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TEC and MAPK Kinase Signalling Pathways in T helper (T_H) cell Development, T_H2 Differentiation and Allergic Asthma

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Abstract

Significant advances in our understanding of the signalling events during T cell development and differentiation have been made in the past few decades. It is clear that ligation of the T cell receptor (TCR) triggers a series of proximal signalling cascades regulated by an array of protein kinases. These orchestrated and highly regulated series of events, with differential requirements of particular kinases, highlight the disparities between αβ⁺CD4⁺ T cells. Throughout this review we summarise both new and old studies, highlighting the role of Tec and MAPK in T cell development and differentiation with particular focus on T helper 2 (T_H2) cells. Finally, as the allergy epidemic continues, we feature the role played by T_H2 cells in the development of allergy and provide a brief update on promising kinase inhibitors that have been tested in vitro, in pre-clinical disease models *in vivo* and into clinical studies.

Introduction

Peripheral αβ⁺CD4⁺ T cell differentiation

Antigen-restricted effector and effector-memory αβ⁺CD4⁺ T cells are a central component of adaptive immunity. Following successful development and activation in the periphery, effector αβ+CD4+ T cells function to rally innate cells, provide help to B cells for antigen-specific immunoglobulin production and stimulate local tissue

responses; while regulatory αβ⁺CD4⁺ T cells control the proliferation of effector αβ⁺CD4⁺ T cells, suppress innate cell activation and prevent autoimmune reactions. These divergent functions are tightly regulated through cell-intrinsic and extrinsic mechanisms. When these regulatory checkpoints fail, effector αβ⁺CD4⁺ T cells can

cause lethal lymphomas or hyper-inflammatory conditions such as autoimmune, allergy and leukaemia. Conversely, if effector αβ⁺CD4⁺ T cells fail to develop mature or differentiate, individuals can be left with insufficient immunological protection with equally catastrophic outcomes, such as life-threatening severe immunodeficiency. Similarly, failure of regulatory αβ⁺CD4⁺ T cell development can allow the activation of

auto-reactive T cells and uncontrolled inflammation [1]. Thus, throughout the development, differentiation, activation, effector/regulatory function and long-term survival, multiple feedback loops are in place regulating αβ⁺CD4⁺ T cell responses.

Once in the periphery, αβ⁺CD4⁺ T cells can reversibly differentiate into a variety of helper (T_H) / effector (T_H1, T_H2, T_H17) T follicular helper (T_{FH}) and regulatory (T_{REG}) populations, often characterized by their cytokine expression profile and up-stream transcription factors (reviewed elsewhere [2-5]). With occasional exceptions, the molecular programs involved in the differentiation of T_H, T_{FH} or T_{REG} cells are mostly well defined. For example, IFNγ and IL-12 stimulate *Tbx21* (T-bet) through activation of STAT-1 and STAT-4 for T_H1 differentiation and IL-4- and IL-2-induce GATA-3 / STAT-6 and STAT-5 for T_H2 differentiation. Similarly, IL-6 and TGFβ promote RORγt and STAT-3 for T_H17 differentiation and IL-4 in combination with TGFβ induces PU-1 for T_H9 differentiation (thoroughly reviewed [6,7]). While, the precise signals required for T_{FH} cell differentiation are unclear, BCL6 has been demonstrated to orchestrate part of the T_{FH} cell developmental program [8,9]. Finally, IL-2 and TGFβ induce STAT-5 and Foxp3, which prescribe natural T_{REG} (nT_{REG}) development in the thymus or inducible T_{REG} (iT_{REG}) development in the periphery [10]. Foxp3, a transcription factor restricted to T_{REG} cells is essential for T_{REG} development, maintenance and function [11-14]. Despite their importance in specifying T_H cell lineage commitment, many of these transcription factors are not self sufficient in coordinating complete transcriptional programs; for example, Bcl6 and PU-1 for T_{FH} and T_H9 cell differentiation respectively [7,8]. This suggests a role for multiple transcriptional regulators functioning together in T_H cell differentiation. Although differentiated αβ⁺CD4⁺ T cells can adopt different effector/regulatory characteristics, there is significant flexibility between their phenotypes [15-18]. In addition to being phenotypically flexible, different αβ⁺CD4⁺ phenotypes share a common activation step via the T cell receptor (TCR).

T cell receptor (TCR) complex and proximal signalling events

The αβ⁺TCR functions as a biological bottleneck, translating peptide-loaded MHCII-delivered messages into cellular responses via signalling modules and a series of inter-dependent signalling cascades. Signals transmitted via these pathways influence T cell fate decisions in the thymus, differentiation and proliferation in the periphery and antigen-induced cell death. The α and β subunits of the TCR, similar to the γ and δ subunits, undergo a series of selection processes during T cell ontogeny in the thymus. Pairing of β subunits with α chain occurs during the double negative 3 (DN3) stage with the emergence of CD4⁺CD8⁺ (double positive, DP) thymocytes. At this stage, αβ⁺T cells are again selected in the thymus by their ability to respond, or not, to antigen. Just as a response generated in the absence of an antigen leads to cell death by neglect, strong antigen-induced responses also result in

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cell death by positive selection. Thymocytes that respond weakly to the antigen undergo further selection into $CD4^+$ or $CD8^+$ single positive cells that mature further to contribute to the peripheral T lymphocyte pool (a detailed review of this process can be found at [19]).

The α and β chains of the TCR encode short cytoplasmic tails and do not signal. Instead, they transduce TCR signals through the non-covalently associated CD3 proteins that exist as a series of hetero (CD3γε and CD3δε) and homodimers (CD3ζζ) along with the αβ chains [20,21]. The interaction between the αβ chains and CD3 is facilitated by positively charged amino acids in the αβ transmembrane domain and the negatively charged residues in the transmembrane regions of the CD3 complex [22]. Successful selection of a mature T lymphocyte involves the expression of all the components of the TCR, with unassembled protein components ubiquitinated and proteolytically degraded by the endoplasmic reticulum [23]. The cytoplasmic domain of CD3 encode immunoreceptor tyrosine-based activation motif (ITAM) that play a crucial role in initiating signalling downstream of the TCR.

Briefly, in response to TCR engagement by antigen, clustering of the TCR and the co-receptors induces protein tyrosine kinases (PTKs) such as Lck, a member of the Src family of tyrosine kinases, to phosphorylate the tyrosine residues in the ITAMs in the cytoplasmic tails of CD3. These phosphorylated ITAMs serve as docking sites for the Src homology 2 (SH2) domain containing tyrosine kinase ZAP-70 (ζ-associated protein of 70kD). Recruited ZAP-70 is then able to phosphorylate adaptor proteins such as LAT and SLP-76, which brings about downstream activation of different signalling pathways like the PI3-kinase pathway, Ras-MAPK pathway, Ca^{2+}-mediated signalling and Vav-1 mediated signalling leading to actin-cytoskeletal reorganization (Figure 1).

The kinase kingdom

Over 500 kinases regulate many aspects of a cell function. By the addition of phosphate groups to substrate proteins, kinases coordinate protein movement, localisation and activity. With regard to T lymphocytes, three PTK families namely, the Src family kinases (Lck, Fyn), the Syk family kinases (Syk, Zap-70) and the Tec family kinases (Tec, Itk, Btk and Rlk), are essential for peripheral T cell development. Spontaneous mutations in any of these kinase families, or genetic ablation in murine models, results in dramatically dysregulated T cell development [24-30]. In addition to regulating the function of these protein kinases, the T cell kinome also regulates processes ranging from metabolism, actin re-organisation, anergy/ tolerance and cytokine secretion [31-36].

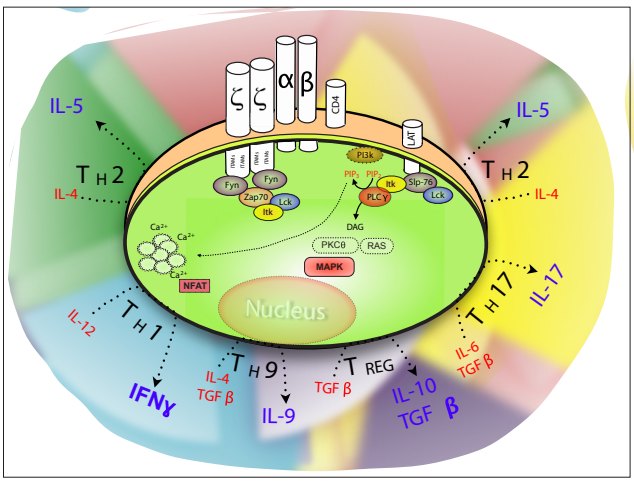

Figure 1: Proximal TCR signaling
Following TCR engagement protein tyrosine kinases (PTKs) such as Lck, phosphorylate the tyrosine residues in the ITAMs in the cytoplasmic tails of CD3ζζ, which serve as docking sites for the Src homology 2 (SH2) domain containing tyrosine kinase ZAP-70. ZAP-70 phosphorylates adaptor proteins, including LAT and SLP-76, activating PI3-kinase pathway, Ras-MAPK pathways and Ca^{2+}-mediated signalling. In the appropriate cytokine environment (red cytokines), $CD4^+$ T cells differentiate into reversible T_H1, T_H2, T_H9, T_H17 and T_{reg} cells and secrete a suite of characteristic cytokines (blue, bold cytokines).

Kinases are subdivided into eight conventional groups and four atypical groups based on sequence similarity, accessory domains and known modes of regulation (Table 1) [37]. The different kinase families share divergent roles in T lymphocyte development, differentiation of T_H cells and their effector functions. Although other kinase families serve important functions in T cells, reviewed elsewhere [38-40], Tec kinase and MAPK (ERK, p38 and JNK) signalling pathways have been intensively studied in $αβ^+CD4^+$ T cells and are the main focus of this review. We have highlighted the unique features of these kinases in T_H2 cells in the context of allergic diseases, and provide an update on current therapeutic strategies using inhibitors to interfere with these signalling pathways in allergic diseases.

Involvement of Kinases in $αβ^+CD4^+$ T helper cell Development and Differentiation

Tec-family Kinases

The Tec family of non receptor tyrosine kinases came into focus when Btk (Bruton's tyrosine kinase), one of the members of the family was identified in severe B cell immunodeficiency, X-linked agammaglobulinemia (XLA) and X-linked immunodeficiency in humans and mice, respectively [64-67]. These reports provided the first evidence of how mutations in tyrosine kinases affect lymphocyte signaling and hence could be linked to primary immunodeficiencies. The Tec family of kinases, of which Tec (tyrosine kinase expressed in hepatocellular carcinoma) was the first to be identified, also consists of: Btk, Itk (interleukin-2 (IL-2)-inducible T-cell kinase; also known as Tsk or Emt), Rlk (resting lymphocyte kinase; also known as Txk) and Bmx (bone-marrow tyrosine kinase gene on chromosome X; also known as Etk) [68]. These proteins expressed primarily in hematopoietic cells are characterized by a common domain organization (Figure 2A). With the exception of Rlk, these proteins encode a pleckstrin homology (PH) domain that binds to phosphatidylinositol 3, 4, 5- triphosphate ($PtdIns(3,4,5)P_3$) on their amino-terminal end [68]. Rlk is an atypical Tec kinase that has a palmitoylated string of cysteine residues at its amino-terminal end [69,70]. This is followed by one or two proline rich regions (PRRs), a SRC homology 3 (SH3), SH2 and a kinase domain at the carboxy terminal end [68]. The presence of the PH domain allows for these proteins to be regulated by phosphatidylinositol 3- kinase (PI3K) mediated signalling, while the cysteine string in Rlk allows for its constitutive association with the plasma membrane independent of PI3K signalling [71-73].

Typically, recruitment of Tec kinases to the plasma membrane through interaction with $PtdIns(3,4,5)P_3$ (generated upon PI3K action on phosphatidylinositol-4,5-bisphosphate ($PtdIns(4,5)P_2$)), is followed by the phosphorylation of a tyrosine residue in the kinase domain by Src-family kinases, which brings about conformational changes that allow for the recruitment of these proteins into antigen-receptor signalling complexes [68,74]. Downstream phosphorylation

Table 1: Kinase families and their functions.

Conventional Family

Family	Description	Reference
AGC	Named after Protein Kinase A, G and C families, consisting of 60 members across 16 families of cytoplasmic serine/threonine kinases, regulated by secondary messengers. E.g.: PKC and PKA. Mutation and dysregulation linked to many conditions such as diabetes and cancer.	[41,42]
CaMK	Named after Calmodulin/Calcium regulated kinases, the CaMK group consists of both calcium and non-calcium regulated kinases. E.g.: CaMK I, CaMK II and CaMK IV. CaMK II is essential for NF-κB activation following TCR ligation.	[43,44]
CK1	Named after Casein kinase 1 (CK1), these monomeric serine/threonine selective kinases are evolutionary conserved within eukaryotes. CK1 kinases regulate diverse functions form circadian rhythm, Wnt signalling, DNA replication and RNA metabolism.	[45]
CMGC	The CMGC group includes the evolutionarily conserved, cyclin-dependent kinases (CDKs), mitogen-activated protein kinases (MAPKs), glycogen synthase kinases (GSKs) and CDK-like kinases (CLKs). Their roles include, cell cycle regulation, immune signalling mediated downstream gene expression, regulating cell proliferation and apoptosis.	[32,46-49]
RGC	The receptor guanylate cyclases (RGCs) synthesize cyclic GMP from GTP. RGCs are membrane bound and are activated by calcium or the binding of atrial naturetic peptide or related peptides. E.g.: GC-A and GC-G.	[50,51]
STE	Serine threonine kinases (STE) are subdivided into 3 main families Ste7, Ste11 and Ste20, which sequentially activate each other and MAPK members. Ste20 kinases (MAP4K) act on Ste11 kinases (MAP3K), which activate Ste7 kinases (MAP2K, MEK, MKKs). These families have distinct roles in T lymphocyte activation.	[32,52]
TK	Tyrosine kinases (TK) evolutionarily conserved in mammals, phosphorylate tyrosine residues. The TK are divided into receptor and non-receptor (cytoplasmic) tyrosine kinases (CTK). The receptor tyrosine kinases are activated by extracellular signals such as growth factors at the cell surface. The CTK include, Src kinases such as Tec and janus kinases (JAK).	[53,54]
TKL	The tyrosine kinases-like (TKL) group has close sequence similarity to the TKs. These diverse, serine/threonine kinases, are subdivided into 7 major families, including the RAF, IRAK and RIPK families. The TKL kinases are involved in a wide range of immune cell processes including cell growth, Toll like receptor (TLR) and IL-1 signalling and cell death.	[55-57]

Atypical Family

Family	Description	Reference
Alpha	α-Kinases have low sequence homology with other conventional kinases and have known functions in protein translation, intracellular transport, cell migration and proliferation. E.g.: elongation factor 2 kinase and Dictyostelium myosin heavy chain kinases	[58]
PIKK	The phosphoinositide 3-kinase-related kinases (PIKK) are involved in DNA damage, nutrient dependent signalling mRNA decay. E.g.: the mammalian target of rapamycin (mTOR), has been well studied in the context of T cell metabolism and differentiation.	[33,59]
PDK	The mitochondrial pyruvate dehydrogenase kinases (PDKs), required for oxidative metabolism mediate the phosphorylation of pyruvate dehydrogenase. There are four known mammalian isoforms; PDHK1-4, and these regulate the balance between glycolysis and lipid metabolism.	[60,61]
RIO	The right open reading frame (RIO) kinases are expressed in archea to humans. Their functions include, ribosome biogenesis and chromosome maintenance, although the precise substrate of the RIO kinases is unknown. RIO kinases from the ruminant nematode, *Haemonchus contortus* have been targeted for nematocidal drugs.	[62,63]

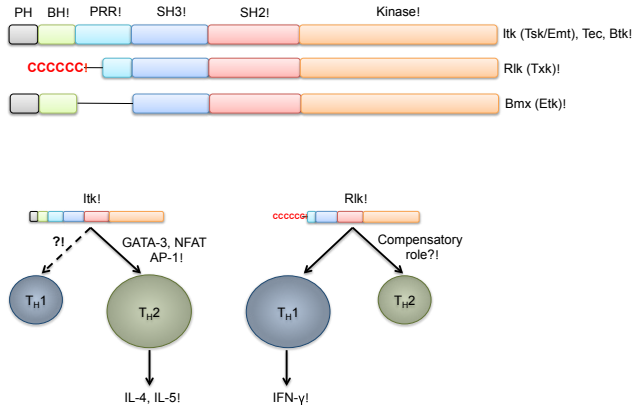

Figure 2: Tec kinases structure and their differential roles
The different members of the Tec kinase family are shown in a schematic. The proteins typically encode a N terminal pleckstrin homology domain (PH), followed by a Btk homology domain (BH), a proline rich region (PRR), a Src homology 3 (SH3) domain, a SH2 domain and a carboxy terminal kinase domain. Rlk, unlike the other members, encodes a palmitoylated string of cysteine residues on its amino terminal domain. Itk is expressed maximally in and is important for T_H2 differentiation, while Rlk has a significant role in regulating T_H1 cells. The role of Itk in T_H1 cells is unclear, while Rlk may compensate for Itk function in $Itk^{-/-}$ cells. BMS-509744 is a pharmacological inhibitor of Itk.

of adaptor molecules, LAT and SLP-76 brings about the recruitment of the protein tyrosine kinase Itk. Itk in turn phosphorylates and activates PLC-γ, which catabolises inositol-1,4,5-triphosphate ($Ins(1,4,5)P_3$) and diacylglycerol (DAG). This activates DAG binding proteins like protein kinase C (PKC) and Ras guanyl releasing proteins (RasGRP), which activate the MAPK family leading to downstream effector responses (Figure 1). Increases in the intracellular Ca^{2+} levels due to $Ins(1,4,5)P_3$ signalling also activates the nuclear factor of activated T cells (NFAT) family of transcription factors, which bring about changes in the gene expression levels.

In addition to being primarily activated in response to TCR signalling, Tec kinases are also involved in chemokine, cytokine, growth factor and co-stimulatory signaling pathways [74]. For example, Itk and Tec interact with the T cell co-stimulatory receptor CD28, where they become phosphorylated and mediate downstream signalling [75-77]. Itk is involved in CD2, CXCR4 and the chemokine, stromal cell derived factor 1a (SDF-1a)/(CXCL12) mediated signalling [78-81]. Tec regulates signalling downstream of cytokine receptors, IL-3, IL-5, prolactin, erythropoietin, GCSF, c-kit and the IL-6 family, while Rlk is involved in CXCL12 and MIP3-β-mediated signalling [74,81].

Tec kinases and T lymphocytes

T lymphocytes express Itk, Rlk and Tec differentially; suggesting their importance in different sub-populations of T lymphocytes, with Itk maximally expressed in naïve T cells [68,74]. T cell activation further increases the expression of Itk, with higher expression of the protein in T_H2 cells compared to T_H1 cells. In this regard, Itk has been implicated in the development of T_H2 responses [82]. In addition to T cell activation, IL-2R signalling can also induce Itk expression [82,83]. Rlk, akin to Itk, is also expressed in thymocytes and mature naïve T

lymphocytes, though its expression levels are 3-10 fold lower in naïve T lymphocytes compared to Itk [84]. Unlike Itk, Rlk is preferentially expressed in T_H1 cells implicating its importance in T_H1 responses [85,86]. Tec has relatively low expression in T lymphocytes compared to Itk and Rlk with 100-fold lower expression than Itk. Tec is increased upon stimulation and, similar to Itk, is expressed higher in T_H2 cells than in T_H1 cells [84,87]. These observations suggest that Tec kinases play an important role in regulating effector T cell differentiation.

Function of Tec kinases in T lymphocytes

In accordance with their roles in TCR activation, mice deficient in Tec kinases demonstrate defective PLC-γ phosphorylation and activation. Consequently, this leads to defective production of $Ins(1,4,5)P_3$, reduced Ca^{2+} influx and activation of downstream transcription factors. Impaired activation of MAPKs like extracellular signal regulated kinase (ERK), JUN N-terminal kinase (JNK) and activated protein-1 (AP-1) is also observed in these cells [68]. Mutations affecting Tec kinases lead to impaired TCR stimulation, proliferation and IL-2 production [27,88,89]. Itk was first demonstrated to be important for the development of conventional T lymphocytes [27]. This was followed by studies demonstrating the role of Itk and Rlk in positive selection and negative selection in response to both MHC class I and class II restricted transgenes [27,28,90]. These roles of Itk and Rlk were attributed to decreased ERK activation and defects in cellular adhesion in the kinase deficient cells [28,91,92]. Among other important roles, Tec kinases have been implicated in the development and differentiation of T lymphocytes. Specifically, Itk deficiency led to impaired recruitment of Vav-1, a guanine nucleotide exchange factor, at the TCR, resulting in disruptions in the nucleation of actin polymerization [68]. Itk also regulates integrin expression and adhesion, following TCR engagement, with Itk-deficiency resulting in reduced TCR activation [93]. Collectively both Itk and Rlk regulate the strength of signal originating from the TCR during T cell development. It is also important to note that beyond $\alpha\beta^+$ T cells, Itk is also required for the development and function of iNKT cells and γδ T cells (reviewed in [94]).

Itk and Rlk have been analysed for their roles in cytokine production, immunity to infection and inflammatory diseases, such as allergy and autoimmunity. These studies have established clear roles for Itk in the differentiation of T_H2 cells (Figure 2B), with $CD4^+$ T cells from *Itk*$^{-/-}$ mice impaired in their ability to produce T_H2 cytokines *in vitro* [82,83,95,96]. Concordant with this, *Itk*$^{-/-}$ mice failed to mount T_H2 responses following infection with *Leishmania major, Nippostrongylus brasiliensis, Schistosoma mansoni,* or allergen-induced airway inflammation [82,83,95]. In contrast, *Itk*$^{-/-}$ mice were capable of generating effective T_H1 responses in these settings. Consistent with its role in regulating T_H2 responses, enhanced Itk expression was observed in patients with atopic dermatitis, a T_H2-associated inflammatory disease of the skin [97]. These defects have been attributed to impaired recruitment of Vav-1 and PKC-θ following TCR activation, with reduced NFAT activation, as well as the requirement of Itk to support the maintenance and amplification of the T_H2 effector responses [98].

Rlk, distinct from Itk, is not only expressed in high levels in T_H1 cells but also bound to the *Ifng* promoter and was required for the production of IFN-γ [86] (Figure 2B). Despite this preferential regulation in T_H1 cells, *Rlk*$^{-/-}$ mice show only minor defects in response to infection by *Toxoplasma gondii*, a T_H1 inducing pathogen [83,89]. Though this observation is yet to be completely understood, it is currently thought that the existence of compensatory mechanisms in these mice could contribute to this effect [68].

Despite the distinct roles of Itk and Rlk in T_H cell differentiation, further examination of Itk/Rlk double knockout mice under different T_H cell regulatory conditions suggests that this dichotomy might be an oversimplified model. Itk/Rlk double knockout (DKO) mice upon initial characterization demonstrated severely impaired T_H1 responses following *T. gondii* infection [89]. In contrast, Itk/Rlk DKO mice mounted an effective T_H2 response following *S. mansoni* infection, despite impaired NFAT activation [83]. These differences have lead to many different ideas about the roles of the individual Tec kinases in T_H cells [68].

The models put forth to explain these differences in the *Itk*$^{-/-}$, *Rlk*$^{-/-}$ and the Itk/Rlk DKO mice include, the idea of "*differential expression of Tec kinases in T_H cells regulate their distinct functions*". This idea is supportive of the individual roles of Itk and Rlk in T_H1 and T_H2 cells respectively, and the compensatory roles of Itk and Rlk, as the expression of an Rlk transgene in the *Itk*$^{-/-}$ mice rescues the impaired T_H2 response upon challenge with T_H2-inducing Schistosome egg antigen (SEA) or in a mouse model of allergic asthma [99].

Another model draws its basis from, "*the TCR signal strength dictates TH-cell development*". This model was put forth to explain the T_H2 biased nature of Itk/Rlk DKO mice upon *S. mansoni* infection. This notion would explain how weak signals, such as low antigen doses or altered peptide ligands, promotes a T_H2 biased response [100], as activated cells down regulate GATA-3 and differentiate into other lineages [83]. Concordant with this, Itk/Rlk DKO mice were severely compromised in signalling downstream of their TCR and T cells from these mice were defective in their ability to repress the expression of GATA-3 [83]. This suggests, that regulation of GATA-3 expression by TCR signalling in Itk/Rlk wild type cells is precedent to the differentiation of the T_H cells to the T_H2 lineage.

Defective activation of the NFAT family members in Itk/Rlk DKO mice might also be responsible for the intact T_H2 responses [83,89]. Inadequate NFAT activation, due to a lack of, or impaired activation of, NFATc2 and NFATc3, members of the NFAT family, is also associated with increased development and activation of T_H2 cells [101,102]. Additionally, the potency of the TCR signal contributes to the differential expression of NFATc1, another NFAT family member, which enhances T_H2 polarization, and transcription of *il4* [103]. Despite these observations, the differential expression of NFAT proteins in Itk/Rlk DKO mice is debated [68,83]. Since impaired Ca^{2+} flux is associated with the development of a T_H2 phenotype and tyrosine mutant LAT is associated with impaired Ca^{2+} flux and a T_H2 bias, the possibility of a similar LAT mutant in Itk/Rlk DKO mice is a plausible hypothesis worth evaluation [104]. Moreover, the role of T cell extrinsic mechanisms such as contributions from Natural killer (NK) cells, Mast cells, Basophils and Eosinophil's in Itk/Rlk DKO mice is another aspect yet to be explored [68].

Although the precise roles of Itk and Rlk in modulating T_H2 development with contributions from the above-mentioned models is yet to be confirmed, the role of Itk in modulating T_H2 responses remains undisputed. These observations have prompted several studies targeting Itk as a potential therapeutic target for asthma and other inflammatory disorders, described in more detail below [105-107].

Itk also regulates T_H17-associated cytokines [108]. $CD4^+$ T lymphocytes from *Itk*$^{-/-}$ mice expressed very low levels of IL-17A when

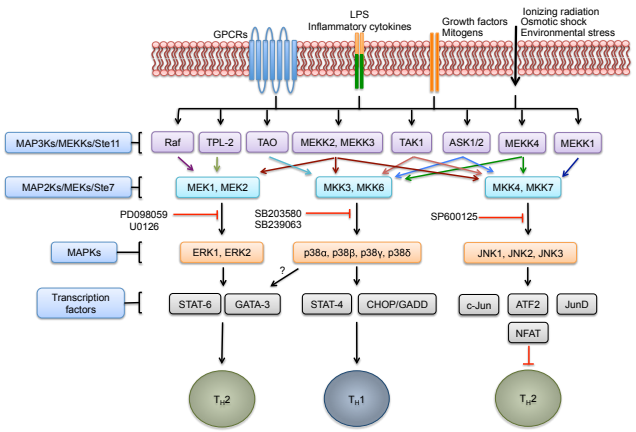

Figure 3: MAPK activation cascade
MAPKs are activated in response to G-protein coupled receptors (GPCRs), growth factors, mitogens, pathogen associated molecular patterns (PAMPs) like LPS, inflammatory cytokines and environmental stresses. The kinase signalling cascade is organized into a three-tier system, with the activated MAP3Ks activating MAP2Ks, which in turn activate the MAPKs. There is significant crosstalk between the pathways and different MAPKs can activate different T_H cell specific transcription factors, that signal the T cells to differentiate accordingly. ERKs and p38 family members are specific for T_H2 and T_H1 cells respectively. While the role of p38 in T_H2 cells remains debated, JNKs are specific inhibitors of T_H2 cells. The different pharmacological inhibitors of the MAPKs are also shown.

polarized under T_H17 conditions *in vitro*. This effect was even more dramatic in T lymphocytes from Itk/Rlk DKO mice, supporting the functional redundancy between the Tec kinases. This role of Itk was once again coupled to its ability to activate NFAT in response to TCR signalling, suggesting activated NFAT binds to the *Il17a* promoter in wild type cells but not in *Itk*$^{-/-}$ cells [108]. Collectively, Tec kinases regulate distinct T lymphocyte development and functions at multiple levels and provide an 'Achilles heel' to target T cell-mediated diseases.

Mitogen Activated Protein Kinases (MAPKs)

MAPKs are one of the most ancient and evolutionarily conserved families of kinases. These proteins are activated in response to cytokines, growth factors, and environmental stresses and are involved in cell proliferation, differentiation and death. The MAPK family includes three major groups in mammalian cells: the extracellular signal regulated kinases (ERKs), the p38 MAPK family and the c-Jun N-terminal kinases (JNK) (Figure 3) [109-111]. Each family of MAPK is activated by dual phosphorylation of threonine and tyrosine residues in their activation loop (Thr- X-Tyr) by evolutionarily conserved upstream kinases, MAPK kinases (MAPKK or MAP2Ks) and MAPK kinase kinases (MAPKKK or MAP3Ks). The MAPKKK are serine/threonine kinases that are typically activated by RAS superfamily GTPases or signalling intermediates in response to cytokines and growth factors. This brings about the phosphorylation of the downstream MAPKK, which activate the MAPKs by dual phosphorylation of the threonine and tyrosine residues in the activation loop. Once activated, the MAPKs phosphorylate their substrates such as transcription factors, phospholipases, cytoskeletal proteins, as well as several additional protein kinase families such as the ribosomal S6 kinases, mitogen and stress activated kinases and MAPK-interacting kinases [49]. This serine/threonine specific phosphorylation of the target substrate is marked by a preceding proline residue. Once phosphorylated, the substrates mediate downstream signalling ranging from regulation of gene expression and cellular differentiation to apoptosis.

The ERK family, the first family of mammalian MAPK to be identified, encodes two classical members, ERK1 and ERK2. These kinases are dual phosphorylated at the activation loop Thr-Glu-Tyr and are classically activated in response to mitogens or insulin resulting in the activation of the proto-oncogene, Ras. Ras recruits and activates the Raf family of MAP3Ks. Raf in turn phosphorylates the MAP2K members, MEK1 and MEK2 which activates the downstream ERK family of kinases [112]. The ERK families of kinases are also activated in response to pro-inflammatory cytokines, such as TNFα, IL-1β and pathogen associated molecular patterns (PAMPs) like lipopolysaccharide (LPS) [49]. The ribosomal S6 kinases (RSKs), mitogen and stress activated kinase family members (MSKs), MSK1 and MSK2 and the MAPK-interacting kinase (MNK), MNK1 and MNK2 are selective substrates for the ERK family [113-117]. ERK can also regulate the activation of Elk1, member of the ternary complex factor (TCF) family, which is an important regulator of the AP-1 family of transcription factors [118].

The p38 family, another group of proteins in the MAPK family, has 4 members; p38α, p38β, p38γ and p38δ. These are activated in response to environmental stresses like ionizing radiation and osmotic shock, inflammatory cytokines and PAMPs. Upon activation, the MAP3Ks phosphorylate the MAP2Ks, MKK3/MKK4/MKK6, which in turn phosphorylate the p38 family members in their activation loop, Thr-Gly-Tyr. The p38 family in addition to regulating the MAPK-interacting kinases (MNK1/2) and the mitogen and stress activated protein kinases (MSK-1/2) also regulate the MAPK-activated protein kinases (MK2) [119-122]. The p38 family members also regulate the activation of the transcription factors, C/EBP homologous protein (CHOP/GADD), myocyte enhancer factor 2 (MEF2) family members MEF2A/C and ATF2 of the AP-1 family [123-125]. The p38s also phosphorylate Sap-1a, a member of the TCF family, which together with serum response factor (SRF) binds to the serum response element (SRE) on the *c-fos* promoter and mediates *c-fos* induction [126,127].

The last group of the MAPK family, the JNK family, has 3 main members in eukaryotic cells; JNK1, JNK2 and JNK3 [111]. The JNK family, in addition to being activated by cytokines and growth factors, is also activated by environmental stresses, genotoxins, mechanical stress, pro-inflammatory cytokines, PAMPs and danger associated molecular patterns (DAMPs). The JNK family members are subject to differential pre-mRNA splicing thus giving rise to 12 different JNK polypeptides. While the functional significance of these isoforms remains unclear, it has been reported some of these isoforms (α and β JNK) differ in their affinities for their substrate [128-130]. A typical signalling cascade brings about the phosphorylation of the MAP2Ks, MKK4 and MKK7 by MAP3Ks. This in turn phosphorylates the JNK family at the activation loop Thr-Pro-Tyr to activate downstream signalling. A major group of JNK substrates are the AP-1 family of transcription factors including, c-Jun, JunD and ATF2 [128,131,132]. JNKs also phosphorylate Elk-1 at the carboxy terminus, contributing to the regulation of *c-fos* [133], NFAT4 and NFAT2 [134,135]. Since NFAT2 activity is important for the polarization of T_H cells to T_H2 cells, JNK mediated inhibition of NFAT2 has been shown to negatively regulate T_H2 differentiation [136,137].

MAPKs in T cell Development, Differentiation and Function

ERK and T lymphocytes

The role of MAPKs, specifically the MEK-ERK pathway, in early

thymocyte development was initially established in fetal thymic organ cultures (FTOC) exhibiting compromised differentiation of immature $CD25^+CD44^-$ thymocytes to the pre-TCR expressing thymocytes [138]. Further support for the functional role of ERK in pre-TCR development came from studies that demonstrated that transfection with a TCR-β chain in *Rag2*$^{-/-}$ FTOC resulted in ERK activation. Furthermore, constitutive activation of ERK using a mutant form of c-Raf-1 induced maturation and expansion of $CD25^-CD44^-$ thymocytes from *Rag2*$^{-/-}$ mice [139,140].

The importance of the Ras-ERK pathway in late thymocyte development was also demonstrated when *Erk1*$^{-/-}$ mice had reduced percentages of mature $CD4^+/CD8^+$ thymocytes [141]. The role of the Ras-ERK pathway in positive selection was substantiated when Delgado and colleagues reported the inability of *Cd3δ*$^{-/-}$ DP thymocytes to undergo positive selection [142]. This report held important implications for the role of ERK in thymocyte maturation and TCR signalling, as the positive selection defect was coupled to impaired ERK activation, due to defective tyrosine phosphorylation of the upstream adaptor LAT and the CD3ζ chain upon engagement of the TCR. This suggested that activation of the LAT-Ras-ERK pathway was important for positive selection. ERK function in TCR signalling was also validated by studies linking the adaptor proteins, SLP76 and Grb2 to the downstream activation of the SOS-Ras-MEK-ERK pathway [143-145]. Simultaneously, studies demonstrated the functional significance of clonal anergy linked to incomplete Ras and ERK activation [146,147].

In addition to TCR signalling, the Ras-ERK pathway also regulates T_H2 differentiation. ERK activation, mediated by MEK, induced phosphorylation of STAT-6 and IL-4Rα in response to IL-4 [148]. Furthermore, TCR activation of the Ras-ERK pathway stabilized the T_H2 specific transcription factor GATA-3 through inhibition of its degradation by the ubiquitin proteasome pathway [149]. ERK activation has also been linked to IL-4 expression during TCR induced T_H differentiation [150,151]. The mechanism mediating this regulation was governed by the level of ERK activation, which in turn, was governed by the strength of signal originating from the TCR. Mechanistically, low strength TCR activation induced IL-4 production by naïve $CD4^+$ T cells through activation of ERK weakly and transiently. Intense and sustained ERK activation in response to strong TCR signals inhibited the expression of early IL-4 from $CD4^+$ T cells and led to T_H1 differentiation [100,150,152].

In addition to the direct reports of ERK regulation in T lymphocytes, the MAP3K, tumour progression locus-2 (TPL-2), has also been linked to T cell differentiation. TPL-2, initially identified as an oncoprotein kinase, is an important regulator of ERK activation in macrophages [153-157]. Deficiency of *tpl2* led to impaired IFN-γ production by T_H1 cells, with compromised control of *T. gondii* [158]. The impaired T_H1 responses in T. gondii infected *Tpl2*$^{-/-}$ mice was suggested to be T cell intrinsic. Furthermore, *Tpl2*$^{-/-}$ mice develop an enhanced inflammatory T_H2 phenotype upon OVA challenge [159]. This exaggerated T_H2 response was linked to the intrinsic inability of the T lymphocytes to differentiate to IFN-γ producing T_H1 cells *in vitro*, as a consequence of poor induction of T-bet and STAT-4 [158,159]. This functional role of TPL-2 in influencing T_H-polarization was further attributed to TPL-2 dependent ERK activation. Contrastingly in a separate study, *Tpl2*$^{-/-}$ mice develop skewed T_H1 responses upon *L. major* infection and OVA immunization, suggesting TPL-2 functions as a negative regulator of T_H1 responses by inhibiting IL-12 production in innate cells [160]. These studies conflicting observations on the role of TPL-2 in T cell differentiation and function may be complicated further as TPL-2 regulates the proteolytic degradation of the NFκB inhibitory protein, p105, which functions to retain the NFκB subunits inactive in the cytosol [161]. Thus, defects in TPL-2 signalling pathway would also encode defects in NFκB signalling.

p38 family in T cell development and differentiation

The p38 kinase is required for the early expansion of immature thymocytes [162]. High levels of p38 kinase activity measured by an *in vitro* kinase assay, are associated with CD4/CD8 DN thymocytes, which are $CD25^-/CD44^+$, $CD25^+/CD44^+$ and $CD25^+/CD44^-$. However, low levels of p38 kinase activity are associated with the $CD25^-/CD44^-$ preTCR thymocytes as well as CD4/CD8 DP thymocytes [162]. While p38 MAPK regulates the early stages of thymocyte development, constitutive activation of p38 MAPK in a constitutively active *mkk6* transgenic mouse, blocks the differentiation of immature thymocytes to the DP stage, and results in a lack of T lymphocytes in the peripheral immune system [162]. The role of p38 MAPK in positive and negative selection was initially addressed using FTOC assays and a pharmacological inhibitor of p38 MAPK. This initial study demonstrated that p38 was not required for positive selection. Instead, forced expression of a constitutively active p38 MAPK caused the deletion of DP thymocytes [163]. Meanwhile, a contrasting study documented an important regulatory role for the p38 family in positive selection, where inhibition of p38 kinase using the pharmacological inhibitor SB 203580 prevented the differentiation of DP cells into single positive cells *in vitro* [164]. This observation was further supported by studies using transgenic mice with dominant negative mutations in *mkk3* and *mkk6* that suggested, complete inhibition of p38 kinase activity lead to impaired positive selection with a decrease in single positive CD4 or CD8 thymocytes [165]. This study provided a definitive role for p38 kinase in positive selection, unlike the previous observations with incomplete inhibition of p38 kinase activity- either due to an ineffective dominant negative mutation or due to compensatory mechanisms from alternative signalling pathways [162,166].

With regard to T cell differentiation, inhibition of p38 MAPK in $CD4^+$ T cells from a transgenic mouse expressing a dominant negative p38, resulted in decreased IFN-γ production [167]. The regulatory role of p38 in IFN-γ production was further supported by the observation that $CD4^+$ T cells from a *mkk6* transgenic mouse with a constitutively active p38 kinase also produced increased levels of IFN-γ [167]. These observations, supported by other independent studies, suggest an important role for the p38 MAPK pathway in regulating TH1 responses both *in vitro* and *in vivo* [168-170]. Additionally, the role of p38 kinase, specifically its α isoform, p38α in TH1 responses was demonstrated to be cytokine dependent with p38α$^{-/-}$ $CD4^+$ T_H1 cells defective in IFNγ secretion upon IL-12 and IL-18 stimulation compared to TCR induced IFNγ production [171]. This suggested that p38 kinase played an important role in maintaining T_H1 cytokine production. Contrasting observations were made with respect to T_H2 cells. While, the $CD4^+$ T cells from the dominant negative *p38* transgenic model showed no change in IL-4 production, $CD4^+$ T cells from the mkk6 transgenic model demonstrated decreased IL-4 production [167]. In an independent study, inhibition of p38 MAPK kinase activity using the inhibitor SB203850, demonstrated a role for p38 MAPK in GATA-3 phosphorylation and positive regulation of T_H2 cytokines [172]. Exactly how this differential regulation by p38 MAPK occurs in T_H1 and T_H2 cells is not completely understood. Common upstream activators like Rac2 and GADD45γ (the stress inducible protein), whose expression is specific to, or higher in, T_H1 cells than T_H2 cells, have been suggested to potentially activate one pathway over the other [170]. In this regard,

expression of GADD45γ or GADD45β in the stress activated MAP3K, MEKK4 sufficient cells, mediates p38 activation and up regulates IFN-γ production in $CD4^+$ T cells [173].

In addition to the canonical pathway of p38 activation by MAP2Ks and MAP3Ks, there exists a non-canonical pathway of p38 activation, which is mediated by phosphorylation of Y323 of the p38 MAPK, catalysed by the TCR signalling adaptor Zap70 [174]. This phosphorylation is followed by the auto phosphorylation at the activation loop T180/Y182 that brings about the activation of the p38 MAPK [174]. The non-canonical pathway has important implications in T cell differentiation as a mutant form of *p38α* in T cells, which is incapable of undergoing non-canonical activation due to a Y323F mutation, has been linked to reduced IFN-γ production [175]. All these studies indicate the p38 MAPK family has important regulatory functions in early thymocyte maturation and differentiation of $CD4^+$ T cells into IFN-γ producing T_H1 cells.

JNK family in T cell development and differentiation

Though there is no strong support for the role of JNKs in the development of $CD4^+$ or $CD8^+$ T cells, several studies have implicated the JNK pathway in the deletion of CD4/CD8 DP thymocytes [176-178]. These studies demonstrated that the JNK pathway is activated in response to signals initiating negative selection in response to MKK7 activation. Experiments with a dominant negative *jnk* transgenic mouse demonstrated that inhibition of the JNK pathway resulted in the deletion of CD4/CD8 DP thymocytes [176]. Of the different isoforms of JNK, JNK2 was demonstrated to have a dominant effect on TCR induced thymocyte apoptosis, which was in turn related to its ability to modulate c-Jun and NFAT [179].

JNKs are very weakly expressed in peripheral lymphoid tissues and their activity is also very low [180]. This low level of activity is also supported by the low level of expression of their kinases MKK4 and MKK7 [170]. However, their activity is significantly upregulated upon TCR activation and peaks 36 - 60h post activation. Despite comparable expression of the JNK proteins in the effector phase of T_H1 and T_H2 cells, JNK kinase activity is dominant in T_H1 cells rather than T_H2 cells [181].

Several groups analysed the role of JNK1 and JNK2 in T cell activation and cytokine production [136,137,177,178,182]. JNK2 deficient 'T_H1' cells produced significantly lower levels of IFN-γ and $CD4^+$ T cells from these mice failed to differentiate into T_H1 cells [182]. Reduced IFN-γ production in *Jnk2*$^{-/-}$ mice was due to reduced expression of the IL-12 receptor β chain (IL-12Rβ2) [182]. JNK1, unlike JNK2, seemed to have a more pronounced role in freshly activated T cells despite comparable protein expression of the two isoforms in these cells [136]. This suggested that total JNK kinase activity in T cells might not always be a result of the cumulative effects of the functional JNK1 and JNK2 isoforms. Indeed, in support of this observation kinase activity by JNK2 is more pronounced in T_H1 effector cells [182]. The role of JNK1 and JNK2 in IL-2 expression was resolved by Dong and colleagues, who generated JNK1/JNK2 double knockout mice and analysed the effect of JNK1/JNK2 absence TCR activation and IL-2 expression [137]. This study revealed JNK1 and JNK2 were not required for the activation of T cells and IL-2 expression but were important for T_H cell, particularly T_H1 differentiation and its corresponding effector cytokine production, beyond IL-2.

The role of JNK1 in T_H cell differentiation comes from studies using *Jnk1*$^{-/-}$ mice, which exhibit exaggerated T_H2 responses in addition to impaired T_H1 responses [136]. This was true even for $CD4^+$ T cells cultured under T_H1 conditions. The enhanced T_H2 response observed in *Jnk1*$^{-/-}$ mice was consistent with their inability to heal skin lesions and ulcers upon infection with *L. major* [183]. Dong and colleagues examined the negative regulatory effect by JNK1 on T_H2 differentiation and showed *Jnk1*$^{-/-}$ mice had elevated levels of NFATc, whose nuclear retention in wild type cells is regulated by JNK mediated phosphorylation [136]. JNK phosphorylation of NFATc prevents it from being dephosphorylated by calcineurin phosphatase that is responsible for the nuclear retention of NFATc [135]. In the absence of JNK signalling, NFATc susceptible to dephosphorylation by calcineurin phosphatase, accumulates in the nucleus and mediates exaggerated transcriptional activity by increasing the expression of T_H2 cytokines.

Additionally, it has been demonstrated that JNK1 mediated suppression of T_H2 effector cytokine production was dependent on the proteolytic turnover of the AP-1 family member, JunB [184-186]. JunB is post-translationally regulated by ubiquitination in $CD4^+$ T cells [187]. This is mediated by the E3 ubiquitin ligase, Itch, which is activated following TCR co-stimulation by JNK1 phosphorylation [187,188]. These observations were corroborated by Enzler and colleagues who demonstrated that the stress induced MAP3K, MEKK1 upon TCR activation recruits Itch to its signalling complex and activates JNK1, thus regulating JunB mediated T_H2 cytokine expression [189]. Itch induced ubiquitination of JunB, mediated by MEKK1-JNK1 signalling has also been shown to be involved in peripheral T_H2 tolerance [190].

The stress activated MAP3Ks and T lymphocytes

The stress activated MAP3Ks consist of a family of five members, MEKK1-4 and the NF-κB-inducing kinase (NIK). This group of proteins can activate multiple members of the MAPK family. Of these family members, MEKK2 and MEKK3 are promiscuous activators as they are non-selective in their target substrates and equally activate ERK, JNK as well as the p38 MAPK family [49]. Their function in T cell activation and homeostasis has been demonstrated using biochemical and genetic studies, where they share the downstream activation of MAPK [191,192]. MEKK3 has also been recently implicated in T cell responsiveness wherein conditional knockout of mekk3 in T cells led to impaired IFN-γ expression in T cells [193]. This impaired TH1 phenotype correlates with decreased clearance of *L. monocytogenes*–OVA infection and is associated with reduced TCR mediated activation of ERK, JNK and p38 MAPKs.

Both MEKK2 and MEKK3 are co-expressed in T cells suggesting they might have overlapping functions. Chang and colleagues generated MEKK2/MEKK3 DKO mice to investigate the role of MEKK2 and MEKK3 in T_H cell differentiation [194]. These mice exhibited increased number of T_{REG} as well as T_H17 cells. This was due to a cell intrinsic phenomena as mixed bone marrow chimeras with equal ratios of wild type or MEKK2/MEKK3 DKO T cells in *Rag2*$^{-/-}$ mice maintained the T_{REG} and T_H17 accumulation in the periphery, identical to the parent MEKK2/MEKK3 DKO mice. The increased numbers of T_{REG} and T_H17 cells was suspected to be due to the enhanced sensitivity of the naïve T cells from the MEKK2/MEKK3 DKO mice to TGF-β (required for T_{REG} and T_H17 differentiation) as pharmacological inhibition of TGF-β receptor signalling *in vivo* reduced this increase in T_{REG} and T_H17 cells. This study also linked the enhanced sensitivity to TGF-β to defective activation of ERK1 and ERK2, which in turn brought about the hypo-phosphorylation of the SMAD2/3 proteins in their linker regions, which is important for suppression of TGF-β signalling [195-198].

Thus this study provided evidence to support the mechanistic role of MEKK2 and MEKK3 mediated inhibition of TGF-β signalling and regulating T_H cell differentiation.

Therapeutically Targeting Kinases in Allergy

Atopy and the manifestation of allergic asthma presents as a heterogeneous clinical disease, mediated in part by $CD4^+$ T_H1 and T_H17 cells, in addition to a dominant role played by T_H2 cells [199]. Briefly, the development of IL-4-secreting allergen-specific T_H2 cells, which promote the class-switching and clonal expansion of allergen- reactive B cells, leads to allergen-reactive IgE and an atopic or sensitized state. Following subsequent exposure to the same allergen, cross-linking of mast cell- and basophil-bound IgE molecules, trigger acute early phase reactions with histamine, prostaglandin and leukotriene release. Re-activation of allergen-specific T cells typify the late phase response, re-enforcing antibody class switching but also mobilizing, maturing and activating eosinophils and promoting goblet cell hyperplasia and mucus hyper secretion, through the actions of IL-5 and IL-13. In chronic cases, resolution of repeated inflammatory events within the tissue can lead to excessive wound healing and the development of fibrotic plaques and smooth muscle thickening. Many cellular events contribute to this inflammatory disease, and therefore targeting of any particular kinase, or kinase family is fraught with complexities. Nevertheless, several kinase inhibitors have been proposed, tested or moved into pre-clinical and clinical trials to treat inflammatory diseases, including asthma. Below we summarize some of the many inhibitors being developed and refer to more focused reviews, where possible, for further reading.

Tec kinase inhibitors

PI3kinase inhibitors: PI3kδ, one member of the PI3K family, is predominantly expressed in hematopoietic cells including mast cells, B cells, T cells and neutrophils. Although not a Tec kinase, PI3kδ converts the membrane phospholipid PtdIns(4,5)P_2 into PtdIns(3,4,5)P_3, allowing the recruitment of Tec family kinases. Concordant with its expression, PI3kδ-dead knock-in mice have significant defects in B and T cell signalling, high affinity IgE receptor, FcεR1, signalling and neutrophil activation [200-202]. IC87114, an inhibitor of PI3Kδ (and to a lesser extent PI3Kγ and β), significantly inhibits the T_H2-associated inflammatory cascade with diminished allergen-induced airway inflammation and hyper-responsiveness (AHR) in a mouse model [203]. Additionally, enhanced T_{REG} cell development has been reported *in vitro* upon PI3K interference, due to premature termination of TCR activation. Despite this observation, this effect does not fully translate in-vivo, as PI3Kδ-knock-in mice develop inflammatory bowels disease (IBD), a condition primarily due to a lack of intestinal T_{REG} cells [200]. Hence, despite an array of PI3K inhibitors in development and phase 2 trials, few seem to have been applied to the allergy arena [204].

Btk inhibitors: Btk is crucial for B cell and IgE receptor (FcεR1) signalling but also appears to play important roles in myeloid cells [205-207]. Btk is neither expressed in T cells, nor capable of activating downstream pathways when over expressed in T cell lines [87]. Nevertheless, inhibiting Btk could disrupt the B cell / IgE / mast cell axis, which is responsible for allergen sensitisation. Indeed, inhibiting Btk with the selective irreversible inhibitor, PCI-32765, significantly blocked IgE-mediated basophil activation, cytokine secretion and degranulation [208]. Many Btk inhibitors (PCI-32765, terreic acid and LFM-A13, GDC-0834, RN486) have been tested in a variety of *in vivo* settings, with minimal toxicity and significant reduction in arthritis and leukaemia [209-213]. However, despite suitable B cell /IgE / mast cell-dependent models of allergic inflammation and an array of Btk inhibitors, there are no studies reporting the impact of Btk inhibitors in allergy models. Currently 27 Phase 1 and 2 clinical studies are investigating the use of Btk inhibitors in a variety of diseases.

Itk inhibitors: Itk, as mentioned above, is significantly expressed in T cells and regulates T_H2 cell development [27,83]. Itk also regulates mast cell cytokine secretion, but not degranulation [214,215]. In animal models, Itk-deficient mice and transgenic mice lacking Itk activity are protected from allergen-induced airway inflammation, supporting the case for therapeutic Itk inhibition [95,216-218]. A significant amount of work has been done optimizing inhibitors of Itk, however very few have made it through to pre-clinical trials, or at least been reported [106,107,219-222]. An Itk inhibitor, BMS-509744, was shown to inhibit airway eosinophilia in a murine model of ovalbumin-induced inflammation, however many of the other airway allergy-associated parameters were not reported in this study [223]. Many other pharmaceutical companies, including Boehringer Ingelheim, AstraZeneca and Sanofi-Aventis have products in the pipeline, but have not published their findings yet.

MAP Kinase inhibitors

The three-tier MAP kinases that phosphorylate serine/threonine MAP3Ks, which in turn phosphorylate MAP2Ks, that phosphorylate MAPKs, have been widely studied and interrogated at every level with small molecule inhibitors [224].

MEK/ERK inhibitors: Of the several MEK/ERK inhibitors, PD098059 and U0126 have received significant attention inhibiting MEK1 and MEK2 with a high degree of specificity [225,226]. Using in vitro guinea pig bronchial rings to test the contraction of airways as a model of airway contraction, PD098059 was shown to inhibit peptide-leukotriene's from mast cells, allow a quicker recovery time post contraction and block IL-1β-induced prostaglandin D_2 release from primary and immortalised airway epithelial cells [227-230]. *In vivo*, U0126 inhibited allergen-induced airway inflammation, although it also increased steroid resistance [231-233]. To date and to our knowledge, neither PD098059 nor U0126 have moved into clinical studies, but have remained invaluable tools for researchers to dissect molecular pathways. Eleven other MEK/ERK inhibitors, including PD184352, have proved promising *in vitro* and *in vivo*, and have been moved into phase 1 and 2 in various clinical trails in cancer patients [234-237]. Although cancer treatment has driven the development of PD184352 and other first and second-generation MEK/ERK inhibitors, their application to other inflammatory diseases, including allergy, will be of significant interest.

p38 inhibitors: p38 regulates many inflammatory pathways involved in respiratory diseases (reviewed by [238-240]). It has been extensively pursued, and unlike many MEK/ERK inhibitors to date, several p38 inhibitors have been tested in allergy models and allergic patients [241,242]. The p38 inhibitors, SD282, SB239063 and a respirable p38α antisense oligonucleotide have all been shown to inhibit experimental allergen-induced airway eosinophilia, IgE and airway hyper-responsiveness in mice [243-246]. T_H2 cell-derived IL-5 mobilises eosinophils from the bone marrow, matures and activates them in the tissue [247]. In this context, the p38 inhibitor SB203580 inhibits IL-5, reduces IL-13 synthesis from human T cells and induces eosinophil apoptosis, thus suggesting that the IL-5/eosinophil axis is sensitive to p38 inhibition [231,248,249]. Additionally, it has been observed that many asthmatic patients are resistant, or insensitive, to

inhaled corticosteroids (ICS) [250-252]. It appears that this insensitivity is mediated, in part, by p38 signalling, and that inhibition of p38 can restore steroid sensitivity [253-256]. Thus, p38 inhibitors hold great promise in interfering with allergen-induced inflammatory axis (IL-5/eosinophilia), and also in hard-to-treat allergic patients. There are currently 3 experimental clinical studies investigating the role of p38 in steroid sensitivity in severe asthmatics, with one phase 2 clinical trial testing the safety of the p38 inhibitor SB-681323.

JNK inhibitors: Several JNK inhibitors have been tested in a variety of *in vitro* and *in vivo* disease models (reviewed in [257]). In particular XG-102 and SP600125 have been successfully tested in pre-clinical *in vivo* models of IBD and have been shown to reduce TNFα expression and disease severity [258-260]. With respect to asthma, SP600125 has been tested in ozone and allergen-induced airway inflammation models in mice and rats, with both studies reporting positive outcomes [261-263]. In mice, 30 mg/kg of SP600125 reduced airway eosinophilia, goblet cell and mucus secretion and airway hyper responsiveness, following ovalbumin airway challenge [262]. However in rats, 90 mg/kg of SP600125 reduced cellular infiltration into the air spaces, but did not impact inflammatory cytokine secretion, IgE or airway hyper responsiveness [263]. To date, several JNK inhibitors (CC-401, CNI-1493, AM-111, XG-102, CC359, CC930 and CEP-1347) have moved into phase 1 and phase 2 clinical trials, but are yet to be tested in allergic individuals [257].

Concluding Comments

In summary, TEC kinase-regulated pathways, which differentially control T_H1, T_H2, and T_H17 responses through regulation of PLC-γ activation, Ca^{2+} influx and transcription factor translocation are attractive therapeutic targets to disable T cell-mediated responses. Specifically in the allergy field, the TEC kinase Itk that is required for Th2 cells, may forestall atopy and Th2-reactivation. Similarly, the MAPKs, which integrate into proximal TCR signalling cascades, required for downstream transcription factor activation, including the phosphorylation of STAT6 and stabilisation of GATA3, are an attractive family of kinases to therapeutically target.

However, despite significant advances in our understanding of the signalling events in T cells, the complexities and abundance of kinase-regulated signalling pathways and a better grasp of the pathogenesis of allergic asthma, broad spectrum non-specific inhaled corticosteroids are still the mainstay of current treatment. This is due to many factors, including; the complexities of CD4 T cell development and differentiation, the apparent differential kinase requirements in different T cells and the emerging appreciation of T cell plasticity. For example, recent studies have identified that fully differentiated T cells maintain a degree of plasticity with the ability to change between different T_H phenotypes and even between helper and regulatory cells [264]. If the kinase-regulated signalling pathways associated with the different T_H phenotypes are also plastic, allowing for dynamic re-organisation, then this would pose a significant challenge for therapeutically targeting any specific T_H population with inhibitors. Furthermore, allergic asthma is now viewed as a heterogeneous disease consisting of T_H1-, T_H2- and T_H17-associated responses, with differential kinase requirements in different T_H cells, as described above [228,245]. This poses an additional obstacle as it considerably expands both the number and expression of the potential targets. For example, targeting Itk, which is the most abundantly expressed TEC kinase and required for T_H2 and T_H17, but not T_H1, cell development, may skew allergen-reactivity to T_H1 cells that may be equally as damaging. The potential off-target effects of kinase inhibitors, whether in non-targeted cells or closely related pathways in the appropriate cell, pose another great difficulty which is yet to be overcome [265]. Thus, despite our current understanding of the substrates and phosphorylated residues involved in these signalling pathways, there are many unknown gaps in the chain, which need to be investigated and identified. Nevertheless, the requirement for kinase signalling pathways in various T_H cells and at various stages is still an appealing therapeutic target. Finally, to tackle the complexities of allergic diseases and in a bid to move our efforts forward, a cross-disciplinary approach between chemists, biochemists, immunologists and *in-vivo* disease biologists may be more fruitful and greater than the sum of our individual parts.

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