Current Allergy and Asthma Reports (2019) 19: 29
https://doi.org/10.1007/s11882-019-0860-8

BASIC AND APPLIED SCIENCE (I LEWKOWICH, SECTION EDITOR)

# Th9 Cells in Allergic Disease

Pornpimon Angkasekwinai [1]

Published online: 26 March 2019


## Abstract

**Purposes of Review** Th9 cells are recognized as a novel subset of effector T helper cells that preferentially produce IL-9. Here, we provide a current update on the reports related to the function of Th9 cells in allergic inflammatory diseases.

**Recent Findings** The effector Th9 cells differentiating from naïve T helper cells have recently been identified. Because of accumulating findings of Th9 cells in many inflammatory diseases, including allergic diseases, diverse functions of Th9 cells in regulating immune responses have been suggested. Related reports indicate multiple sources of IL-9 besides Th9 cells and their association with the pathogenesis of allergic rhinitis, asthma, atopic dermatitis, contact dermatitis, and food allergy. More recently, elements of the epigenetic landscape involving in the regulation of IL-9 by Th9 cells have been identified to be the potential target for allergic inflammation.

**Summary** This review provides the most recent information about Th9 cells and their contribution in airway allergic disease, skin, and food allergy.

**Keywords** Th9 · IL-9 · T helper cell · Allergy · Allergic diseases · Asthma

## Introduction

Diversity of T helper cell differentiation has significant impact on the functional contribution of effector T helper cells in immune responses to pathogens and immune-mediated diseases, including allergic disease. Many effector T helper cell lineages have been identified, and the Th9 subset that has been described to mainly produce IL-9 is one of them. Development and function of Th9 cells and Th2 cells are closely related because IL-9 was originally found to be produced by Th2 cells, and Th2 cells can be further driven to Th9 cells in the presence of TGF-β [1, 2]. Clustering analysis also indicated the close relationship between Th2 and Th9 cells [3]. Th9 cells have a gene signature that is distinct from Th2 and Treg subsets [3]. More recent studies have proposed that human Th9 cells are indeed a subpopulation of Th2 cells that briefly upregulated IL-9 expression and appeared transiently in skin allergic inflammation [4••, 5]. Th9 cells function to enhance the protective immune responses to intestinal helminth infection and exacerbate allergic disease by promoting the function of mast cells, epithelial cells, and smooth muscle cells [6–10]. In addition, they have unique roles in promoting tumor progression of hematological tumor and enhancing tumor immunity for solid tumor by activating function of mast cells and cytotoxic T lymphocytes as well as driving direct cytotoxicity to tumor cells [11]. A more recent study related the function of Th9 cells in driving intestinal inflammation in colitis [12]. In this review, we aim to provide current information on the role of Th9 cells in allergic diseases, emphasizing on the recent evidence of Th9 cells in airway allergic disease, skin, and food allergy in humans and murine models.

## The Role of T Helper Cells in Allergic Diseases

Allergic diseases are chronic inflammatory diseases caused by dysregulated immune responses to allergens. The exposure to innocuous environmental antigens influences the activation of naïve to effector T helper cells. It has been appreciated for many years that the increase of cytokines associated to Th2 cells correlates with the clinical pathology of various allergic diseases [13]. The Th2-derived cytokines, including IL-4, IL-

This article is part of the Topical Collection on *Basic and Applied Science*

✉ Pornpimon Angkasekwinai
upornpim@tu.ac.th; p.akswn@gmail.com

[1] Department of Medical Technology, Faculty of Allied Health Sciences, Thammasat University, Pathumthani 12120, Thailand

5, and IL-13, function to promote the production of IgE, eosinophil survival, epithelial cell hyperplasia, and airway hyperresponsiveness. Recently, type 2 innate lymphoid cells have been discovered to mirror the function of adaptive counterpart of Th2 cells to produce type 2 cytokines [14–16] and the cooperation of ILC2 and Th2 cells thus contributed to the exacerbation of allergic diseases [17, 18]. Moreover, since the discovery of the diversity of T helper cell subsets in the past decade, there has been increasing evidence for the influence of other T helper cell lineages on regulating allergic diseases. Th17, the third T helper cell lineage, was found to participate in the progression of certain forms of allergic diseases, such as neutrophilic allergic asthma and allergic contact dermatitis by promoting neutrophil recruitment and tissue inflammation [19]. Th9 cells, a distinct T helper cell lineage that produced IL-9 but not IL-4, IL-5, and IL-13, were reported to enhance allergic lung inflammation, particularly through the activation of mast cell function [8, 10, 20]. In addition to these effector CD4+ T cells, extensive data have demonstrated the suppressive effect of regulatory T cells in the control of allergic responses, and the induction of these cell types may be an alternative approach for treatment of established allergic diseases [21]. Therefore, the balance between the inductions of effector T helper subsets and regulatory T cells plays critical roles in determining allergic disease outcomes.

## Th9 Cells

Activation of naïve T helper cells in the presence of IL-4 and TGF-β was identified to promote development of Th9 cells [1, 2]. Several cytokines and costimulatory molecules were found to be involved in the regulation of Th9 cells. While IFN-γ strongly inhibited IL-9 expression [22], IL-1β [23], IL-2 [24], TSLP [25], IL-21 and IL-25 promoted IL-9 production by Th9 cells [9, 26]. Moreover, costimulatory molecules, including OX40 [27], PDL-2 [28], GITR [29, 30], Notch receptor [31], and TL1A [32], were shown to enhance the differentiation of Th9 cells. The activation of transcription factors STAT6 [33], IRF4 [34], and GATA3 [2] is critical for the development of Th9 cells. In addition, transcription factors PU.1 [10], BAFT [3], NF-kB [35], NFAT1 [36], STAT5 [24], SMAD2/3 [37], IRF8 [38], and FOXO1 [39] also promoted Th9 cell polarization and IL-9 production. More recently, the regulatory element super-enhancer (SE) region and element 25 kb upstream from the *Il9* gene (*Il9* CNS-25) were identified involving in the regulation of IL-9 [40••, 41••].

Besides Th9 cells, IL-9 can be produced by innate immune cells, including mucosal mast cell precursor (MMC9) [42], type 2 innate lymphoid cells [43, 44], and adaptive immune cells such as CD8+ T (Tc9) cells [45] and Th17 cells [46, 47], depending on the disease settings. The most recent study using ex vivo human T helper cells proposes that Th9 cells are a Th2 cell subpopulation [4]. By analyzing chemokine receptors on memory T helper cells, it was found that Th9 cells expressed Th2 chemokine receptor CCR4 but with unique expression of skin-homing receptor CCR8, indicating shared characteristics of Th2 and Th9 cells. However, both Th2 and Th9 clones, generated from isolated memory T helper cells, expressing $CCR4^{+}CCR8^{-}$ and $CCR4^{+}CCR8^{+}$, respectively, were capable of producing IL-9, and Th9 clones after activation can also secrete IL-5 and IL-13. This study termed IL-$9^{+}$Th2 cells as the subpopulation of Th2 cells that produced IL-9. Moreover, it suggested that the phenotype of Th9 cell lineage indeed derived from the transient down-regulation of signature Th2 cytokines in IL-$9^{+}$Th2 cells. Using transcriptome analysis to analyze Th1, Th2, Th17, and IL-$9^{+}$Th2 clones, the principal component analysis demonstrated that Th9 cells are related to Th2 cells and identified PPAR-γ as transcriptional factor involved in the regulation of IL-9-producing Th2 cells [4]. IL-9 production by human-naïve T helper cells induced by IL-4 and TGF-β as well as by Th2 cells was dependent on transcription factor PPAR-γ [4••]. How Th9 cells are derived from Th2 cells in vivo and their differential function in different disease settings awaits further investigation.

## Th9 Cells in Airway Allergic Diseases

Indeed, the up-regulation of IL-9 and its contribution to allergic lung diseases have been reported even before the discovery of Th9 cells. The characterization of genetic factors in asthma and atopy demonstrated the contribution of the IL-9 locus on the level of serum IgE but not bronchial hyperresponsiveness [48, 49]. Chemokine and mucin expression by lung epithelial cells and eosinophil chemotaxis in the lungs was observed after both exogenous IL-9 treatment and in IL-9 transgenic mice [50–52]. More importantly, deficiency of IL-9 and IL-9 blockade reduced goblet cell hyperplasia and mast cell proliferation in response to lung challenge to allergens [53–55]. The roles of IL-9 in promoting the function of lung epithelial cells, eosinophil, mast cells, and the production of IgE may thus account for its involvement in the pathogenesis of airway allergic diseases.

## Evidence for Th9 Cell Involvement in Allergic Lung Diseases in Mice

Numerous studies in mice indicate the importance of IL-9 and Th9 cells in exacerbating allergic lung diseases [50–52]. After Th9 cells were identified [1, 2], the contribution of IL-9-producing T helper cells in allergic disease was then further investigated by characterizing antigen-specific IL-9 production by ELISA, analyzing the

frequency of Th9 cells by flow cytometry, evaluating Th9 cell function by in vivo adoptive transfer of Th9 cells, and by using mice deficient in IL-9-promoting transcription factors in T cells [56]. Th9 cells were found to express IL-17 receptor B, the cognate IL-25 receptor, and antigen-specific IL-9 responses that were enhanced by IL-25 in mice with allergic airway inflammation [9]. Deficiency of transcription factor PU.1 specifically in T cells reduced IL-9 expression in association with the attenuated allergic lung inflammation [10]. To evaluate the role of Th9 cells in allergic airway disease, Th9 cells were adoptively transferred to Rag2-deficient mice and these mice were then exposed to allergen ovalbumin [34]. Compared with adoptively transferred Th2 cells, Th9 cells induced similar asthma symptoms characterized by increased airway hyperresponsiveness, eosinophils, and mucus-producing goblet cells. A neutralizing IL-9 antibody ameliorated these symptoms in Th9 cell but not Th2 cell-recipient mice [34], suggesting the in vivo source of IL-9 derived from Th9 cells and the critical contribution of Th9 cells in mediating asthma pathogenesis [34]. However, it has been demonstrated that ILC2s are the major source of IL-9 in papain-induced allergic inflammation in the lungs [57]. These data suggest that IL-9 may be produced by different cell types in response to different allergens and Th9 cells may contribute to some types of allergic airway diseases.

Chronic allergic airway diseases seem to potentiate the induction of Th9 cells more than acute allergic airway diseases. Stimulation of chronic airway hyperresponsiveness (AHR) using *Aspergillus fumigatus* lysate resulted in induction of pulmonary Th2 by week 4 and was followed by accumulation of Th9 cells by week 6 [28]. This study showed a direct correlation between the presence of Th9 cells and the severity of lung inflammation, mucus production and AHR, and strengthened the importance of Th9 cells in chronic allergic airway responses. Furthermore, adoptive transfer of Th9 cells showed the obligate contribution of Th9 cells to the enhanced allergic inflammation by promoting mast cell accumulation [20]. In murine models, exposure to HDM-induced Th9 cells was the major cellular source of IL-9 in response to allergen challenge [8]. Adoptive transfer of in vitro generated Th9 cells followed by HDM exposure enhanced inflammatory cell infiltration and mast cell activation [8]. Moreover, neutralization of IL-9 in murine model of allergic bronchopulmonary aspergillosis ameliorated allergic lung pathology in response to fungus. Indeed, the production of IL-9 by Th9 cells and IL9$^+$ILC2s was found to drive IL-2 production by mast cells, resulting in the expansion of ILC2, which in turn activate Th9 cells, leading to an amplified allergic lung inflammation [58••]. This study emphasizes the cooperative function of Th9 cells, mast cells, and ILC2s in promoting lung inflammation.

## Evidence for Th9 Cell Involvement in Allergic Lung Diseases in Humans

Research in human mostly evaluated the expression of IL-9, either by detecting serum IL-9 or IL-9 mRNA in peripheral blood mononuclear cells of patients with allergy. In earlier studies in bronchial biopsy of patients with asthma, the amount of IL-9 transcript was found to be higher than in atopic non-asthmatic controls and in healthy subjects [59, 60]. The expression of IL-9 was also increased in bronchoalveolar lavage cells after allergen challenge in atopic asthma subjects [60]. Before discovery of Th9 cells, it had been demonstrated that T lymphocytes in bronchoalveolar lavage cells were the major cellular source of IL-9 [60] and more than 60% of cells expressing IL-9 mRNA in asthmatic tissue were CD3+ lymphocytes [59], suggesting that IL-9 originated from T cells. In patients with allergic rhinitis, the nasal mucosal IL-9$^+$ cells were increased during pollen season and the increase was inhibited after immunotherapy [61]. This study found that several cell types, including T cells, eosinophils, and mast cells, were potential cellular sources of IL-9 [61]. In an in vitro study of PBMCs restimulated with allergen, the cells isolated from atopic patients and individuals with asthma showed higher IL-9 production than those isolated from non-atopic and rhinitis individuals [62]. The level of IL-9 derived from adult atopic patient PBMCs was strongly correlated with the allergen-specific IgE titer [62]. Furthermore, CD4 depletion completely abolished IL-9 production by PBMCs, suggesting that the principal source of IL-9 was from T helper cells [63].

A few studies reported direct evidence for the existence of Th9 cells during allergic responses in humans. In vitro differentiation of human naïve T helper cells (CD4$^+$CD45RA$^+$ T cells) stimulated with TGF-β and IL-4 showed greater induction of Th9 cells in atopic patients than non-atopic patients [3, 20, 64]. The number of Th9 cells in PBMCs isolated from house dust mite (HDM) or pollen allergic atopic subjects was significantly higher than non-allergic subjects and was correlated with IgE levels [8]. These studies demonstrated that Th9 cells can be detected in atopic subject peripheral blood. In patients with chronic rhinosinusitis, a significant number of Th9 cells were detected in nasal polyp tissue but not in PBMCs and a higher number of Th9 cells were found in eosinophilic chronic rhinosinusitis than in non-eosinophilic subjects [65]. These data suggest that the presence of Th9 cells may be more localized in tissue and important in the pathogenesis of certain subtypes of allergic airway diseases.

However, it appeared that the percentage of Th9 cells and IL-9 level in patients with allergic asthma were correlated [66]. In this study, the peripheral blood Th9 cells in patients with allergic asthma were also compared with allergic rhinitis and healthy subjects [66]. Consistent with other studies, greater frequencies of Th9 cells were observed in the allergic asthma group than in the allergic rhinitis and healthy groups [66],

suggesting a substantial role of Th9 cells in the contribution of the pathogenesis of allergic asthma. In children with HDM allergic asthma, significantly higher IL-9-expressing $CD4^+$ T cells were detected compared with healthy children and these cells did not express IL-4 [67]. More importantly, the presence of IL-9 may contribute to lung pathology by promoting lung inflammation, IgE production, and mast cell activation as demonstrated in the study of cystic fibrosis patients who overproduced IL-9 [58••]. Altogether, these data suggest that the cooperation of type 2 cytokines and IL-9 derived from innate ILC2 and adaptive Th9 cells thus plays critical roles in exacerbating allergic lung inflammation.

## Th9 Cells in Skin Allergic Diseases

The two most common allergic skin disorders are atopic dermatitis and contact dermatitis. Atopic dermatitis, often called eczema, is a chronic allergic skin disease caused by skin barrier dysfunction, followed by inflammation of the skin. Children with atopic dermatitis tend to develop food allergy, allergic rhinitis, and asthma in later age [68]. Recent evidence indicated that allergic sensitization of the skin may be a cause of subsequent elevation in food allergy and allergic airway diseases [69]. The initiation of atopic dermatitis has been associated with the activation of effector T helper cells with predominant expression of Th2 cytokines. Blocking cytokines derived from Th2 cells using a human monoclonal antibody directed against IL-4 receptors (Dupilumb) attenuated atopic dermatitis activities in patients, indicating the critical function of Th2 cells in pathogenesis of atopic dermatitis [70, 71••]. Moreover, it has been demonstrated that novel effector T helper cells, including Th17 and Th22 cells, may also contribute to the disease progression, particularly in the chronic stage [72]. Because IL-9 was originally considered to be a Th2 cell-derived cytokine, the activation of Th9 cells may thus contribute to the pathophysiology underlying atopic dermatitis development.

In support of this, it has been demonstrated that epicutaneous sensitization with ovalbumin promotes the induction of Th9 cells in draining lymph nodes [73]. Genotypic analysis in humans found an association of polymorphisms of the IL-9 gene with development of atopic dermatitis [74], suggesting the potential participation of this cytokine in disease pathogenesis. Moreover, the transcript amounts of IL-9 and IL-9 receptor genes were significantly higher in skin biopsies of atopic dermatitis lesion than normal control [75]. This study suggested that the overexpression of IL-9 in skin caused activation of mast cells in atopic dermatitis. Furthermore, the level of serum IL-9 in patients with atopic dermatitis was also evaluated and shown to be related with the clinical severity using Scoring Atopic Dermatitis (SCORAD) index [76]. In a recent study, increased IL-9 and PU.1 expression in atopic dermatitis was found significantly correlated with the SCORAD index [77]. In early-onset pediatric atopic dermatitis, increased expression of genes associated with activation of Th2, Th9, and Th17 was observed [78], suggesting a contribution of multiple T helper cell responses in skin allergic inflammation.

Many studies demonstrated the contribution of IL-9 that may not directly be derived from Th9 cells in atopic dermatitis. By investigating memory T cells in human blood and tissue, it has been suggested that human Th9 cells express cutaneous homing receptor and are tropic for the skin [79]. Ma et al. observed a higher level of Th9 cells in peripheral circulation of patients with atopic dermatitis than in those with psoriasis and healthy controls [80]. The number of Th9 cells in PBMCs was associated with serum IL-9, transcript amount of IL-9 and PU.1 in PBMCs, as well as IL-9 and VEGF expression in atopic dermatitis lesions. Furthermore, the percentage of Th9 cells was correlated with clinical severity graded by SCORAD index and with the level of serum IgE in atopic dermatitis patients [80], suggesting the direct function of Th9 cell activation in contributing to the pathogenesis of atopic dermatitis.

Besides the involvement in atopic dermatitis, Th9 cells were also reported to function in contact dermatitis. Allergic contact dermatitis is the allergic reaction in the skin that occurs upon contact with substances such as cosmetics, latex gloves, and nickel. Using PBMCs from individuals allergic to the *p*-phenylenediamine (PPD), hair dye component allergen, PPD stimulation increased the expression of IL-5, IL-9, and IL-13 in allergic but not tolerant individuals [81, 82]. Furthermore, expression of IL-9 and Th9 associated genes was found to be elevated in allergic contact dermatitis skin compared to normal skin [83]. Using immunohistochemical analysis, $PU.1^+CD4^+$ double positive T cells were identified in cellular infiltrates of allergic contact dermatitis but not detectable in normal skin samples [83]. Moreover, deficiency of IL-9 in mice attenuated contact hypersensitivity with diminished Th2 and enhanced Th1 responses [83], suggesting the balance of effector T helper cells involving in regulating allergic contact dermatitis. In PPD-allergic patients, Th9 cells were found to be increased in both skin and PBMCs and the expression of IL-9 correlated with the severity of inflammatory reaction after patch test [82]. However, it should be noted that a protective role for Th9 cells has been suggested in the context of contact hypersensitivity induced by PPD because IL-9 receptor-deficient mice exhibited severe development of contact hypersensitivity with more ear swelling [82]. The relative importance of Th9 cell involvement in contact hypersensitivity may be dependent on the experimental model since less pronounced ear swelling in IL-9-deficient mice was observed in contact hypersensitivity induced by 2,4-dinitrofluorobenzene (DNFB) [83]. Moreover, the balance between effector and regulatory T cells is a critical determinant for the development of disease pathogenesis. It has been suggested that IL-9 is also a key mediator of regulatory T cells in immunosuppression

because the absence of IL-9 receptor can weaken the suppressive activity of regulatory T cells in inflammatory diseases [84]. Future studies focusing on the mechanisms by which effector and regulatory T cells involve in chemically induced dermatitis in different models in depth will provide important clues and enhance our understanding.

More recently, it has been demonstrated that IL-9 induction was highest in an acute contact dermatitis reaction to nickel compared with other skin allergic reactions [4••]. The level of IL-9 in the skin was positively correlated with the disease severity of patients with chronic atopic dermatitis and acute contact dermatitis [4••]. This recent study suggested that IL-9 was predominantly expressed by Th2 cells and not a bona fide Th9 cell lineage in the skin [4]. Taken together, accumulating lines of evidence suggest the contribution of IL-9 and Th9 cells in exacerbating the pathogenesis of atopic dermatitis and acute contact dermatitis. The function of multiple cell types and cytokine network derived from T helper cells, including Th9 cells, may be required to integrate in the full development of allergic skin diseases.

## Th9 Cells in Food Allergies

Food allergy is often associated with activation of Th2 immune responses and enhanced production of allergen-specific IgE. After IgE crosslinking, the activation of sensitized mast cells and basophils causes the release preformed inflammatory mediators that result in the immediate allergic reaction upon food intake [85]. IL-9 has been known to be required for oral antigen sensitization and experimental intestinal anaphylaxis by activating mast cell responses that enhance intestinal permeability [86, 87]. Intestinal-specific IL-9 transgenic mice were prone to develop experimental food allergy [86, 87]. Moreover, IL-9$^{+}$IL-10$^{+}$ T helper cells were found to be activated in the small intestine of Th2 inflammation mouse model and responsible for the pathogenesis of the late-phase allergic reaction [88].

To investigate the contribution of Th9 cells in food allergy in humans, PBMCs of peanut allergic (both symptomatic and clinically tolerant) and non-allergic individuals were stimulated with peanut allergen in vitro and the mRNA and protein expression of IL-9 were compared [89]. Indeed, IL-9 was found to be the cytokine that most robustly discriminated between clinical phenotypes of peanut allergic and clinically tolerant individuals [89]. This study suggested using IL-9 as a biomarker to differentiate peanut allergic from tolerant individuals [89]. Following this study, IL-9 was detected in the peanut-specific memory T helper cells sorted from PMBCs of children with peanut allergy by using microarray analysis [90]. The expression of IL-5, IL-9, and IL-13 was higher in PBMCs from individuals with peanut allergy than in those from non-allergic donors. By using a random forest algorithm to determine the efficiency of classifier, IL-9 expression was found to be the best differentiated gene between memory T helper cells from patients with peanut allergy and those from patients with peanut sensitization (tolerant to peanut but having peanut-specific IgE) as well as atopic children without peanut allergy [90]. Using mouse strains having differential susceptibility to IgE-mediated experimental food allergy, the frequency of IL-9-producing T cells in lamina propria of BALB/c mice was higher than those of C57Bl/6 mice that failed to develop experimental food allergy [42]. However, this study suggested that the innate multifunctional IL-9-producing mucosal mast cells (MMC9s) in lamina propria cells were the major cellular source of IL-9 secretion and responsible for promoting intestinal mastocytosis and food allergy symptoms in the experimental food allergic mice [42, 91]. Altogether, these studies emphasize important roles of IL-9 that may be derived from innate mucosal mast cells and adaptive Th9 cells in promoting the development of food allergy.

## Targeting IL-9 and Th9 Cells in Allergic Diseases

As IL-9 has been demonstrated to play important roles in exacerbating allergic airway diseases, an IL-9 antagonist (MEDI-258), a humanized mAb against IL-9 has been generated by MedImmune LLC under license from Genaera Corporation to investigate the potential treatment of asthma in human [92]. Unlike in murine studies, administration of this antibody in asthma patients in phase I and II studies was not efficient in alleviating pulmonary function although positive trends for improvement of asthma symptom have been observed [93, 94]. It has been suggested that the phenotypic heterogeneity of asthma patients may be responsible for the failure of IL-9 blockade. Searching for the correlation of IL-9 expression and Th9 cells in different phenotypes of allergic disease, including asthma, may lead to better understanding in the roles of IL-9 and Th9 cells in mediating allergic disease pathogenesis. Using personalized medicine based on the phenotypes of allergic patients may be the future approach for the management of patients with chronic allergic diseases [95]. Moreover, a recent study found the regulatory elements super-enhancer that regulated the expression of IL-9 by Th9 cells during airway inflammation [40••]. By using inhibitor JQ1 for BET protein that is important for the assembly of IL-9 super-enhancer in allergic lung inflammation in mice, marked reduction of IL-9 expression, inflammatory cell infiltration and mucus hyper-production were found [40]. Interestingly, a recent finding showed the effect of retinoic acid in repressing Th9 epigenome, resulting in reduced pulmonary inflammation [96]. It is likely that a novel therapeutic option targeting epigenetic marks to fine tune cytokine production might be a future strategy for the treatment of allergic diseases [40••, 96••, 97]. Based on a recent finding [4••], it is

also possible that Th9 cells may be transiently induced during allergic inflammation. Biologic therapies that targeted type 2 cytokines showed more promising results in treatment of asthma than targeting IL-9 [98]; therefore, combination therapies that suppress multiple cytokines derived from Th2 and Th9 cells may be a better therapeutic approach for chronic allergic diseases that are regulated by a complex network of cytokines.

## Conclusions and Current Challenges

The induction of Th9 cells and IL-9 in airway, skin, and food allergy in human and murine studies emphasizes their important contribution in allergic diseases. The presence of Th9 cells in chronic airway allergic diseases suggests a function in potentiating inflammation in the late stage of allergic disease development. As neutralization of IL-9 in clinical study of asthma was not effective, inhibiting multiple cytokines derived from Th2 and Th9 cells may provide better results for effective treatment. Because of the heterogeneous phenotypes of asthma, future studies to search for the correlation between T helper cell subset and asthma phenotypes will provide better understanding of the roles of Th9 cells. The induction of Th9 and IL-9 may be used as biomarker to differentiate different phenotypes of allergy and help to advance the usage of personalized medicine in therapeutic intervention in individuals. Additional studies on the cellular sources of IL-9, their function, and regulation are needed to bring more insights and may pave the way for the improvement of therapeutics of allergic diseases. Because IL-9 regulatory elements and Th9 epigenome have recently been reported, a novel interesting target might be modulating agents that target regulatory regions involving in the regulation of IL-9 and cytokines derived from Th2 cells.

### Compliance with Ethical Standards

**Conflict of Interest** The authors declare no conflicting financial interests.

**Human and Animal Rights** This article does not contain any studies with human or animal subjects performed by any of the authors.

## References

Papers of particular interest, published recently, have been highlighted as:
•• Of major importance


1. Veldhoen M, Uyttenhove C, van Snick J, Helmby H, Westendorf A, Buer J, et al. Transforming growth factor-beta 'reprograms' the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset. Nat Immunol. 2008;9(12):1341–6. https://doi.org/10.1038/ni.1659.
2. Dardalhon V, Awasthi A, Kwon H, Galileos G, Gao W, Sobel RA, et al. IL-4 inhibits TGF-beta-induced Foxp3(+) T cells and, together with TGF-beta, generates IL-9(+) IL-10(+) Foxp3(−) effector T cells. Nat Immunol. 2008;9(12):1347–55. https://doi.org/10.1038/ni.1677.
3. Jabeen R, Goswami R, Awe O, Kulkarni A, Nguyen ET, Attenasio A, et al. Th9 cell development requires a BATF-regulated transcriptional network. J Clin Invest. 2013;123(11):4641–53. https://doi.org/10.1172/JCI69489.
4.•• Micosse C, von Meyenn L, Steck O, Kipfer E, Adam C, Simillion C, et al. Human "TH9" cells are a subpopulation of PPAR-gamma(+) TH2 cells. Sci Immunol. 2019;4(31). https://doi.org/10.1126/sciimmunol.aat5943 **COMMENT: Study showing that Th9 subset transiently induced during skin inflammation is a subpopulation of Th2 lineage.**
5. Clark RA, Schlapbach C. TH9 cells in skin disorders. Semin Immunopathol. 2017;39(1):47–54. https://doi.org/10.1007/s00281-016-0607-8.
6. Licona-Limon P, Henao-Mejia J, Temann AU, Gagliani N, Licona-Limon I, Ishigame H, et al. Th9 cells drive host immunity against gastrointestinal worm infection. Immunity. 2013;39(4):744–57. https://doi.org/10.1016/j.immuni.2013.07.020.
7. Angkasekwinai P, Srimanote P, Wang YH, Pootong A, Sakolvaree Y, Pattanapanyasat K, et al. Interleukin-25 (IL-25) promotes efficient protective immunity against *Trichinella spiralis* infection by enhancing the antigen-specific IL-9 response. Infect Immun. 2013;81(10):3731–41. https://doi.org/10.1128/IAI.00646-13.
8. Jones CP, Gregory LG, Causton B, Campbell GA, Lloyd CM. Activin a and TGF-beta promote T(H)9 cell-mediated pulmonary allergic pathology. J Allergy Clin Immunol. 2012;129(4):1000–10 **e3**. https://doi.org/10.1016/j.jaci.2011.12.965.
9. Angkasekwinai P, Chang SH, Thapa M, Watarai H, Dong C. Regulation of IL-9 expression by IL-25 signaling. Nat Immunol. 2010;11(3):250–6. https://doi.org/10.1038/ni.1846.
10. Chang HC, Sehra S, Goswami R, Yao W, Yu Q, Stritesky GL, et al. The transcription factor PU.1 is required for the development of IL-9-producing T cells and allergic inflammation. Nat Immunol. 2010;11(6):527–34. https://doi.org/10.1038/ni.1867.
11. Vargas TR, Humblin E, Vegran F, Ghiringhelli F, Apetoh L. T(H)9 cells in anti-tumor immunity. Semin Immunopathol. 2017;39(1):39–46. https://doi.org/10.1007/s00281-016-0599-4.
12. Gerlach K, McKenzie AN, Neurath MF, Weigmann B. IL-9 regulates intestinal barrier function in experimental T cell-mediated colitis. Tissue Barriers. 2015;3(1–2):e983777. https://doi.org/10.4161/21688370.2014.983777.
13. Romagnani S. Immunologic influences on allergy and theT(H)1/T(H)2 balance. J Allergy Clin Immunol. 2004;113(3):395–400. https://doi.org/10.1016/j.jaci.2003.11.025.
14. Moro K, Yamada T, Tanabe M, Takeuchi T, Ikawa T, Kawamoto H, et al. Innate production of T(H)2 cytokines by adipose tissue-associated c-kit(+)Sca-1(+) lymphoid cells. Nature. 2010;463(7280):540–4. https://doi.org/10.1038/nature08636.
15. Price AE, Liang HE, Sullivan BM, Reinhardt RL, Eisley CJ, Erle DJ, et al. Systemically dispersed innate IL-13-expressing cells in type 2 immunity. Proc Natl Acad Sci U S A. 2010;107(25):11489–94. https://doi.org/10.1073/pnas.1003988107.
16. Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, Langford TK, et al. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. Nature. 2010;464(7293):1367–70. https://doi.org/10.1038/nature08900.
17. Halim TY, Steer CA, Matha L, Gold MJ, Martinez-Gonzalez I, McNagny KM, et al. Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. Immunity. 2014;40(3):425–35. https://doi.org/10.1016/j.immuni.2014.01.011.

18. Liu B, Lee JB, Chen CY, Hershey GK, Wang YH. Collaborative interactions between type 2 innate lymphoid cells and antigen-specific CD4+ Th2 cells exacerbate murine allergic airway diseases with prominent eosinophilia. J Immunol. 2015;194(8):3583–93. https://doi.org/10.4049/jimmunol.1400951.
19. Choy DF, Hart KM, Borthwick LA, Shikotra A, Nagarkar DR, Siddiqui S, et al. TH2 and TH17 inflammatory pathways are reciprocally regulated in asthma. Sci Transl Med. 2015;7(301):301ra129. https://doi.org/10.1126/scitranslmed.aab3142.
20. Sehra S, Yao W, Nguyen ET, Glosson-Byers NL, Akhtar N, Zhou B, et al. TH9 cells are required for tissue mast cell accumulation during allergic inflammation. J Allergy Clin Immunol. 2015;136(2):433–40 **e1**. https://doi.org/10.1016/j.jaci.2015.01.021.
21. Kim BS, Kim IK, Park YJ, Kim YS, Kim YJ, Chang WS, et al. Conversion of Th2 memory cells into Foxp3+ regulatory T cells suppressing Th2-mediated allergic asthma. Proc Natl Acad Sci U S A. 2010;107(19):8742–7. https://doi.org/10.1073/pnas.0911756107.
22. Murugaiyan G, Beynon V, Da Cunha AP, Joller N, Weiner HL. IFN-gamma limits Th9-mediated autoimmune inflammation through dendritic cell modulation of IL-27. J Immunol. 2012;189(11):5277–83. https://doi.org/10.4049/jimmunol.1200808.
23. Anuradha R, George PJ, Hanna LE, Chandrasekaran V, Kumaran P, Nutman TB, et al. IL-4-, TGF-beta-, and IL-1-dependent expansion of parasite antigen-specific Th9 cells is associated with clinical pathology in human lymphatic Filariasis. J Immunol. 2013;191(5):2466–73. https://doi.org/10.4049/jimmunol.1300911.
24. Liao W, Spolski R, Li P, Du N, West EE, Ren M, et al. Opposing actions of IL-2 and IL-21 on Th9 differentiation correlate with their differential regulation of BCL6 expression. Proc Natl Acad Sci U S A. 2014;111(9):3508–13. https://doi.org/10.1073/pnas.1301138111.
25. Yao WG, Zhang YL, Jabeen R, Nguyen ET, Wilkes DS, Tepper RS, et al. Interleukin-9 is required for allergic airway inflammation mediated by the cytokine TSLP. Immunity. 2013;38(2):360–72. https://doi.org/10.1016/j.immuni.2013.01.007.
26. Wong MT, Ye JJ, Alonso MN, Landrigan A, Cheung RK, Engleman E, et al. Regulation of human Th9 differentiation by type I interferons and IL-21. Immunol Cell Biol. 2010;88(6):624–31. https://doi.org/10.1038/icb.2010.53.
27. Xiao X, Balasubramanian S, Liu WT, Chu XF, Wang HB, Taparowsky EJ, et al. OX40 signaling favors the induction of T(H)9 cells and airway inflammation. Nat Immunol. 2012;13(10):981–90. https://doi.org/10.1038/ni.2390.
28. Kerzerho J, Maazi H, Speak AO, Szely N, Lombardi V, Khoo B, et al. Programmed cell death ligand 2 regulates TH9 differentiation and induction of chronic airway hyperreactivity. J Allergy Clin Immunol. 2013;131(4):1048–57, 57 e1–2. https://doi.org/10.1016/j.jaci.2012.09.027.
29. Kim IK, Kim BS, Koh CH, Seok JW, Park JS, Shin KS, et al. Glucocorticoid-induced tumor necrosis factor receptor-related protein co-stimulation facilitates tumor regression by inducing IL-9-producing helper T cells. Nat Med. 2015;21(9):1010–7. https://doi.org/10.1038/nm.3922.
30. Xiao X, Shi X, Fan Y, Zhang X, Wu M, Lan P, et al. GITR subverts Foxp3(+) Tregs to boost Th9 immunity through regulation of histone acetylation. Nat Commun. 2015;6:8266. https://doi.org/10.1038/ncomms9266.
31. Elyaman W, Bassil R, Bradshaw EM, Orent W, Lahoud Y, Zhu B, et al. Notch receptors and Smad3 signaling cooperate in the induction of Interleukin-9-producing T cells. Immunity. 2012;36(4):623–34. https://doi.org/10.1016/j.immuni.2012.01.020.
32. Richard AC, Tan CY, Hawley ET, Gomez-Rodriguez J, Goswami R, Yang XP, et al. The TNF-family ligand TL1A and its receptor DR3 promote T cell-mediated allergic immunopathology by enhancing differentiation and pathogenicity of IL-9-producing T cells. J Immunol. 2015;194(8):3567–82. https://doi.org/10.4049/jimmunol.1401220.
33. Goswami R, Jabeen R, Yagi R, Pham D, Zhu JF, Goenka S, et al. STAT6-dependent regulation of Th9 development. J Immunol. 2012;188(3):968–75. https://doi.org/10.4049/jimmunol.1102840.
34. Staudt V, Bothur E, Klein M, Lingnau K, Reuter S, Grebe N, et al. Interferon-regulatory factor 4 is essential for the developmental program of T helper 9 cells. Immunity. 2010;33(2):192–202. https://doi.org/10.1016/j.immuni.2010.07.014.
35. Zhu YX, Kang LY, Luo W, Li CC, Yang L, Yang YC. Multiple transcription factors are required for activation of human interleukin 9 gene in T cells. J Biol Chem. 1996;271(26):15815–22.
36. Jash A, Sahoo A, Kim GC, Chae CS, Hwang JS, Kim JE, et al. Nuclear factor of activated T cells 1 (NFAT1)-induced permissive chromatin modification facilitates nuclear factor-kappaB (NF-kappaB)-mediated interleukin-9 (IL-9) transactivation. J Biol Chem. 2012;287(19):15445–57. https://doi.org/10.1074/jbc.M112.340356.
37. Tamiya T, Ichiyama K, Kotani H, Fukaya T, Sekiya T, Shichita T, et al. Smad2/3 and IRF4 play a cooperative role in IL-9-producing T cell induction. J Immunol. 2013;191(5):2360–71. https://doi.org/10.4049/jimmunol.1301276.
38. Humblin E, Thibaudin M, Chalmin F, Derangere V, Limagne E, Richard C, et al. IRF8-dependent molecular complexes control the Th9 transcriptional program. Nat Commun. 2017;8(1):2085. https://doi.org/10.1038/s41467-017-01070-w.
39. Malik S, Sadhu S, Elesela S, Pandey RP, Chawla AS, Sharma D, et al. Transcription factor Foxo1 is essential for IL-9 induction in T helper cells. Nat Commun. 2017;8(1):815. https://doi.org/10.1038/s41467-017-00674-6.
40.•• Xiao X, Fan Y, Li J, Zhang X, Lou X, Dou Y, et al. Guidance of super-enhancers in regulation of IL-9 induction and airway inflammation. J Exp Med. 2018;215(2):559–74. https://doi.org/10.1084/jem.20170928 **COMMENT: Study identifying the elements for the epigenetic landscape regulating IL-9 expression by Th9 cells that could be another target for allergic airway inflammation.**
41.•• Koh B, Abdul Qayum A, Srivastava R, Fu Y, Ulrich BJ, Janga SC, et al. A conserved enhancer regulates Il9 expression in multiple lineages. Nat Commun. 2018;9(1):4803. https://doi.org/10.1038/s41467-018-07202-0 **COMMENT: Study identifying a conserved IL-9 regulatory element, CNS-25 as enhancer for IL-9 expression in multiple cell types, including Th9 cells.**
42. Chen CY, Lee JB, Liu B, Ohta S, Wang PY, Kartashov AV, et al. Induction of Interleukin-9-producing mucosal mast cells promotes susceptibility to IgE-mediated experimental food allergy. Immunity. 2015;43(4):788–802. https://doi.org/10.1016/j.immuni.2015.08.020.
43. Turner JE, Morrison PJ, Wilhelm C, Wilson M, Ahlfors H, Renauld JC, et al. IL-9-mediated survival of type 2 innate lymphoid cells promotes damage control in helminth-induced lung inflammation. J Exp Med. 2013;210(13):2951–65. https://doi.org/10.1084/jem.20130071.
44. Rauber S, Luber M, Weber S, Maul L, Soare A, Wohlfahrt T, et al. Resolution of inflammation by interleukin-9-producing type 2 innate lymphoid cells. Nat Med. 2017;23(8):938–44. https://doi.org/10.1038/nm.4373.
45. Visekruna A, Ritter J, Scholz T, Campos L, Guralnik A, Poncette L, et al. Tc9 cells, a new subset of CD8(+) T cells, support Th2-mediated airway inflammation. Eur J Immunol. 2013;43(3):606–18. https://doi.org/10.1002/eji.201242825.
46. Nowak EC, Weaver CT, Turner H, Begum-Haque S, Becher B, Schreiner B, et al. IL-9 as a mediator of Th17-driven inflammatory disease. J Exp Med. 2009;206(8):1653–60. https://doi.org/10.1084/jem.20090246.

47. Elyaman W, Bradshaw EM, Uyttenhove C, Dardalhon V, Awasthi A, Imitola J, et al. IL-9 induces differentiation of TH17 cells and enhances function of FoxP3+ natural regulatory T cells. Proc Natl Acad Sci U S A. 2009;106(31):12885–90. https://doi.org/10.1073/pnas.0812530106.
48. Doull IJ, Lawrence S, Watson M, Begishvili T, Beasley RW, Lampe F, et al. Allelic association of gene markers on chromosomes 5q and 11q with atopy and bronchial hyperresponsiveness. Am J Respir Crit Care Med. 1996;153(4 Pt 1):1280–4. https://doi.org/10.1164/ajrccm.153.4.8616554.
49. Ulbrecht M, Eisenhut T, Bonisch J, Kruse R, Wjst M, Heinrich J, et al. High serum IgE concentrations: association with HLA-DR and markers on chromosome 5q31 and chromosome 11q13. J Allergy Clin Immunol. 1997;99(6 Pt 1):828–36.
50. Dong Q, Louahed J, Vink A, Sullivan CD, Messler CJ, Zhou Y, et al. IL-9 induces chemokine expression in lung epithelial cells and baseline airway eosinophilia in transgenic mice. Eur J Immunol. 1999;29(7):2130–9. https://doi.org/10.1002/(SICI)1521-4141(199907)29:07<2130::AID-IMMU2130>3.0.CO;2-S.
51. Longphre M, Li D, Gallup M, Drori E, Ordonez CL, Redman T, et al. Allergen-induced IL-9 directly stimulates mucin transcription in respiratory epithelial cells. J Clin Invest. 1999;104(10):1375–82. https://doi.org/10.1172/JCI6097.
52. Temann UA, Ray P, Flavell RA. Pulmonary overexpression of IL-9 induces Th2 cytokine expression, leading to immune pathology. J Clin Invest. 2002;109(1):29–39. https://doi.org/10.1172/JCI13696.
53. Townsend JM, Fallon GP, Matthews JD, Smith P, Jolin EH, McKenzie NA. IL-9-deficient mice establish fundamental roles for IL-9 in pulmonary mastocytosis and goblet cell hyperplasia but not T cell development. Immunity. 2000;13(4):573–83.
54. Kung TT, Luo B, Crawley Y, Garlisi CG, Devito K, Minnicozzi M, et al. Effect of anti-mIL-9 antibody on the development of pulmonary inflammation and airway hyperresponsiveness in allergic mice. Am J Respir Cell Mol Biol. 2001;25(5):600–5. https://doi.org/10.1165/ajrcmb.25.5.4533.
55. Cheng G, Arima M, Honda K, Hirata H, Eda F, Yoshida N, et al. Anti-interleukin-9 antibody treatment inhibits airway inflammation and hyperreactivity in mouse asthma model. Am J Respir Crit Care Med. 2002;166(3):409–16. https://doi.org/10.1164/rccm.2105079.
56. Angkasekwinai P. Allergic inflammation and atopic disease: role of Th9 cells. Methods Mol Biol. 2017;1585:189–99. https://doi.org/10.1007/978-1-4939-6877-0_15.
57. Wilhelm C, Hirota K, Stieglitz B, Van Snick J, Tolaini M, Lahl K, et al. An IL-9 fate reporter demonstrates the induction of an innate IL-9 response in lung inflammation. Nat Immunol. 2011;12(11):1071–7. https://doi.org/10.1038/ni.2133.
58.•• Moretti S, Renga G, Oikonomou V, Galosi C, Pariano M, Iannitti RG, et al. A mast cell-ILC2-Th9 pathway promotes lung inflammation in cystic fibrosis. Nat Commun. 2017;8:14017. https://doi.org/10.1038/ncomms14017 **COMMENT: Study indicating the collaborative network of Th9, ILC2 and mast cells in amplifying lung inflammation.**
59. Shimbara A, Christodoulopoulos P, Soussi-Gounni A, Olivenstein R, Nakamura Y, Levitt RC, et al. IL-9 and its receptor in allergic and nonallergic lung disease: increased expression in asthma. J Allergy Clin Immunol. 2000;105(1 Pt 1):108–15.
60. Erpenbeck VJ, Hohlfeld JM, Volkmann B, Hagenberg A, Geldmacher H, Braun A, et al. Segmental allergen challenge in patients with atopic asthma leads to increased IL-9 expression in bronchoalveolar lavage fluid lymphocytes. J Allergy Clin Immunol. 2003;111(6):1319–27.
61. Nouri-Aria KT, Pilette C, Jacobson MR, Watanabe H, Durham SR. IL-9 and c-kit(+) mast cells in allergic rhinitis during seasonal allergen exposure: effect of immunotherapy. J Allergy Clin Immunol. 2005;116(1):73–9. https://doi.org/10.1016/j.jaci.2005.03.011.
62. Devos S, Cormont F, Vrtala S, Hooghe-Peters E, Pirson F, Van Snick J. Allergen-induced interleukin-9 production in vitro: correlation with atopy in human adults and comparison with interleukin-5 and interleukin-13. Clin Exp Allergy. 2006;36(2):174–82. https://doi.org/10.1111/j.1365-2222.2006.02422.x.
63. Umezu-Goto M, Kajiyama Y, Kobayashi N, Kaminuma O, Suko M, Mori A. IL-9 production by peripheral blood mononuclear cells of atopic asthmatics. Int Arch Allergy Immunol. 2007;143:76–9. https://doi.org/10.1159/000101410.
64. Yao WG, Tepper RS, Kaplan MH. Predisposition to the development of IL-9-secreting T cells in atopic infants. J Allergy Clin Immunol. 2011;128(6):1357–60. https://doi.org/10.1016/j.jaci.2011.06.019.
65. Iinuma T, Okamoto Y, Yamamoto H, Inamine-Sasaki A, Ohki Y, Sakurai T, et al. Interleukin-25 and mucosal T cells in noneosinophilic and eosinophilic chronic rhinosinusitis. Ann Allergy Asthma Immunol. 2015;114(4):289–98. https://doi.org/10.1016/j.anai.2015.01.013.
66. Hoppenot D, Malakauskas K, Lavinskiene S, Bajoriuniene I, Kalinauskaite V, Sakalauskas R. Peripheral blood Th9 cells and eosinophil apoptosis in asthma patients. Medicina-Lithuania. 2015;51(1):10–7. https://doi.org/10.1016/j.medici.2015.01.001.
67. Jia L, Wang Y, Li J, Li S, Zhang Y, Shen J, et al. Detection of IL-9 producing T cells in the PBMCs of allergic asthmatic patients. BMC Immunol. 2017;18(1):38. https://doi.org/10.1186/s12865-017-0220-1.
68. Bieber T. Atopic dermatitis. N Engl J Med. 2008;358(14):1483–94. https://doi.org/10.1056/NEJMra074081.
69. Lowe AJ, Leung DYM, Tang MLK, Su JC, Allen KJ. The skin as a target for prevention of the atopic march. Ann Allergy Asthma Immunol. 2018;120(2):145–51. https://doi.org/10.1016/j.anai.2017.11.023.
70. Beck LA, Thaci D, Hamilton JD, Graham NM, Bieber T, Rocklin R, et al. Dupilumab treatment in adults with moderate-to-severe atopic dermatitis. N Engl J Med. 2014;371(2):130–9. https://doi.org/10.1056/NEJMoa1314768.
71.•• Thaci D, Simpson EL, Beck LA, Bieber T, Blauvelt A, Papp K, et al. Efficacy and safety of dupilumab in adults with moderate-to-severe atopic dermatitis inadequately controlled by topical treatments: a randomised, placebo-controlled, dose-ranging phase 2b trial. Lancet. 2016;387(10013):40–52. https://doi.org/10.1016/S0140-6736(15)00388-8 **COMMENT: Clinical trial study indicating the effect of targeting Th2 cytokine IL-4 and IL-13 in atopic dermatitis.**
72. Werfel T, Allam JP, Biedermann T, Eyerich K, Gilles S, Guttman-Yassky E, et al. Cellular and molecular immunologic mechanisms in patients with atopic dermatitis. J Allergy Clin Immunol. 2016;138(2):336–49. https://doi.org/10.1016/j.jaci.2016.06.010.
73. Lin JY, Chen JS, Hsu CJ, Miaw SC, Liu CY, Lee SJ, et al. Epicutaneous sensitization with protein antigen induces Th9 cells. J Invest Dermatol. 2012;132(3 Pt 1):739–41. https://doi.org/10.1038/jid.2011.382.
74. Namkung JH, Lee JE, Kim E, Park GT, Yang HS, Jang HY, et al. An association between IL-9 and IL-9 receptor gene polymorphisms and atopic dermatitis in a Korean population. J Dermatol Sci. 2011;62(1):16–21. https://doi.org/10.1016/j.jdermsci.2011.01.007.
75. Sismanopoulos N, Delivanis DA, Alysandratos KD, Angelidou A, Vasiadi M, Therianou A, et al. IL-9 induces VEGF secretion from human mast cells and IL-9/IL-9 receptor genes are overexpressed in atopic dermatitis. PLoS One. 2012;7(3):e33271. https://doi.org/10.1371/journal.pone.0033271.
76. Ciprandi G, De Amici M, Giunta V, Marseglia A, Marseglia G. Serum Interleukin-9 levels are associated with clinical severity in children with atopic dermatitis. Pediatr Dermatol. 2013;30(2):222–5. https://doi.org/10.1111/j.1525-1470.2012.01766.x.

77. Hamza AM, Omar SS, Abo El-Wafa RA, Elatrash MJ. Expression levels of transcription factor PU.1 and interleukin-9 in atopic dermatitis and their relation to disease severity and eruption types. Int J Dermatol. 2017;56(5):534–9. https://doi.org/10.1111/ijd.13579.
78. Esaki H, Brunner PM, Renert-Yuval Y, Czarnowicki T, Huynh T, Tran G, et al. Early-onset pediatric atopic dermatitis is TH2 but also TH17 polarized in skin. J Allergy Clin Immunol. 2016;138(6):1639–51. https://doi.org/10.1016/j.jaci.2016.07.013.
79. Schlapbach C, Gehad A, Yang C, Watanabe R, Guenova E, Teague JE, et al. Human TH9 cells are skin-tropic and have autocrine and paracrine proinflammatory capacity. Sci Transl Med. 2014;6(219):219ra8. https://doi.org/10.1126/scitranslmed.3007828.
80. Ma L, Xue HB, Guan XH, Shu CM, Zhang JH, Yu J. Possible pathogenic role of T helper type 9 cells and interleukin (IL)-9 in atopic dermatitis. Clin Exp Immunol. 2014;175(1):25–31. https://doi.org/10.1111/cei.12198.
81. Coulter EM, Jenkinson C, Farrell J, Lavergne SN, Pease C, White A, et al. Measurement of CD4+ and CD8+ T-lymphocyte cytokine secretion and gene expression changes in p-phenylenediamine allergic patients and tolerant individuals. J Invest Dermatol. 2010;130(1):161–74. https://doi.org/10.1038/jid.2009.187.
82. Baeck M, Herman A, de Montjoye L, Hendrickx E, Cheou P, Cochez PM, et al. Increased expression of interleukin-9 in patients with allergic contact dermatitis caused by p-phenylenediamine. Contact Dermatitis. 2018;79(6):346–55. https://doi.org/10.1111/cod.13123.
83. Liu J, Harberts E, Tammaro A, Girardi N, Filler RB, Fishelevich R, et al. IL-9 regulates allergen-specific Th1 responses in allergic contact dermatitis. J Invest Dermatol. 2014;134(7):1903–11. https://doi.org/10.1038/jid.2014.61.
84. Eller K, Wolf D, Huber JM, Metz M, Mayer G, McKenzie AN, et al. IL-9 production by regulatory T cells recruits mast cells that are essential for regulatory T cell-induced immune suppression. J Immunol. 2011;186(1):83–91. https://doi.org/10.4049/jimmunol.1001183.
85. Valenta R, Hochwallner H, Linhart B, Pahr S. Food allergies: the basics. Gastroenterology. 2015;148(6):1120–31 **e4**. https://doi.org/10.1053/j.gastro.2015.02.006.
86. Forbes EE, Groschwitz K, Abonia JP, Brandt EB, Cohen E, Blanchard C, et al. IL-9- and mast cell-mediated intestinal permeability predisposes to oral antigen hypersensitivity. J Exp Med. 2008;205(4):897–913. https://doi.org/10.1084/jem.20071046.
87. Osterfeld H, Ahrens R, Strait R, Finkelman FD, Renauld JC, Hogan SP. Differential roles for the IL-9/IL-9 receptor alpha-chain pathway in systemic and oral antigen-induced anaphylaxis. J Allergy Clin Immunol. 2010;125(2):469–76 **e2**. https://doi.org/10.1016/j.jaci.2009.09.054.
88. He SH, Liu ZQ, Chen X, Song CH, Zhou LF, Ma WJ, et al. IL-9(+) IL-10(+) T cells link immediate allergic response to late phase reaction. Clin Exp Immunol. 2011;165(1):29–37. https://doi.org/10.1111/j.1365-2249.2011.04394.x.
89. Xie J, Lotoski LC, Chooniedass R, Su RC, Simons FE, Liem J, et al. Elevated antigen-driven IL-9 responses are prominent in peanut allergic humans. PLoS One. 2012;7(10):e45377. https://doi.org/10.1371/journal.pone.0045377.
90. Brough HA, Cousins DJ, Munteanu A, Wong YF, Sudra A, Makinson K, et al. IL-9 is a key component of memory TH cell peanut-specific responses from children with peanut allergy. J Allergy Clin Immunol. 2014;134(6):1329–38 **e10**. https://doi.org/10.1016/j.jaci.2014.06.032.
91. Shik D, Tomar S, Lee JB, Chen CY, Smith A, Wang YH. IL-9-producing cells in the development of IgE-mediated food allergy. Semin Immunopathol. 2017;39(1):69–77. https://doi.org/10.1007/s00281-016-0605-x.
92. Antoniu SA. MEDI-528, an anti-IL-9 humanized antibody for the treatment of asthma. Curr Opin Mol Ther. 2010;12(2):233–9.
93. Parker JM, Oh CK, LaForce C, Miller SD, Pearlman DS, Le C, et al. Safety profile and clinical activity of multiple subcutaneous doses of MEDI-528, a humanized anti-interleukin-9 monoclonal antibody, in two randomized phase 2a studies in subjects with asthma. BMC Pulm Med. 2011;11:14. https://doi.org/10.1186/1471-2466-11-14.
94. Oh CK, Leigh R, McLaurin KK, Kim K, Hultquist M, Molfino NA. A randomized, controlled trial to evaluate the effect of an anti-interleukin-9 monoclonal antibody in adults with uncontrolled asthma. Respir Res. 2013;14:93. https://doi.org/10.1186/1465-9921-14-93.
95. Muraro A, Lemanske RF Jr, Hellings PW, Akdis CA, Bieber T, Casale TB, et al. Precision medicine in patients with allergic diseases: airway diseases and atopic dermatitis-PRACTALL document of the European academy of allergy and clinical immunology and the American Academy of Allergy, Asthma & Immunology. J Allergy Clin Immunol. 2016;137(5):1347–58. https://doi.org/10.1016/j.jaci.2016.03.010.
96.•• Schwartz DM, Farley TK, Richoz N, Yao C, Shih HY, Petermann F, et al. Retinoic Acid Receptor Alpha Represses a Th9 Transcriptional and Epigenomic Program to Reduce Allergic Pathology. Immunity. 2019;50(1):106–20 e10. https://doi.org/10.1016/j.immuni.2018.12.014 **COMMENT: Study indicating the effect of retinoic acid on transcriptome of Th9 cells and repressing Th9 epigenome can control allergic lung pathology.**
97. Lloyd CM, Harker JA. Epigenetic control of Interleukin-9 in asthma. N Engl J Med. 2018;379(1):87–9. https://doi.org/10.1056/NEJMcibr1803610.
98. McGregor MC, Krings JG, Nair P, Castro M. Role of biologics in asthma. Am J Respir Crit Care Med. 2018;199:433–45. https://doi.org/10.1164/rccm.201810-1944CI.