and eosinophils are the direct target leukocytes of the novel IL-1 family member IL-33. *Blood* 2009;113:1526–1534. [PubMed: 18955562]
8. Schneider E, Petit-Bertron AF, Bricard R, Levasseur M, Ramadan A, Girard JP, Herbelin A, Dy M. IL-33 activates unprimed murine basophils directly in vitro and induces their in vivo expansion indirectly by promoting hematopoietic growth factor production. *J Immunol* 2009;183:3591–3597. [PubMed: 19684081]
9. Silver MR, Margulis A, Wood N, Goldman SJ, Kasaian M, Chaudhary D. IL-33 synergizes with IgE-dependent and IgE-independent agents to promote mast cell and basophil activation. *Inflamm Res*. 2009 in press.
10. Suzukawa M, Iikura M, Koketsu R, Nagase H, Tamura C, Komiya A, Nakae S, Matsushima K, Ohta K, Yamamoto K, Yamaguchi M. An IL-1 cytokine member, IL-33, induces human basophil activation via its ST2 receptor. *J Immunol* 2008;181:5981–5989. [PubMed: 18941187]
11. Barrett NA, Austen KF. Innate cells and T helper 2 cell immunity in airway inflammation. *Immunity* 2009;31:425–437. [PubMed: 19766085]
12. Xu D, Jiang HR, Kewin P, Li Y, Mu R, Fraser AR, Pitman N, Kurowska-Stolarska M, McKenzie AN, McInnes IB, Liew FY. IL-33 exacerbates antigen-induced arthritis by activating mast cells. *Proc Natl Acad Sci U S A* 2008;05:10913–10918. [PubMed: 18667700]
- 13*. Sokol CL, Barton GM, Farr AG, Medzhitov R. A mechanism for the initiation of allergen-induced T helper type 2 responses. *Nat Immunol* 2008;9:310–318. Shows that basophils are activated by a model protease allergen. [PubMed: 18300366]
14. Mikhak Z, Luster AD. The Emergence of Basophils as Antigen-Presenting Cells in Th2 Inflammatory Responses. *J Mol Cell Biol*. 2009 in press.
- 15**. Denzel A, Maus UA, Rodriguez Gomez M, Moll C, Niedermeier M, Winter C, Maus R, Hollingshead S, Briles DE, Kunz-Schughart LA, Talke Y, Mack M. Basophils enhance immunological memory responses. *Nat Immunol* 2008;9:733–742. Indicates that basophils participate in memory humoral immune responses. [PubMed: 18516038]
- 16**. 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. [PubMed: 19465907]
- 17**. Perrigoue JG, Saenz SA, Siracusa MC, Allenspach EJ, Taylor BC, Giacomini PR, Nair MG, Du Y, Zaph C, van Rooijen N, Comeau MR, Pearce EJ, Laufer TM, Artis D. MHC class II-dependent basophil-CD4⁺ T cell interactions promote T(H)2 cytokine-dependent immunity. *Nat Immunol* 2009;10:697–705. [PubMed: 19465906]
- 18**. Yoshimoto T, Yasuda K, Tanaka H, Nakahira M, Imai Y, Fujimori Y, Nakanishi K. 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. Demonstrate that basophils present peptide-MHC II complexes to CD4⁺ T cells. [PubMed: 19465908]
- 19*. Charles N, Watford WT, Ramos HL, Hellman L, Oettgen HC, Gomez G, Ryan JJ, O'Shea JJ, Rivera J. Lyn kinase controls basophil GATA-3 transcription factor expression and induction of Th2 cell differentiation. *Immunity* 2009;30:533–543. Shows that Lyn functions to inhibit the function of basophils. [PubMed: 19362019]
20. Rivera J, Fierro NA, Olivera A, Suzuki R. New insights on mast cell activation via the high affinity receptor for IgE. *Adv Immunol* 2008;98:85–120. [PubMed: 18772004]
21. Scapini P, Pereira S, Zhang H, Lowell CA. Multiple roles of Lyn kinase in myeloid cell signaling and function. *Immunol Rev* 2009;228:23–40. [PubMed: 19290919]
22. Gilfillan AM, Rivera J. The tyrosine kinase network regulating mast cell activation. *Immunol Rev* 2009;228:149–169. [PubMed: 19290926]
23. Mocsai A, Abram CL, Jakus Z, Hu Y, Lanier LL, Lowell CA. Integrin signaling in neutrophils and macrophages uses adaptors containing immunoreceptor tyrosine-based activation motifs. *Nat Immunol* 2006;7:1326–1333. [PubMed: 17086186]
- 24*. Van Ziffle JA, Lowell CA. Neutrophil specific deletion of Syk kinase results in reduced host defense to bacterial infection. *Blood*. 2009 in press.

- 25*. Robinson MJ, Osorio F, Rosas M, Freitas RP, Schweighoffer E, Gross O, Verbeek JS, Ruland J, Tybulewicz V, Brown GD, Moita LF, Taylor PR, Reis e Sousa C. Dectin-2 is a Syk-coupled pattern recognition receptor crucial for Th17 responses to fungal infection. *J Exp Med* 2009;206:2037–2051. [PubMed: 19703985]
- 26*. Gross O, Poeck H, Bscheider M, Dostert C, Hanneschlager N, Endres S, Hartmann G, Tardivel A, Schweighoffer E, Tybulewicz V, Mocsai A, Tschopp J, Ruland J. Syk kinase signalling couples to the Nlrp3 inflammasome for anti-fungal host defence. *Nature* 2009;459:433–436. These studies illustrate new roles for Syk in responses to pathogens. [PubMed: 19339971]
27. Grochowy G, Hermiston ML, Kuhny M, Weiss A, Huber M. Requirement for CD45 in fine-tuning mast cell responses mediated by different ligand-receptor systems. *Cell Signal* 2009;21:1277–1286. [PubMed: 19332117]
28. Hermiston ML, Xu Z, Weiss A. CD45: a critical regulator of signaling thresholds in immune cells. *Annu Rev Immunol* 2003;21:107–137. [PubMed: 12414720]
29. Akimoto M, Mishra K, Lim KT, Tani N, Hisanaga SI, Katagiri T, Elson A, Mizuno K, Yakura H. Protein tyrosine phosphatase epsilon is a negative regulator of FcepsilonRI-mediated mast cell responses. *Scand J Immunol* 2009;69:401–411. [PubMed: 19508371]
30. Nakata K, Yoshimaru T, Suzuki Y, Inoue T, Ra C, Yakura H, Mizuno K. Positive and negative regulation of high affinity IgE receptor signaling by Src homology region 2 domain-containing phosphatase 1. *J Immunol* 2008;181:5414–5424. [PubMed: 18832698]
31. McPherson VA, Sharma N, Everingham S, Smith J, Zhu HH, Feng GS, Craig AW. SH2 domain-containing phosphatase-2 protein-tyrosine phosphatase promotes Fc(epsilon)RI-induced activation of Fyn and Erk pathways leading to TNF(alpha) release from bone marrow-derived mast cells. *J Immunol* 2009;183:4940–4947. [PubMed: 19786542]
32. Kim MS, Radinger M, Gilfillan AM. The multiple roles of phosphoinositide 3-kinase in mast cell biology. *Trends Immunol* 2008;29:493–501. [PubMed: 18775670]
33. Huber M, Helgason CD, Damen JE, Liu L, Humphries RK, Krystal G. The src homology 2-containing inositol phosphatase (SHIP) is the gatekeeper of mast cell degranulation. *Proc Natl Acad Sci U S A* 1998;95:11330–11335. [PubMed: 9736736]
34. Langdon JM, Schroeder JT, Vonakis BM, Bieneman AP, Chichester K, Macdonald SM. Histamine-releasing factor/translationally controlled tumor protein (HRF/TCTP)-induced histamine release is enhanced with SHIP-1 knockdown in cultured human mast cell and basophil models. *J Leukoc Biol* 2008;84:1151–1158. [PubMed: 18625911]
35. Leung WH, Bolland S. The inositol 5'-phosphatase SHIP-2 negatively regulates IgE-induced mast cell degranulation and cytokine production. *J Immunol* 2007;179:95–102. [PubMed: 17579026]
36. Saini SS, Paterniti M, Vasagar K, Gibbons SP Jr, Sterba PM, Vonakis BM. Cultured peripheral blood mast cells from chronic idiopathic urticaria patients spontaneously degranulate upon IgE sensitization: Relationship to expression of Syk and SHIP-2. *Clin Immunol* 2009;132:342–348. [PubMed: 19477690]
37. Furumoto Y, Brooks S, Olivera A, Takagi Y, Miyagishi M, Taira K, Casellas R, Beaven MA, Gilfillan AM, Rivera J. Cutting Edge: Lentiviral short hairpin RNA silencing of PTEN in human mast cells reveals constitutive signals that promote cytokine secretion and cell survival. *J Immunol* 2006;176:5167–5171. [PubMed: 16621980]
38. Soroosh P, Doherty TA. Th9 and allergic disease. *Immunology* 2009;127:450–458. [PubMed: 19604299]
39. King C, Tangye SG, Mackay CR. T follicular helper (TFH) cells in normal and dysregulated immune responses. *Annu Rev Immunol* 2008;26:741–766. [PubMed: 18173374]
40. Hamid Q, Tulic M. Immunobiology of asthma. *Annu Rev Physiol* 2009;71:489–507. [PubMed: 19575684]
41. Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, Corrigan C, Durham SR, Kay AB. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *New England J Med* 1992;326:298–304. [PubMed: 1530827]
42. Lloyd CM, Hawrylowicz CM. Regulatory T cells in asthma. *Immunity* 2009;31:438–449. [PubMed: 19766086]

43. Wang YH, Liu YJ. The IL-17 cytokine family and their role in allergic inflammation. *Curr Opin Immunol* 2008;20:697–702. [PubMed: 18832032]
44. Smith-Garvin JE, Koretzky GA, Jordan MS. T cell activation. *Annu Rev Immunol* 2009;27:591–619. [PubMed: 19132916]
45. Au-Yeung BB, Deindl S, Hsu LY, Palacios EH, Levin SE, Kuriyan J, Weiss A. The structure, regulation, and function of ZAP-70. *Immunol Rev* 2009;228:41–57. [PubMed: 19290920]
46. Deindl S, Kadlecsek TA, Brdicka T, Cao X, Weiss A, Kuriyan J. Structural basis for the inhibition of tyrosine kinase activity of ZAP-70. *Cell* 2007;129:735–746. [PubMed: 17512407]
- 47*. Hsu LY, Tan YX, Xiao Z, Malissen M, Weiss A. A hypomorphic allele of ZAP-70 reveals a distinct thymic threshold for autoimmune disease versus autoimmune reactivity. *J Exp Med* 2009;206:2527–2541. Provides evidence that Zap-70 functions as an adapter in the TCR signaling pathway. [PubMed: 19841086]
48. Siggs OM, Miosge LA, Yates AL, Kucharska EM, Sheahan D, Brdicka T, Weiss A, Liston A, Goodnow CC. Opposing functions of the T cell receptor kinase ZAP-70 in immunity and tolerance differentially titrate in response to nucleotide substitutions. *Immunity* 2007;27:912–926. [PubMed: 18093540]
49. Picard C, Dogniaux S, Chemin K, Maciorowski Z, Lim A, Mazerolles F, Rieux-Laucat F, Stolzenberg MC, Debre M, Magny JP, Le Deist F, Fischer A, Hivroz C. Hypomorphic mutation of ZAP70 in human results in a late onset immunodeficiency and no autoimmunity. *Eur J Immunol* 2009;39:1966–1976. [PubMed: 19548248]
- 50*. Levin SE, Zhang C, Kadlecsek TA, Shokat KM, Weiss A. Inhibition of ZAP-70 kinase activity via an analog-sensitive allele blocks T cell receptor and CD28 superagonist signaling. *J Biol Chem* 2008;283:15419–15430. Reports the creation of Zap-70 variants that are sensitive to a small molecule inhibitor. [PubMed: 18378687]
51. Fowell DJ. Signals for the execution of Th2 effector function. *Cytokine* 2009;46:1–6. [PubMed: 19237299]
52. Gomez-Rodriguez J, Sahu N, Handon R, Davidson TS, Anderson SM, Kirby MR, August A, Schwartzberg PL. Differential expression of interleukin-17A and -17F is coupled to T cell receptor signaling via inducible T cell kinase. *Immunity* 2009;31:587–597. [PubMed: 19818650]
53. Readinger JA, Mueller KL, Venegas AM, Horai R, Schwartzberg PL. Tec kinases regulate T-lymphocyte development and function: new insights into the roles of Itk and Rlk/Txk. *Immunol Rev* 2009;228:93–114. [PubMed: 19290923]
54. Mueller C, August A. Attenuation of immunological symptoms of allergic asthma in mice lacking the tyrosine kinase ITK. *J Immunol* 2003;170:5056–5063. [PubMed: 12734350]
55. Ferrara TJ, Mueller C, Sahu N, Ben-Jebria A, August A. Reduced airway hyperresponsiveness and tracheal responses during allergic asthma in mice lacking tyrosine kinase inducible T-cell kinase. *J Allergy Clin Immunol* 2006;117:780–786. [PubMed: 16630934]
- 56*. Qi Q, Xia M, Hu J, Hicks E, Iyer A, Xiong N, August A. Enhanced development of CD4+ gamma delta T cells in the absence of Itk results in elevated IgE production. *Blood* 2009;114:564–571. [PubMed: 19443662]
- 57*. Felices M, Yin CC, Kosaka Y, Kang J, Berg LJ. Tec kinase Itk in gamma delta T cells is pivotal for controlling IgE production in vivo. *Proc Natl Acad Sci U S A* 2009;106:8308–8313. These studies help explain the increases in serum IgE seen in Itk-deficient mice despite impaired Th2 responses. [PubMed: 19416854]
58. Iyer AS, August A. The Tec family kinase, IL-2-inducible T cell kinase, differentially controls mast cell responses. *J Immunol* 2008;180:7869–7877. [PubMed: 18523250]

Figure 1.

Simplified schematic of the IgE receptor (FcεRI) signaling pathway. FcεRI engagement results in phosphorylation of receptor ITAMs by Lyn and Fyn, thus generating docking sites for these proteins as well as for Syk. Binding to ITAMs results in the upregulation of Fyn, Lyn and Syk kinase activity and causes phosphorylation of downstream signaling molecules. As a consequence of these events multi-protein complexes containing various signaling molecules are formed that induce activation of MAPK pathways and the generation of second messenger molecules, leading to the induction of cell activation. Sphks - sphingosine kinases.

Figure 2.

Simplified schematic of the T cell receptor signaling pathway. TCR engagement induces the kinase activity of Lck, which phosphorylates ITAMs in the CD3 zeta chains. Zap-70 is recruited to these phosphorylated ITAMs resulting activation of kinase activity and phosphorylation of downstream signaling molecules. Multi-protein complexes nucleated by the adapter protein LAT are formed. Components of these complexes mediate activation of MAPK pathways and generate second messenger molecules. These signals activate various transcription factors and induce T cell proliferation and differentiation.