

EXPERT OPINION

1. Introduction
2. Pim kinases
3. Runx
4. The Pim1-Runx3 axis regulates development of peanut allergy
5. Expert opinion

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healthcare

Targeting Pim1 kinase in the treatment of peanut allergy

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Introduction: Peanut sensitization is the most significant food allergen associated with life-threatening allergic reactions. Unlike many food sensitivities, peanut allergy often persists into adulthood. Presently, the only effective therapy is peanut avoidance. Effective preventative therapy requires an understanding of the pathways that lead to anaphylaxis. IgE and mast cell activation are essential contributors. The responsible pathways upstream are driven by pro-allergic T helper 2 differentiation and release of cytokines including interleukin-4 (IL-4) and IL-13.

Areas covered: The research utilized an experimental model of peanut-induced anaphylaxis in mice that mimics many of the responses seen in the human disease. Following peanut sensitization and challenge, clinical responses, intestinal inflammatory and immune cell interactions, and genetic and molecular events were monitored. For the first time, evidence for Pim1 kinase involvement was demonstrated in association with the downregulation of Runx3, a known silencer of the IL-4 gene locus. Evidence for Pim1 kinase involvement was shown through the use of a small molecule inhibitor of Pim1 kinase.

Expert opinion: Activation of Pim1 kinase and downregulation of Runx3 were essential to the development of peanut-induced intestinal anaphylaxis. Targeting of this Pim1 kinase-Runx3 axis may provide new therapeutic options in the prevention of life-threatening reactions to peanut.

Keywords: anaphylaxis, peanut, Pim1 kinase, Runx3

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1. Introduction

Peanut allergy is one of the most common food allergies and the prevalence is increasing in the United States and Western countries. It is estimated that the prevalence of peanut allergy is 1% in children and 0.6% in adults in the United States [1] and 1.5% among children in the UK [2]. Unlike other food allergies such as cow's milk, egg and fruit, peanut allergy often persists throughout life and is the leading cause of fatal, food-induced anaphylaxis in Western Europe and the United States [3,4]. Despite the increasing numbers of peanut-sensitive individuals and risks of severe allergic reactions, peanut allergy research has not defined new therapeutic options to date, especially for prevention. Avoidance of ingestion of peanut remains the only effectively preventive measure. Therefore, identifying potential, new therapeutic strategies for the treatment and prevention of peanut-induced allergic reactions is a priority.

Peanut-induced allergic reactions are mediated by both IgE-dependent and IgE-independent (IgG1) pathways [5,6]. Clinical and experimental studies suggest that initiation of food-induced intestinal allergy is regulated by numerous inflammatory cells and mediators, including mast cells, eosinophils [7,8], T cells [9], and T helper 2 (Th2)-cytokines [10,11]. Increased numbers of activated T cells have been correlated with elevated levels of Th2 cytokines as well as the degree of gastrointestinal inflammation and dysfunction in patients with food allergy [12,13]. *In vitro*,

Article highlights.

- Allergen sensitization and challenge leads to an increase in Pim1 kinase levels in mice.
- This is accompanied by a downregulation of Runx3, an important silencer of IL-4 transcription and IL-13 production in mice.
- Inhibition of Pim1 kinase activation using a small molecule inhibitor attenuates the full spectrum of allergic responses in mice.
- The inhibition of Pim1 kinase activation following sensitization and challenge results in a normalization of Runx3 levels and downregulation of IL-13 production in the small intestine of mice.
- The Pim1 kinase-Runx3 axis is a critical regulator of pro-allergic T lymphocyte differentiation in mice.

This box summarizes key points contained in the article.

allergen-stimulated T cells and T cell clones generated from peanut allergic patients produced elevated levels of Th2-cytokines (IL-4, IL-5, and IL-13) [14]. These cytokines activate immunological pathways associated with the initiation of allergic responses, including Th2 cell differentiation, IgE synthesis, and mast cell and eosinophil recruitment and activation [11].

2. Pim kinases

2.1 Pim1 kinase characteristics and function

Provirus integration site for Moloney murine leukemia virus (Pim) is a proto-oncogene encoding a family of serine/threonine protein kinases. The Pim family includes three members: Pim1, Pim2 and Pim3 [15]. Pim1 and Pim2 are expressed in most hematopoietic cells; Pim3 is expressed in brain, kidney and mammary tissue. Pim2 and Pim3 show a high degree of homology to Pim1. At the amino acid level, Pim2 and Pim3 show 61 and 71% identity to Pim1 [16]. Pim1 genes are located on chromosome 6p21 and chromosome 17 in human and mouse, respectively [17]. In the mouse, Pim1 encodes two isoforms with molecular weights of 34 and 44 kDa, respectively [18]. The 44 kDa protein contains an amino-terminal extension of the 34 kDa protein. The 34 and 44 kDa proteins are synthesized by alternative translational initiation at AUG and CUG codons, respectively [18]. Pim1 34 kDa is monomeric *in vivo* whereas Pim1 44 kDa is a complex. Both proteins contain the kinase domain and exhibit comparable kinase activities *in vitro*; the Pim1 44 kDa protein is relatively more stable than the 34 kDa protein [18]. In humans, initially the *Pim1* gene was thought to only encode a 34 kDa protein [17]. Subsequently, it was shown that the human *Pim1* gene, like the mouse *Pim1* gene, also encodes a 44 kDa protein [19,20]. The 44 kDa Pim1 is translated efficiently and upregulated significantly in human cancer cell lines as well as in human cancers [20]. Cellular localization of Pim1 kinase was found

in the nucleus, cytoplasm and membrane of the cells [17,18,20]. The cellular localization of the 34 kDa Pim1 is in the cytoplasm and nucleus, while the 44 kDa isoform is present in cytoplasm and cell membranes [20]. Pim1 kinase has multiple functions involved in cell survival, proliferation, differentiation, apoptosis and tumorigenesis and has been implicated in cytokine-dependent signaling in hematopoietic cells [17]. Pim1 has been widely used as a target in various cancers [21,22].

2.2 Pim1 kinase and allergic inflammation

Several cell types implicated in allergic reactions express Pim1 kinase including eosinophils [23] and CD4 T cells [24,25]. Pim1 expression was increased in eosinophils from bronchoalveolar lavage (BAL) fluid compared to blood eosinophils from asthmatic patients after allergen provocation [26]. Pim1 kinase mRNA and protein expression were increased in the lung of sensitized and challenged mice in an experimental model of asthma [27] and Pim1 kinase expression was enhanced during T cell activation [28]. Pim1 kinase increases T cell proliferation by enhancing the activity of nuclear factor of activated T cells (NFAT) c1, increasing IL-2 production in T cells [29]. Pim kinases are also reported to control T cell activation and function [15,25]. A recent study identified and demonstrated that Pim1 kinase was upregulated and functioned as a novel effector molecular, sufficient to drive CD4 T cell development and survival in the absence of cytokine receptor signals [30].

2.3 Regulation and activation of Pim1 kinase

In terms of immune/inflammatory responses, the consequences of Pim1 kinase activation are pleiotropic. Pim1 kinase expression can be induced by a variety of cytokines (IL-2, IL-3, IL-5, IL-6, IL-7, IL-12, IL-15), various mitogens and hormones such as GM-CSF, erythropoietin, interferon (IFN)- γ and prolactin [19]. Pim1 kinase plays an important role in key signal transduction pathways and promotes cell survival and proliferation through the PI3K/AKT and JAK/STATs signaling pathways [31] and modification and phosphorylation of cell cycle regulators including CDC25A and CDC25C [32]. In addition, Pim1 regulates cell cycle progression through phosphorylation and inhibition of proapoptotic proteins (BAD, MAP3K5, FOXO3, SOCS1/3) [32]. Phosphorylation of BAD induces release of the anti-apoptotic protein Bcl-X(L)/Bcl2. Phosphorylation of MAP3K5 by Pim1 kinase inhibits MAP3K5-mediated phosphorylation of JNK and JNK/p38MAPK, which reduces caspase-3 activation and cell apoptosis [33]. In another mode of activity, Pim1 kinase regulates homing and migration of hematopoietic cells through modification of CXCR4 expression [34]. A novel finding was that Pim1 kinase was involved in the differentiation of Th2 and Th17 cells through the regulation of Runt-related transcription factor (Runx) expression [8].

3. Runx

3.1 Runx family members and disease-associations

Runx, a novel family of transcription factors, play essential roles in embryonic development and the members are key regulators of lineage-specific gene expression responsible for the development of allergic responses [35-38]. There are three mammalian Runx genes: *Runx1*, *Runx2* and *Runx3*. *Runx1* is required for hematopoiesis, [39], and mutations of *Runx1* have been demonstrated in human leukemia [40,41]. *Runx2* is critical regulator of osteogenesis [42,43] and mutations of *Runx2* have been demonstrated in cleidocranial dysplasia patients [44]. The *Runx3* gene resides on human chromosome 1p36.1 [45], which maps to a region containing susceptibility genes for asthma [46] and on mouse chromosome 4 [47], which is a susceptibility gene for atopic dermatitis [48]. It has been reported that *Runx3* is required for neurogenesis, thymopoiesis and the control of gastric epithelial cell proliferation [49-51]. Loss of *Runx3* results in spontaneous development of inflammatory bowel disease [36], gastric cancer [51,52], as well as asthma [37,38,53]. A genetic association between *Runx3* and immune-mediated ulcerative colitis has been reported [54]. Thus, genetic alterations in the *Runx3* gene are associated with several immune/inflammatory diseases, including allergic disease.

3.2 Runx3 functions

Importantly, Runx3 protein regulates CD4/CD8 T cell lineage decision. Taniuchi *et al.* have shown that Runx3 is essential for establishing epigenetic CD4 silencing and for specification of functional cytotoxic T cells in thymocytes [55]. Loss of Runx3/core binding factor (Cbf)β complex function in CD4⁺ T cells resulted in spontaneous development of asthma, at least in part, through failure of IL-4 silencing [56]. The Runx complexes are composed of two subunits, the Runx protein, which possesses a conserved DNA-binding domain and a unique Cbfβ protein. Cbfβ stabilizes Runx protein-DNA interactions and prevents ubiquitin-mediated Runx degradation [57,58]. Runx complexes act together with other transcription factors to regulate immune responses. Runx3 cooperates with T-bet to repress the production of IL-4 by binding to the IL4 silencer in the Th2 cytokine locus and promotes the production of IFN-γ in Th1 cells [59,60]. Runx3 enhances Th1 and decreases Th2 differentiation by interacting with GATA3 [61,62]. Together, these findings suggest that *Runx3* plays a critical role in regulating T-cell development, differentiation of Th1/Th2 cells and Th1/Th2 cytokine production.

4. The Pim1-Runx3 axis regulates development of peanut allergy

Direct interactions between Pim1 kinase and Runx3 have been reported and catalytically-active Pim1 kinase regulates

the transcriptional activity of Runx3 [63]. However, some of the results appear discordant [64]. In a mouse model of peanut allergy, levels of Pim 1 kinase expression were increased in the small intestine of peanut-sensitized and challenged mice, while levels of Runx3 expression were decreased. Inhibition of Pim1 kinase using a specific small molecule inhibitor (AR460770) [27] prevented peanut-induced allergic responses including diarrhea, improved symptom scores and significantly reduced the accumulation of mast cells and eosinophils in the jejunum. In the peanut-sensitized and challenged mice, inhibition of Pim1 kinase was associated with an upregulation of Runx3 mRNA and protein expression in the small intestinal tissues [8]. In *Runx3*^{+/-} mice, the inhibitor exhibited reduced effects on all of these parameters, supporting the notion of an interaction (negative) between Pim1 kinase and Runx3. *In vitro*, inhibition of Pim1 kinase attenuated wild-type CD4⁺ Th2 and Th17 cell differentiation, at the same time maintaining Runx3 levels. In *Runx3* heterozygote-deficient (*Runx3*^{+/-}) mice, inhibition of Pim1 kinase had significantly lower effects *in vivo* and *in vitro*, confirming the interaction of Pim1 kinase and Runx3 in modulating the T cell-mediated responses to peanut sensitization and challenge [8].

These data support a novel regulatory pathway involving the inverse relationship of Pim1 kinase and Runx3 in the control of peanut allergic reactions through the regulation of Th2 (and Th17) differentiation (Figure 1). Targeting this novel regulatory axis involving Pim1 kinase and Runx3 may offer new therapeutic opportunities for the control of peanut-induced allergic reactions.

5. Expert opinion

Peanut allergy has become increasingly more common in recent decades in the United States. Unlike other food allergies, peanut allergy often persists throughout life and is the leading cause of fatal, food-induced anaphylaxis in Western Europe and the United States. Currently, there are no effective therapies for food allergy other than to avoid the allergenic food. In spite of attempts to avoid allergenic foods, accidental exposures are the major causes of allergic reactions to foods and result in food-induced anaphylaxis. As a result, there is a critical need to find a means to prevent and treat these life-threatening events. Clinical and experimental analyses suggest that initiation of food-induced intestinal allergy is regulated by numerous inflammatory cells and mediators, including T cells, mast cells, eosinophils and Th2-cytokines. CD4 T cells are among the predominant allergic inflammatory cells, and are recruited into the intestine in experimental peanut allergy and in patients with peanut allergy.

Pim1 kinase is a serine/threonine kinase implicated in cytokine-induced cell signaling. Recently the roles of Pim1 kinase and Runx3 in the pathogenesis of peanut-induced intestinal allergy have been defined. Pim1 kinase

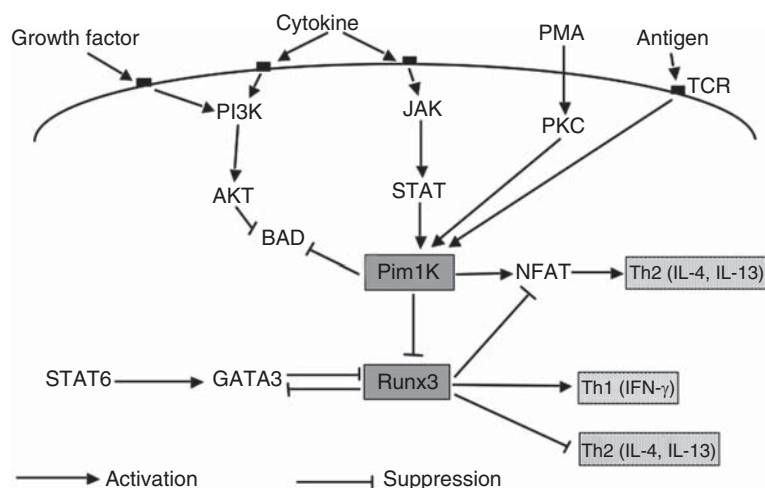


Figure 1. Pim1 kinase/Runx3 regulation of Th1 and Th2 cytokine expression. Pim1 kinase expression is induced primarily through JAK/STAT and PI3K/AKT signaling pathways. Pim1 kinase induces Th2 cytokine expression through its activation of nuclear factor of activated T-cells (NFAT). Runx3 cooperates with T-box transcription factor (T-bet) to repress the production of IL-4 by binding to the IL-4 silencer in the Th2 cytokine locus and promotes the production of interferon (IFN)- γ in Th1 cells. Runx3 enhances Th1 cytokine expression and downregulates Th2 cytokine expression by inhibiting GATA3, a major transcription factor for Th2 differentiation. Activation of Pim1 kinase suppresses Runx3 expression, resulting in the upregulation of Th2 cytokine expression.

activation and downregulation of Runx3 are essential components of intestinal anaphylaxis induced by peanut sensitization. Inhibition of Pim1 kinase by a small molecule inhibitor prevented peanut-induced intestinal anaphylaxis and was associated with an increase in Runx3 expression and a decrease in CD4 T cell numbers and function in the small intestinal mucosa *in vivo* and *in vitro*. Therefore, targeting Pim1 kinase activation by small molecule inhibitors has the potential to prevent life-threatening anaphylactic reactions. The findings in mice now must be translated to man with the demonstration that activation of Pim1 kinase is important in the human equivalent. Currently, development of small molecule and specific inhibitors of Pim1 kinase remain a challenge as witnessed in targeting other kinases. The field is rapidly evolving through innovative medicinal chemistry

approaches to target kinases with greater specificity and reduction in the potential for serious adverse events. Although targeting Pim kinases has primarily been used for cancer therapy, the selective involvement of Pim1 kinase in driving allergic reactions may open avenues for novel drug design. Indeed, unmasking the importance of the Pim1 kinase-Runx3 control of pro-allergic T cell differentiation opens up many avenues of research in the wide spectrum of allergic diseases.

Declaration of interest

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