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JAK and Src tyrosine kinase signaling in asthma

Kavita Tundwal[1], Rafeul Alam[1]

[1]Division of Allergy and Immunology, Department of Medicine, National Jewish Health, University of Colorado Denver, 1400 Jackson Street, Denver, CO 80206

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1. ABSTRACT

Tyrosine kinases play a critical role in transducing intracellular signals from the receptors. Many receptors do not have intrinsic tyrosine kinase activity, so they rely on cytosolic and/or membrane-associated tyrosine kinases for initial signal generation. The Src and JAK family kinases are frequently associated with receptors and generate the initial cytosolic signals. These signals are then transduced to other compartments of the cytosol and to the nucleus to elicit a specific cellular response. In this review we focus on these two families of tyrosine kinases and review their involvement in activation of cells that are involved in the pathogenesis of asthma. A Th2-type immune response dominates the processes that lead to the phenotype of asthma. For this reason we give special attention to the tyrosine kinases that are involved in a Th2 response. Further we examine the involvement of tyrosine kinases in activation of mast cells, eosinophils and other cells.

2. INTRODUCTION TO TYROSINE KINASES

Tyrosine kinases can be broadly categorized into two groups: (i) Receptor tyrosine kinases (RTKs) such as Epidermal Growth Factor (EGF), Fibroblast Growth Factor (FGF), Platelet Derived Growth Factor (PDGF) etc. (ii) Non receptor tyrosine kinases which associate with receptors usually upon ligand stimulation. There are about 90 known tyrosine kinases in human genome, of which 58 are RTKs and 32 are non-receptor tyrosine kinases. The non-receptor tyrosine kinases are further classified into 10 families namely, ABL, ACK, CSK, FAK, FES, FRK, JAK, SRC, TEC, and SYK (1).

In this review, we will discuss only Src and JAK family of tyrosine kinases and their role in development and pathogenesis of asthma.

Src family kinases were first of the non-receptor tyrosine kinases to be characterized structurally and

biochemically. The domain structure of Src kinases is conserved and consists of an N-terminal membrane localization motif, a Src homology3 (SH3) domain, a Src homology2 (SH2) domain, a kinase domain and a C-terminal regulatory tail. Fgr, Fyn, Src, Yes, Blk, Hck, Lck and Lyn belong to the Src family of tyrosine kinases (1-3).

JAK family kinases include JAK-1, JAK-2, JAK-3 and TYK-2 (tyrosine kinase-2). JAK tyrosine kinases are constitutively associated with the intracytoplasmic domain of cytokine receptors. Upon ligand binding and receptor dimerization, JAKs are activated and phosphorylate specific tyrosine residues on the intracytoplasmic portion of the cytokine receptor. These phosphorylated tyrosine residues then serve as docking site for SH2 domain containing protein like Signal Transducers and Activators of Transcription (STATs), Src kinases, adaptor proteins etc. STATs are transcription factors which, following tyrosine phosphorylation by JAKs, dimerize and translocate to nucleus and act as gene regulators (3- 5). Some studies have also shown that JAK-1, JAK-2 and JAK-3 can directly phosphorylate IRS-1 *in vitro* (7). Cells lacking JAK-1 fail to induce IRS-1 phosphorylation following IL-4 stimulation. JAK-2 and TYK-2 are also shown to phosphorylate IRS-1 in certain cell lines (6-9). IRS-1 then leads to activation of phosphoinositide3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathway.

3. TYROSINE KINASES IN ASTHMA

Asthma is a chronic inflammatory disease characterized by airway hyperreactivity, inflammation and remodeling. Each of these phenotypes is mediated by co-ordinated action of several inflammatory and structural cells. Upon allergen stimulation, histamine, leukotrienes, cytokines, chemokines, growth factors, and other inflammatory mediators are secreted by different cell types, which in turn induce signaling cascade in recipient cells thereby leading to the specific biological outcomes (3, 5, 10, 11). Tyrosine kinase-mediated signaling pathways play an important role in inflammatory responses. Receptor tyrosine kinases, for example, are critical in airway remodeling. Non-receptor tyrosine kinsases, on the other hand, are one of the earliest activated signaling components in response to stimulation of immune receptors e. g. T cell receptor (TCR), B cell receptor (BCR), FC-ε-R1, cytokine receptors and chemokine receptors. Activated tyrosine kinases then phosphorylate and activate several downstream signaling pathways including MAPK, PI3K, STAT, calcium signaling and nuclear factor-κB (NF-κB), leading to cell recruitment via chemotaxis, survival, proliferation, differentiation and degranulation (5, 10). Activation of the initial tyrosine kinase and subsequent signaling pathway is specific to cell type and the stimulating ligand.

4. TYROSINE KINASES IN T CELLS

$CD4^+$ T cells have been recognized as a major player in asthma. Studies in both humans and mice have shown importance of the Th2 cell in pathophysiology of asthma. Mouse model of asthma studies showed that adoptive transfer of antigen-specific Th2 cells, but not Th1 cells can induce asthma phenotypes, like airway hyperreactivity, mucus production, and airway eosinophilia following subsequent allergen challenge (3).

4.1. The Src family kinases in T cells

Src kinases play an important role in activation and transduction of signaling pathways following TCR stimulation (Figure 1). Thus Src kinases are crucial in T cell development, differentiation, proliferation and survival. Lck and Fyn are the first ones to be activated following TCR engagement. These Src kinases then phosphorylate Immunoreceptor tyrosine-based activation motif (ITAMs) located in the CD3ζ subunits, creating docking site for other kinases and adaptor proteins (4, 5, 10). Expression of dominant-negative Lck inhibited Th2 development (12). Lck activation subsequently leads to activation of interleukin-2 inducible tyrosine kinase (Itk), a member of the Tec family kinases. The activation of this kinase is important for Th2 cytokine secretion, especially during the effector phase (13). Itk is essential for generation of NFAT(c). NFAT, in cooperation with GATA3, induces IL-4 gene transcription in effector Th2 cells. For this reason an understanding of the mechanism of Lck activation is important.

Uncoordinated protein 119 (Unc-119, also known as HRG4 for human retinal gene protein 4) is an adaptor protein, originally cloned as a retina-specific gene. It was also discovered as an IL-5Rα interacting protein using yeast two hybrid studies (14). Gorska *et al.* have shown that Unc119 plays a crucial role in regulating Src kinase (Lck in particular) activation dynamics following TCR stimulation, thereby contributing to Th2 differentiation. Unc-119 levels are higher in $CD4^+$ T-cells of asthma patients as compared to normal subjects, patients with allergic rhinitis (restricted Th2 inflammation) or patients with rheumatoid arthritis (Th1/Th17 dominant inflammation) (15). Following TCR stimulation, Unc-119 interacts with Lck and/Fyn via its SH2 and SH3 binding domains, thereby activating these Src kinases by releasing their self inhibitory linker region (2, 16). CD45, a transmembrane tyrosine phosphatase, also plays an important role in this activation process by dephosphorylating the inhibitory C-terminal phosphotyrosine (17, see Figure 1). In addition to Lck activation Unc-119 is also involved in targeting Lck to plasma membrane. Lck traffics through the Rab-11 compartment and its exit from the Rab-11 compartment to the plasma membrane is regulated by Unc-119 (18). Further studies showed that Unc-119 plays an important role in Th2 differentiation, by regulating the Src kinase activation dynamics. Unc-119 levels increased at later time points following TCR stimulation and this increase was much higher in Th2 cells compared to Th1 differentiated cells. Several studies showed that prolonged TCR stimulation favors Th2 commitment (15, 19).

4.2. The JAK family kinases in T cells

Cytokine signaling, along with TCR stimulation, directs the T cell differentiation. The JAK-STAT pathway is one of the major signaling pathways activated following cytokine stimulation (Figure 1). They also play an important role in Th2 differentiation.

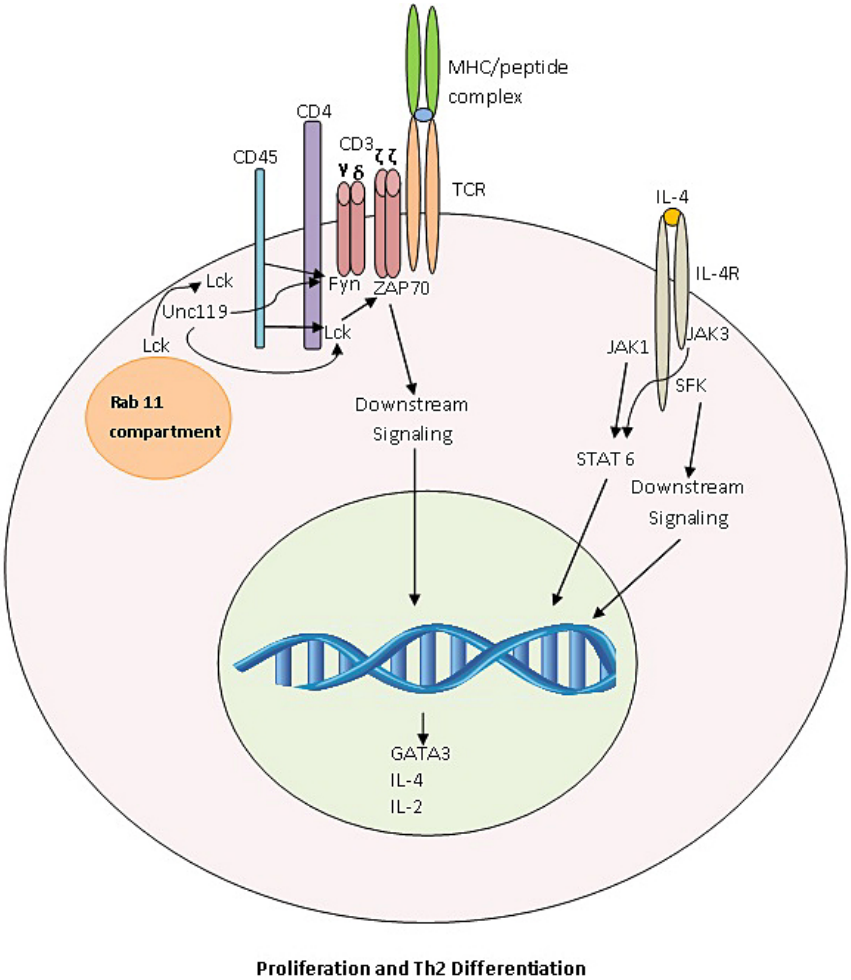

Figure 1. A diagrammatic presentation of Src and JAK kinase signaling in T cell. (a) Activation of tyrosine kinases (Lck, Fyn and ZAP70) in the T cell receptor complex. Lck is basally inactive because of a C-terminal tyrosine phosphorylation (Y505) and an intramolecular interaction. CD45 partially activates Lck by dephosphorylating the C-terminal tyrosine phosphorylation. The activation of the TCR and the engagement of CD4 lead to the interaction of the receptor-bound Unc119 with Lck. This results in further activation of Lck. Lck then phosphorylates the ITAM tyrosine residues of the zeta chain. The phosphorylation of the zeta chains allows docking and subsequent phosphorylation of ZAP70, which then transduces signals downstream. (b) IL-4 stimulation activates IL-4 receptor bound JAK1 and JAK3 which subsequently activate STAT6. Src family kinases (SFK) are also activated at the receptor, resulting in activation of downstream signaling cascade.

IL-2 and IL-4 are both required for efficient Th2 induction *in vitro*. IL-2 activates STAT-5α and STAT-5β, resulting in increased expression of IL-4Rα. IL-2 thus helps in stabilization of Th2 lineage by increasing IL-4 production as well as responsiveness to IL-4 during Th2 differentiation. Furthermore, IL-2 is also required for T cell proliferation and survival (20, 21). IL-4 signaling promotes Th2 differentiation by inducing transcription factor GATA-3 and Th2 cytokines. IL-4 induced signaling suppresses the gene expression profile for Th1 differentiation. IL-4 activates JAK-1 and JAK-3 leading to STAT-6 activation (3, 22). STAT-6 is not only necessary but also sufficient to drive Th2 differentiation, since introduction of constitutively active STAT-6, even in Th1 differentiated cells, results in TH2 cytokine production (23-25). *Stat6* knockout mice do not develop AHR and asthma phenotype. *Stat6* knockout studies have also shown its importance in Th2 differentiation, Th2 memory cell development and survival (3). STAT-6 also plays an important role in IL-4 induced T cell proliferation, by regulating expression of cell cycle–dependent kinase inhibitor p27kip in response to IL-4 stimulation (26).

The Th1 cytokine IL-12, on the other hand, activates JAK-2 and Tyk-2 which leads to STAT-4 activation. STAT-4 induces T-bet and Th1 cytokines. Like IL-4 suppresses Th1 differentiation, IL-12 suppresses Th2 differentiation (3, 12).

RANTES signals through its receptor CCR5 on T cells. CCR5 stimulation activates JAK-2 and JAK-3 leading to formation of STAT-1 and STAT-3 dimers.

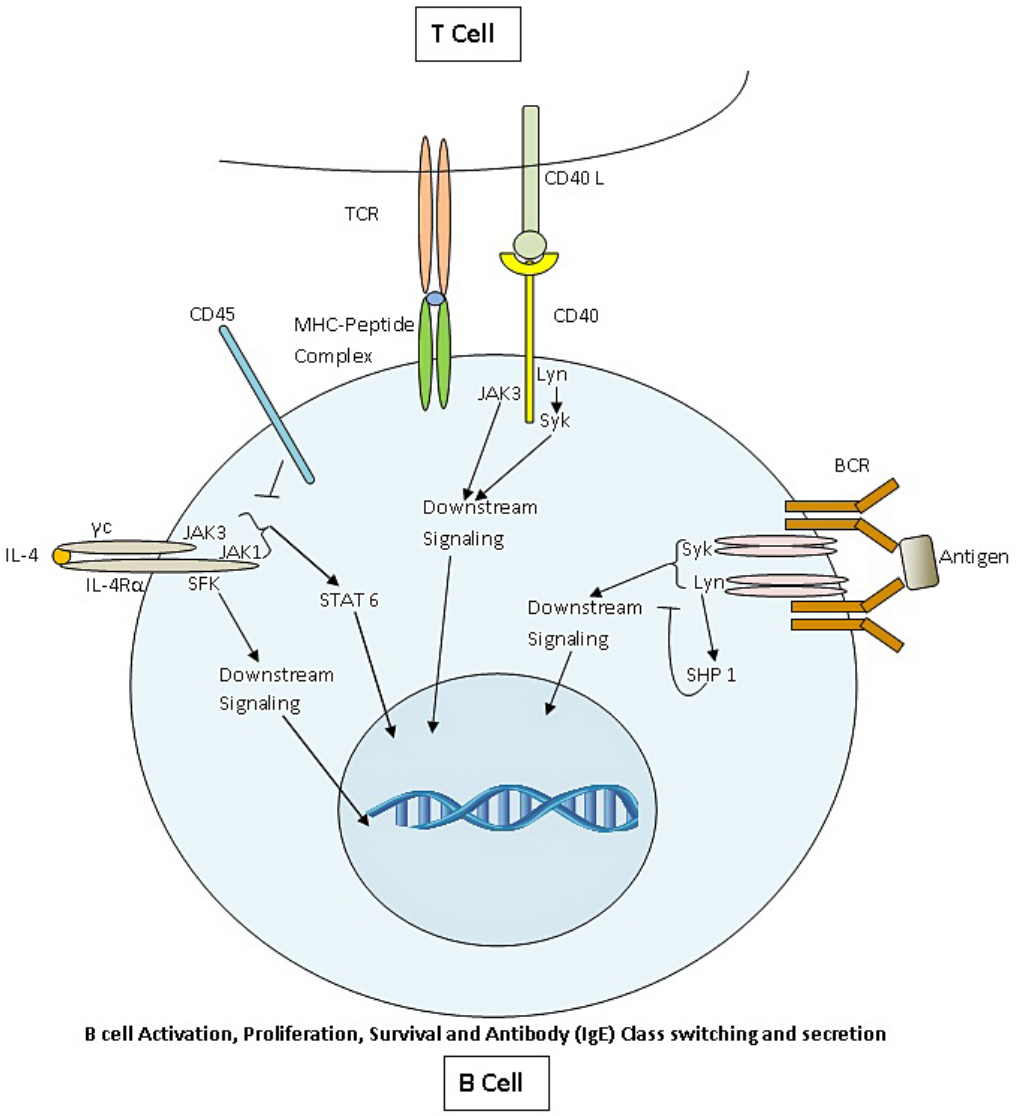

Figure 2. A diagrammatic representation of various B cell signaling events resulting in IgE class switching. The binding of an antigen to the B cell membrane IgM causes the activation of the B cell receptor (BCR)-associated Lyn and Syk kinases, which then transducer downstream signals. Lyn regulates the duration and strength of this signal by activating the phosphatase SHP1. The engagement of the IL4R and CD40 on B cells induces Jak-STAT signaling and the activation of Src family kinases (SFK). The BCR signal induces B cell proliferation, survival and immunoglobulin secretion. The IL4R and CD40 signaling induces immunoglobulin class switching.

Studies in human T cell lines have showed that the stroma cell-derived factor-1α (SDF-1α) signaling via CXCR4 receptor activates JAK-2 and JAK-3 kinases, leading to trans-endothelial migration (5, 27, 28).

The JAK-STAT pathway induces suppressor of cytokine signaling (SOCS) proteins, which are also important signaling regulators in $CD4^+$ T cells. SOCS are adaptor proteins known to be negative regulators of the JAK-STAT pathway. SOCS-3 is preferentially expressed in Th2 cells and is also known to modulate Th17 differentiation. Overexpression of SOCS-3 in T cells inhibits activation of IL-12 signaling and gene activation, leading to impaired Th1 differentiation. IL-13 induces SOCS-1 and provides a negative feedback loop for IL-13 signaling (29, 30).

5. TYROSINE KINASES IN B CELLS

5.1. The Src family kinases in B cells

Following BCR activation, the Src family kinases such as Lyn, Fyn and Blk phosphorylate ITAMs on the cytoplasmic tail of BCR (Figure 2). Syk is then recruited to the ITAMs and activated. Syk generates downstream signals via Bruton's tyrosine kinase (Btk; a member of Tec family tyrosine kinases), PLCγ2 and PI3K (5, 31, 32).

Although Lyn appears to be important in initiating signaling pathways through BCR stimulation, studies on Lyn deficient cells have identified Lyn as both positive and negative regulator of signaling through BCR. Early responses to BCR stimulation are delayed in Lyn deficient B cells, confirming the role of Lyn in initiating BCR signaling. However, once activated, Lyn deficient B cells show enhanced MAPK activation and prolonged calcium mobilization. Lyn deficient B cells also exhibit anti-IgM induced hyperproliferation. Thus Lyn also acts as negative regulator of BCR induced signaling. Lyn is also shown to downregulate BCR expression on cell surface (33-35).

In addition to BCR signaling, CD40 interaction with the CD40 ligand (CD40L) on activated T cells is also important in B cell function and Ig class switching. Upon ligand binding the CD40 receptor activates Lyn and Syk, which subsequently activates PI3K and PLCγ inducing calcium mobilization (11, 36).

5.2. The JAK family kinases in B cells

IgE is one of the important mediators in asthma pathogenesis. The class switching in B cells and IgE synthesis are regulated by multiple signaling pathways. IL-4 plays an important role in IgE class switching by activating STAT-6. STAT-6 activation is mediated by a GC-associated nuclear protein (GANP) which interacts with the JAK-binding protein, arginine methyl transferase (PRMT) 5 to modulate JAK-1/JAK-3 signaling to STAT-6 (37). STAT-6 then induces transcription of germline ε in IgM-positive B cells, thereby contributing to the IgE class switching event (6, 11). In addition, signaling induced by CD40 is also required for IgE synthesis. Both IL-4 and CD40 signaling pathways involve JAK-3 activation as a crucial step in IgE synthesis. JAK-3 is shown to be constitutively associated with the intracellular domain of CD40 in resting B cells (11, 36-40).

Yamada *et al.* showed that activation of CD45 negatively regulates IgE class switching in human B cells by dephosphorylating JAK-1, JAK-3 and STAT-6 but not stress-activated/mitogen activated protein kinases (MAPK) (41, 42).

The interferon-γ (IFNγ) signaling, on the other hand, inhibits transcription of germline Igε and Igγ1, thereby blocking IgE and IgG1 production by B cells. IFNγ induces SOCS-1, which can bind to all JAKs and inhibit STAT activation. Thus IFNγ inhibits IL-4 signaling via SOCS-1 induction leading to suppression of STAT-6 activation (11, 43).

6. TYROSINE KINASES IN DENDRITIC CELLS AND MACROPHAGES

Dendritic cells (DCs) are the most important antigen presenting cells (APCs) in the development of allergic asthma. Their location along the basement membrane of the airway epithelium is ideal for capturing inhaled allergens. In addition to antigen presentation to T cells, DCs prime helper T cell differentiation by secretion of specific cytokines (5, 11, 44).

Macrophages are the most common cells found in the bronchoalveolar lavage of asthma patients. Like DCs, they function as APCs. In addition, they mediate inflammatory responses by release of nitric oxide and reactive oxygen species (5, 11).

6.1. The Src family kinases in dendritic cells and macrophages

In monocyte-derived DCs, the CD40 interaction with CD40L on T cells, results in activation of Lyn and Syk. Lyn in turn then activates the MAPK pathway for downstream signal transduction (45, 46). Src kinases are also activated following TLR stimulations. Src family kinases are involved in Lippolysaccharide (LPS) induced cytokine production via regulation of the AP1 complex formation or cAMP-protein kinase A dependent pathway. Lyn associates with CD14 and is activated by LPS stimulation in monocytes (47, 48). Lyn deficient DCs exhibit inefficient maturation, defective inhibitory signaling pathways and decreased IL-12 production. This suggests a positive regulatory role for Lyn in DC maturation and antigen presentation capability. *Lyn*$^{-/-}$ DCs are able to induce delayed T cell proliferation *in vivo*, but fail to prime for Th1 differentiation (47-49). Napolitani *et al*, using Src inhibitor PP1, showed that Src plays important role in LPS induced DC maturation and cytokine production, via cJun mediated AP1 complex formation. However, the upregulation of co-stimulatory molecules is independent of the Src pathway. Thus, Src inhibition in DCs, still allows them to induce T cell proliferation, however impairs their ability to induce Th1 differentiation (48). Similarly, following TLR-3 or TLR-8 stimulation, Src kinases regulate c-Jun and IRF1 activation without affecting NFkB and Extracellular signal-regulated kinase (ERK) pathways. This study also showed that Src inhibition resulted in impaired production of IL-12, IL-6 and TNFα but not IL-23. As a consequence Src inhibition in DCs leads to impaired Th1 differentiation but not Th17 (49). Similar studies in macrophages, using the Src kinase inhibitor PP2 (pyrazolo[3,4-d]-Pyrimidines), have shown that Src kinases play a crucial role in TLR induced cytokine production (50). Src kinases also participate in LPS induced NADPH oxidase activation via the PI3K pathway in murine macrophage RAW264.7 cells (51). Src is also required for activation of TNF-related activation induced cytokine receptor (TRANCE-R) in dendritic cells, leading to the activation of NF-kB and Akt (5).

Similar to its role in other hematopoietic cells, Lyn exhibits both positive and negative regulatory role in DCs. *In vitro* and *in vivo* studies have shown that Lyn negatively regulates differentiation, proliferation and survival of monocyte derived DCs (52). Adoptive transfer of antigen pulsed *lyn*$^{-/-}$ DCs to naïve wild type mice is shown to induce airway inflammation. *Lyn*$^{-/-}$ mice also exhibit more severe airway inflammation and asthma phenotype following airway challenge (53). Keck *et al* showed negative regulatory role of Lyn kinase in murine macrophage response to TLR-2 and TLR-4. Using *lyn*$^{-/-}$

macrophages, they showed increased production of cytokines (IL-6, TNFα and IFN α/β) by macrophages in response to LPS (TLR-4 agonist) and FSL-1 (TLR-2 ligand), but not in response to TLR-3 and TLR-9 ligand (54). Such negative regulation is suggested to be mediated by activation of negative regulators like SHP-1 (53-55). The Src family kinases also modulate signaling by G protein coupled receptors. Fgr is a negative regulator of chemokine induced signaling pathways in DCs, including MAPK, Ca^{2+} influx and actin polymerization, leading to reduced chemotaxis *in vitro* and *in vivo* (55).

FcR activation on macrophages by IgG immune complexes induces Src and Syk kinase activities. This leads to phagocytosis, antigen presentation and gene transcription (5). Macrophages from *hck*$^{-/-}$ *lyn*$^{-/-}$ *fgr*$^{-/-}$ mice exhibit diminished or delayed downstream signaling. FcγR induced phagocytosis in these macrophages was delayed (56). On the contrary, Fgr has been shown to negatively regulate FcγR and CR3 mediated phagocytosis, but not macropinocytosis or receptor mediated endocytosis (57). Further studies are required to address these discrepancies.

6.2. The JAK family kinases in dendritic cells and macrophages

CD40 ligation activates JAK-3 in monocyte derived DCs. Saemenn *et al.* showed that JAK-3 inhibition resulted in decreased CD40 mediated maturation of DCs, defective antigen presentation and cytokine production. This indicates the importance of JAK-3 in dendritic cell maturation and T cell stimulation. In addition, since immune cells influence each other, JAK-3 signaling in other cells can also indirectly affect DCs function. For instance, TNFα aids dendritic cell maturation and migration from periphery to lymph nodes. Since the TNFα release by mast cells is regulated by JAK-3, inhibition of JAK-3 can indirectly suppress DC maturation and antigen presentation (11, 58).

Several studies using Tyk-2 deficient mice have established role of Tyk-2 in DC function. Tokumasa *et al* showed that *tyk2*$^{-/-}$ splenic DCs fail to produce Th1 promoting cytokines (such as IL-12 and IFNγ) in response to TLR agonists like CpG olegonucleotides and LPS. Thus Tyk-2 expression is required in DCs for induction of antigen specific Th1 differentiation but not Th2 differentiation (59). Others have shown a role of Tyk-2 in macrophage function following LPS stimulation (60, 61). These studies indicate that cytokine signaling (via IL-12 and IFNs) affects the sensitivity of TLRs thereby affecting antigen specific response of DC and macrophages. Furthermore, LPS activates JAK-2 in murine macrophages, which in turn regulates JNK and STAT-5 activation. JAK-2 thus regulates LPS induced IL-1β and IL-6 production in murine macrophages (62, 63). In DCs, however, JAK kinases selectively regulate IL-10 but not IL-6 production, in response to multiple TLR stimulation (64).

7. TYROSINE KINASES IN EOSINOPHILS

Eosinophils are key effector cells in human asthma. Eosinophils cause tissue damage only when they are activated, such as when stimulated with cytokines (like IL-3, IL-5, GM-CSF) or chemokines (like Eotaxin and RANTES). Once activated, eosinophils release inflammatory mediators (e.g. major basic protein, eosinophil cationic protein, eosinophil-derived neurotoxin), which cause damage to airway epithelium, stimulation of underlying sensory nerves and change in osmolarity of mucosal surface, resulting in AHR. Eosinophils secrete inflammatory cytokines, chemokines and lipid mediators that amplify various inflammatory circuits. They play an important role in airway remodeling via secretion of TGF-α and TGF-β(65, 66).

IL-5 signaling is important for the growth, terminal differentiation, recruitment, activation and survival of eosinophils. Like other cytokines, IL-5 stimulation activates the JAK-STAT pathway (JAK-2, STAT-5 and STAT-1), Src kinase (Lyn) and downstream kinases (Syk) and adaptor proteins (e.g. Shc/Grb2) (67, 68). Lyn, Syk and JAK-2 have been implicated in GM-CSF induced anti-apoptotic effects in human eosinophils. IL-5 and GMCSF activates STAT-3 and STAT-5, which in turn induces Pim-1 expression and regulation of Cyclin D3, resulting in suppression of eosinophil apoptosis (69-72). Although Lyn and JAK-2 are known to be important in eosinophil differentiation and survival, they appear to have no role in eosinophil degranulation or surface adhesion molecule expression. Ras-MAPK pathway seems to be regulating the cell functions such as degranulation (73-75). JAK-2 however, was shown to be critical for eosinophil chemokinesis and adhesiveness. JAK-2 inhibition using AG490 inhibited antigen induced eosinophil recruitment in mouse airways (76). *Lyn* knockout mice are eosinopenic and show decreased eosinopoiesis in bone marrow. This confirms role of Lyn kinase in eosinophil growth and differentiation. JAK is shown to be required for ubiquitination of the βc cytoplasmic domain and proteosome degradation, thereby regulating receptor downregulation following ligand binding (67, 68, 74, 75).

Studies in human eosinophils showed that eotaxin activates Src family kinases like Hck, Fgr and Lyn. These kinases in turn activate Syk and further downstream pathways resulting in chemotaxis and respiratory burst (77).

8. TYROSIN E KINASES IN MAST CELLS

Mast cells play an important role in asthma. FcεR1 crosslinking via antigen bound IgE is one of the major mechanisms of mast cell activation. During early response (within minutes), activated mast cells release inflammatory mediators like histamine, prostaglandins, and leukotrienes resulting in biological outcomes like vasodilation, smooth muscle contraction, and increased mucus production. The late (within hours) effects of mast cell activation include secretion of cytokines and chemokines (5, 11, 78). Since mast cells deficient mice are still able to develop airway inflammation and AHR under certain conditions, mast cells may not be essential for late asthmatic pathology. However, mast cells are still important in augmenting the airway inflammatory response (79-81).

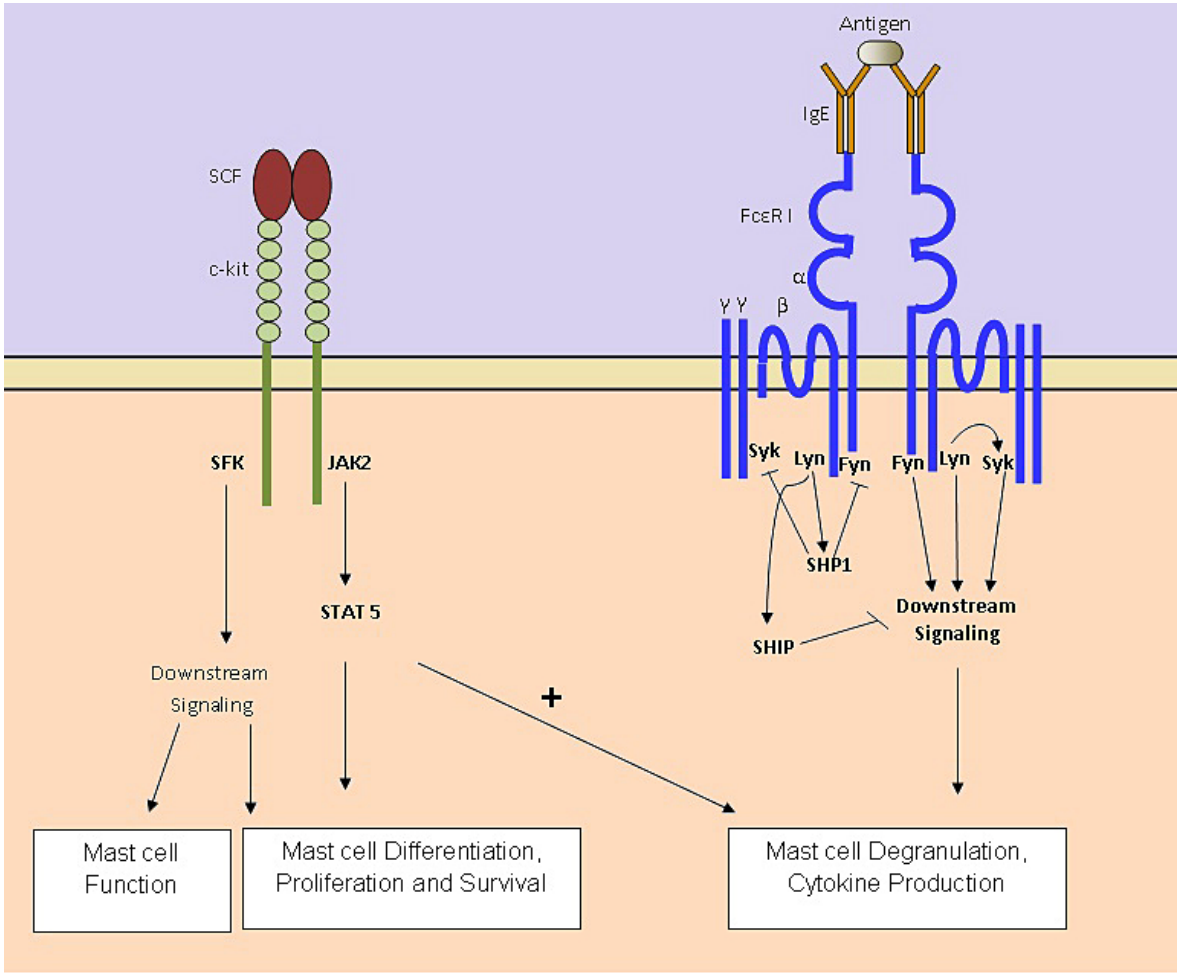

Figure 3. The binding of the stem cell factor (SCF) to c-Kit induces Src family kinase (SFK) and Jak2 activation. The latter activates STAT5, which promotes mast cell proliferation and survival. The engagement of the high affinity FceRI/IgE by multivalent allergens activates Lyn and Fyn kinases, which transducer signals via Syk. The phosphatases SHP-1 and SHIP regulate the strength and duration of this signaling event.

8.1. The Src family kinases in mast cells

Src family kinases are known to associate with FcεRI and important for mast cell activation. Lyn kinase and lipid rafts are crucial for the initial phosphorylation of ITAMs in the cytoplamic domain of FcεRI (Figure 3). This initial step is critical for recruitment and activation of Syk which subsequently activates several downstream signaling pathways (5, 78, 82, 83). However, *lyn* knockout studies have shown that in addition to its positive regulatory role during initial activation stage, it can also have a negative regulatory role in mast cell effector responses. *Lyn*$^{-/-}$ mast cells exhibit delayed activation, but increased degranulation, once they are activated, in comparison to wild type (WT) cells (82, 84, 85). *Lyn*$^{-/-}$ mast cells also show sustained activation of signaling components like Syk, Akt, PI3K and phospho-lipase C-γ (PLC-γ) and calcium mobilization. Degranulation in Lyn deficient mast cells is hyperresponsive regardless of decreased phosphorylation of FcεRI. This could be explained by increased activity of Fyn and decreased activity of SRC Homology 2 Domain–containing Inositol-5-Phosphatase (SHIP) in *lyn*$^{-/-}$ bone marrow derived mast cells (BMMC) (84-87). WT and *lyn*$^{-/-}$ cells also differentially express the transient receptor potential channels (Trpc), which may partially account for altered Ca^{2+} mobilization in *lyn*$^{-/-}$ BMMCs. *Lyn*$^{-/-}$ mice exhibit several allergic phenotypes including increased serum IgE, increased histamine, increased number of mast cells in skin and peritoneum and increased expression of mast cell FcεRI (84). BMMCs from *lyn*$^{-/-}$ mice showed increased Fyn kinase activity which could be partly explained by the loss of appropriate targeting of COOH-terminal Src kinase (Csk), which phosphorylates and inactivates Src family protein kinases (84, 85, 88).

In contrast, Fyn positively regulates mast cell responses by regulating activation of PI3K and other downstream pathways. In addition to activating signaling events, Fyn also induces Gab2-dependent and Ca^{2+} independent microtubule formation, thereby mediating

mast cell degranulation (84, 88). Another Src family kinase, Hck is a positive regulator of FcεRI induced mast cell activation, probably by phosphorylating ITAMs and suppressing Lyn kinase activity. Thus, together these Src family kinases exhibit a hierarchical relationship where Hck inhibits Lyn and Lyn inhibits Fyn (88).

8.2. The JAK family kinases in mast cells

The cytokine activation of the JAK-STAT pathway plays an important role in mast cell proliferation and gene regulation. Stem cell factor (SCF) binds to c-kit on mast cells (Figure 3) and activates JAK-2, which subsequently activates STAT-5 and STAT-6 (11, 89). SCF is required for mast cell differentiation, proliferation and survival. *In vitro* and *in vivo* studies have shown that SCF induces mast cell survival via suppression of apoptosis. SCF can also induce mast cell degranulation as well as potentiate IgE induced degranulation. SCF deficient mice show decreased IL-4 production compared to wild type during local inflammation. IL-3 activates STAT-3 and STAT-5 via JAK-2, which regulates mast cell response against intestinal pathogens (89, 90). Hundley *et al* showed that c-Kit (stem cell factor receptor) signaling can synergize with antigen induced FcεR signaling to enhance mast cell degranulation and cytokine production (91). Suzuki *et al* showed that the IL-4 and IL-9 induced Jak-3 activation via γc chain is required for mast cell proliferation and survival in mice (92). JAK-3 can also be activated by FcεRI stimulation via yet unidentified mechanisms. Studies indicate a role of JAK-3 in fine tuning of FcεRI induced and Syk mediated signaling in mast cells (93).

9. TYROSINE KINASES IN FIBROBLASTS AND AIRWAY SMOOTH MUSCLES

Fibroblasts play an important role in tissue homeostasis. Under pathological conditions, they differentiate into myofibroblasts and contribute to the pathophysiology of disease. An increase in myofibroblasts is observed in airway biopsies from asthma patients (94). Myofibroblasts play an important role in collagen deposition and airway remodeling (95). TGF-β and PDGF can induce myofibroblasts *in vitro* (96). Both these growth factors induce signal transduction via Src family kinases. The adaptor protein Unc-119 regulates TGF-β induced myofibroblast differentiation in human lung fibroblasts. This is mediated by regulation of Fyn activation by Unc-119 during early signaling events following TGF-β stimulation (96).

Airway smooth muscle (ASM) volume contributes significantly to airway hyperresponsiveness (95). Asthma patients show an increase in ASM volume by 3-4 folds in severe cases. Mitogens like PDGF and TGF-β, which are known to induce ASM proliferation and hence airway remodeling, are reported to be elevated in lungs of asthmatic patients (95, 97). JAK, STAT and Src are shown to be required for mitogen-induced signaling in human airway smooth muscle cells (HASMC) *in vitro*, and in mouse model of allergic asthma *in vivo*. While the role of MAPK and PI3K signaling pathways in mitogen-induced smooth muscle cell proliferation have been well characterized, the role of STAT-3 has been studied only recently (96, 97). In addition to proliferation, smooth muscle cell migration contributes to airway remodeling. ASM cell migration can be induced by growth factors and other inflammatory mediators. PDGF stimulation leads to prolonged Src activation, which in turn is required for ASM cell migration. Src kinases are shown to be necessary and sufficient for ASM cell proliferation and migration. Src activates PI3K pathway downstream for signal transduction. IL-13 can augment HASMC migration through the Src kinase and leukotriene dependent pathways (98-101). PDGF induces activation of JAK-2 and subsequently STAT-1 and STAT-3 in human airway smooth muscle cells. Recently, thrombin has been shown to stimulate PI3K and Ras activation, via Src kinase mediated pathways (5, 97, 102).

Growth factor induced STAT activation can be mediated by JAK and/or Src kinases. Inhibitors of JAK and Src prevented STAT-3 activation, resulting in decreased HASMC proliferation by PDGF. Furthermore, siRNA mediated knockdown of STAT-3 resulted in decreased PDGF-induced proliferation of HASMC (97). PDGF induced STAT-3 activation also requires the small GTP binding protein Rac1. STAT-3/Rac1 interaction is important in STAT-3 nuclear translocation and its transcriptional activity. PDGF induced STAT-3 also regulates cell cycle regulators such as p27 and cyclin D3 (97). Studies have associated single nucleotide polymorphism of STAT-3 with decreased lung function in asthma patients (103).

Besides mitogens, Th2 cytokines also affect the function of the airway smooth muscle. While STAT-6 is a common feature of IL-4 and IL-13 signaling, STAT-1 and STAT-3 are known to be activated in restricted cell types, such as lung fibroblasts, following IL-4/IL-13 stimulations (101, 104). Although both IL-4 and IL-13 activate STAT-6 and MAPK pathway in ASM cells, kinetics of STAT-6 activation is different. IL-4 induced STAT-6 activation peaks at 15 min while the peak time is 1hr following IL-13 stimulation (104, 105). JAK-3 has been reported to play an important role in signaling and biological outcomes of these cytokines. Compared to normal fibroblasts, myofibroblasts constitutively express the common γc chain in association with JAK-3. The γc chain forms heterodimer with the pre-phosphorylated IL-4Rα and CD40 chain. This receptor protein complex controls phosphorylation of STAT-3 and Tyk-2 and hence expression of IL-4Rα, IL-13Rα1 and IL-13Rα2 (11, 105). Doucet *et al.* reported that myofibroblasts constitutively express JAK-3, while normal fibroblasts only express JAK-3 following IL-4 or IL-13 stimulation. These studies have shown that JAK-3 is activated in myofibroblasts following stimulation with thrombin, LPS or TNFα via induction of CD40 expression, thus activating the IL-4Rα/CD40/JAK-3 complex. The studies suggest that myofibroblasts are primed to respond to cytokine stimulation, and hence may respond differently than normal fibroblasts under inflammatory conditions (105).

10. TYROSINE KINASES IN EPITHELIAL CELLS

Airway epithelial cells (AEC) form a physical barrier to protect the tissue underneath. However, epithelial

cells also play a dynamic role in the inflammatory response to external stimuli. AECs have been shown to upregulate surface molecules and secrete inflammatory mediators in response to stimulations and infections, resulting in recruitment and activation of immune cells like T cells, eosinophils, and mast cells (106). AECs also secrete mediators promoting airway remodeling which involves migration and proliferation of airway smooth muscles, epithelial cells themselves (106-109).

Diverse signaling pathways are activated following allergen and cytokine/growth factor stimulation of airway epithelial cells, including ERK, p38, NF-kB, JAK/STAT and Src kinases. STAT-6 has been most studied pathway in relation with asthma due to its implication in Th2 development. IL-13 and IL-4 induce STAT-6 activation via JAK-1 and JAK-2. Although further investigation is needed to understand the mechanism of STAT activation in a particular inflammatory condition, both JAK and Src family kinases are known to activate STATs (110-112). Studies using *stat6* knockout mice have established a role for airway epithelial STAT-6 signaling in asthma pathogenesis.*Stat6*$^{-/-}$ mice do not exhibit goblet cell hyperplasia and have reduced mucus secretion in experimental models of asthma (113, 114). Furthermore, transfer of antigen-specific Stat-6$^{+/+}$ T cells fails to overcome this defect, suggesting that Stat6 signaling within the airways is required for mucus production (115). STAT-6 expression and activation is upregulated in bronchial epithelium of asthma patients as compared with normal subjects (116). Lee *et al* showed differential and overlapping effect of STAT-6 and ERK signaling on IL-13 induced asthma features using transgenic mice (117). *In vitro* and *in vivo* studies have shown that STAT-6 activation via JAK is required for IL-13 mediated mucin induction (goblet cell differentiation) in airway epithelial cells (118). STAT-1 has been shown to be constitutively active in airway epithelium from asthmatic patients as compared to normal subjects and patients with chronic bronchitis (119). Recently *stat3* knockout mice have been shown to be resistant to house dust mite induced airway inflammation (120). CD40 is expressed on AECs and this expression increases under inflammatory conditions (121). Cagnoni *et al.* demonstrated that JAK-3 interacts with CD40 in cultured human AECs, indicating a role of JAK-3 in release of IL-6 and IL-8 by AECs. Thus JAK-3 may serve as the initial tyrosine kinase activated during allergic inflammation in AECs and hence crucial for AEC proliferation (hypertrophy) and mediator release (121-123).

The role of Src kinases in airway epithelial cell, especially, in regards to asthma has not been studied extensively. Since Src kinases are among the first tyrosine kinases to be activated following receptor stimulations, they are likely to be involved. Shibichakravarthy *et al* showed that Lyn is required for MCP-1 secretion by alveolar epithelial type II cells, via the NF-kB pathway during *Pseudomonas aeruginosa* infection. MCP-1 subsequently plays important role in activation of alveolar macrophages resulting in clearance of the infection (124). Zhao *et al* showed that Lyn is activated by PKCδ following lysophosphatidic acid (LPA) stimulation, and is involved in mediating autocrine EGF signaling (125). Src kinases and JAK are also associated with LPS induced lung injury (126).). Mucin secretion is an important function of goblet cells. The Src family kinases are involved in mucin secretion. They play an essential role in mucin secretion induced by pathogens such as rhinovirus (127) and *Pseudomonas aeruginosa* (128). Src family kinases regulate calcium activated chloride channel in airway epithelial cells (129).

11. CONCLUSION AND FUTURE PERSPECTIVE

Tyrosine kinases are crucial for generating intracellular signaling cascades following inflammatory stimulation and hence in pathophysiology of asthma. Both Src and JAK kinases are one of the first kinases to be activated upon receptor stimulation. They play a critical role in activating downstream signaling pathways. Small molecule inhibitors for both these kinase families are available and have been tested in animal model. Manipulation of these kinases by use of small molecule inhibitors holds promise for therapeutic benefits in asthma. Some Src kinases play both negative and positive roles in activating various cell types. Thus, it would be important to define the *in vivo* role of some of these kinases and then develop targeted therapies.

12. ACKNOWLEDGEMENT

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Send correspondence to: Rafeul Alam, Division of Allergy and Immunology, National Jewish Health, 1400 Jackson Street, Denver, CO 80206, Tel: 303-398-1656, Fax: 303-270-2180, E-mail: alamr@njhealth.org