

T_H9 cells: In front and beyond T_H2

Ariel Munitz, PhD,^a and Paul S. Foster, PhD^b Ramat Aviv, Israel, and Newcastle, Australia

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Asthma is a chronic and heterogeneous disorder of the airways that has become alarmingly common. Asthma is typically characterized by intermittent episodes of shortness of breath, wheezing, airway occlusion, and airway hyperresponsiveness of airway smooth muscle. These key clinical features of disease are thought to be underpinned by mucus hypersecretion, inflammatory infiltrates, and the induction of airway wall lesions (eg, subepithelial fibrosis).

Ongoing research to identify molecular and cellular mechanisms that contribute to the pathogenesis of asthma have revealed key roles for CD4⁺ T_H2 lymphocytes (T_H2 cells) and their secreted cytokines, namely IL-4, IL-5, IL-9, and IL-13. For example, IL-4 can promote the differentiation of T_H2 cells and regulates B cells to undergo isotype class-switching to synthesize allergen-specific IgE molecules.¹ IL-5 is a key factor promoting the differentiation, maturation, survival, and priming of eosinophils.² IL-13 is a critical effector cytokine mediating many of the major clinical features of asthma, including the induction of airway hyperresponsiveness, goblet cell hyperplasia, mucin overproduction, and fibrosis.³ Finally, IL-9 promotes allergic inflammation through regulation of mast cell differentiation, growth, and activation (eg, production of IL-1 β , IL-5, IL-6, IL-13, and TGF- β).⁴

IL-9 was initially associated with T_H2 cells because increased IL-9 production was found concomitantly with the expression of IL-4, IL-5, and IL-13 in animal models of nematode infection and allergic inflammation.⁵ Recently, the production of IL-9 has been linked to a specific subset of CD4⁺ T cells termed T_H9 cells. These cells are distinct from other T_H cells because they predominantly produce IL-9 but not other signature cytokines of T_H2 cells (eg, IL-4, IL-5, and IL-13), T_H1 cells (eg, IFN- γ), and T_H17 cells (eg, IL-17). Furthermore, the development of T_H9 cells is critically regulated by the transcription factor PU.1, whereas

the differentiation of other T_H cells requires other transcription factors, such as GATA3 (for T_H2), T-box transcription factor (for T_H1), and retinoic acid–related orphan receptor γ t (for T_H17).^{6,7} *In vitro* murine studies have shown that efficient generation of T_H9 cells is dependent on exposure to TGF- β and IL-4.^{6,7} Interestingly, the addition of IL-25 concurrently with TGF- β and IL-4 can further promote IL-9 expression by T_H9 cells.⁸

Importantly, the cellular source for IL-9 is not restricted to T_H9 cells because other immune cells, such as mast cells (usually studied as an IL-9–targeted cell), innate lymphoid cells, natural killer T cells, and regulatory T cells, can also produce this cytokine.⁴ Moreover, the specific contribution of T_H9 cells to the development of allergic airway disease is unclear, and the *in vivo* factors promoting T_H9 cell development require further investigation.

Activin A is a member of the TGF- β superfamily, which was initially described as a positive regulator of follicle-stimulating hormone from the pituitary gland.⁹ However, compelling evidence has also demonstrated that activin A regulates nonreproductive processes, including activation of immune cell function. Interestingly, activin A promotes and synergizes with TGF- β in the conversion of naive murine T cells into forkhead box protein 3–positive regulatory T cells.¹⁰ Furthermore, activin A has a critical role in murine allergic airway disease by promoting airway hyperresponsiveness and remodeling through interactions with IL-25.¹¹ However, whether activin A can synergize with TGF- β , IL-25, or both in T_H9 development is unknown.

In this issue Jones et al¹² describe the functional role of T_H9 cells in a murine model of allergic airway disease. This study is of particular interest because recent studies reinforce the importance of IL-9 in asthma and asthma-associated remodeling and might have significant clinical implications.¹³ For example, it has been shown that *in vivo* neutralization of IL-9 was capable of suppressing allergen-induced mastocytosis, tissue remodeling, and T_H2 cytokine expression (namely IL-5 and IL-13).^{13,14} Furthermore, recent studies assessing the safety profile and potential efficacy of a humanized anti-IL-9 mAb (MEDI-528) revealed that anti-IL-9 therapy might have clinical benefit in asthmatic patients.¹⁵ Thus the functional role of T_H9 cells is clearly relevant to asthma pathogenesis. Jones et al¹² demonstrate that T_H9 cells are present in human peripheral blood and that allergic patients have higher circulating numbers of T_H9 cells when compared with nonallergic control subjects. Furthermore, the authors demonstrate that T_H9 cells have a key role in the development of allergic airway inflammation, which is distinct from that of T_H2 cells by promoting mast cell recruitment and activation within the lung (Fig 1).

Using flow cytometry, the authors assessed the prevalence of circulating T_H9 cells (as defined by CD4⁺/IL-9⁺/IL-13[–]/IFN- γ [–]) in PBMCs obtained from allergic and nonallergic donors. Significantly higher levels of T_H9 cells were identified in the blood of allergic donors and were positively correlated with levels of plasma IgE. T_H9 cells were also readily and rapidly detectable in the lungs of house dust mite–challenged mice.

From ^athe Department of Microbiology and Clinical Immunology, Sackler School of Medicine, Tel-Aviv University, Ramat Aviv, and ^bthe School of Biomedical Sciences and Pharmacy, Faculty of Health, University of Newcastle and Hunter Medical Research Institute.

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Corresponding author: Ariel Munitz, PhD, Department of Microbiology and Clinical Immunology, Sackler School of Medicine, Tel-Aviv University, Ramat Aviv, 69978, Israel. E-mail: arielm@post.tau.ac.il. Or: Paul S. Foster, PhD, School of Biomedical Sciences and Pharmacy, DMB523, David Maddison Clinical Sciences Building and Hunter Medical Research Institute, University of Newcastle, Newcastle, Australia. E-mail: Paul.Foster@newcastle.edu.au.

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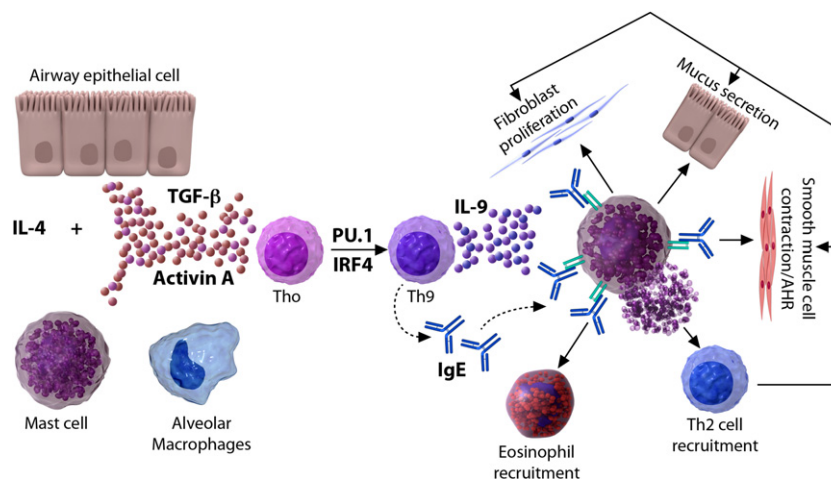


FIG 1. Proposed mechanism for TH9-induced allergic airway inflammation. Activin A and TGF- β promote the differentiation of naive CD4⁺ T cells into TH9 cells. Allergen-specific TH9 cells secrete IL-9, which stimulates mast cell recruitment, survival, and differentiation. Allergen-induced mast cell degranulation brings on the early- and late-phase reactions of allergic airway inflammation characterized by cellular infiltration, contraction of smooth muscle cells, increased mucus production, and fibroblast proliferation. AHR, Airway hyperresponsiveness.

Kinetically, the accumulation of TH9 cells preceded the accumulation of TH2 cells.

Given the role of IL-25 and activin A in regulating allergic airway inflammation and the findings that IL-25 can enhance IL-9 production from TH9 cells *in vitro*, the authors aimed to define whether activin A could regulate TH9 production as well. For this, CD3⁺/CD4⁺ cells were obtained from naive mice and stimulated *in vitro* in the presence of IL-4 in conjunction with either TGF- β 1 or activin A. Addition of activin A to these T-cell cultures resulted in the development of TH9 cells, and the addition of IL-25 to activin A/IL-4-stimulated cell cultures augmented the generation of TH9 cells. Activin A- or TGF- β 1-driven TH9 cells were adoptively transferred to naive mice that subsequently underwent allergen challenge to define a functional role for TH9 cells *in vivo*. Transfer of either activin A- or TGF- β 1-driven TH9 cells to mice followed by exposure to allergen resulted in enhanced allergic airway disease characterized by increased cellular recruitment to the airways lumen and lung tissue by comparison to mice given just allergen alone or TH2 cells as a positive control. Mast cells are a major target for IL-9 activity. Thus mast cell degranulation and accumulation in the lung were also assessed. Indeed, histologic analyses revealed increased serum levels of mast cell-specific serine protease 1 and intraepithelial mast cell numbers in lung tissue after TH9 cell transfer.

TGF- β 1 and activin A levels were also increased after allergen exposure, and both were able to drive differentiation of TH9 cells *in vitro*. Neutralizing antibodies were administered to aeroallergen-treated mice to further define the role of these 2 molecules in TH9 differentiation/function *in vivo*. The combination of anti-TGF- β and anti-activin A antibodies significantly reduced *in vivo* differentiation of TH9 cells. By contrast, neither anti-TGF- β nor anti-activin A antibodies used alone affected the development of TH9 cells. The reduction in allergen-induced TH9 cells correlated with decreased levels of serum mast cell-specific serine protease 1 and decreased mast cell recruitment to the lung. Finally, the authors assessed the role of TH9 cells in patients with chronic allergic airway inflammation. Using a chronic murine

model of allergic airway disease in which features of remodeling are induced, the authors demonstrate that administration of a combination of neutralizing antibodies to activin A and TGF- β resulted in decreased mucus production and mast cell accumulation. Moreover, aeroallergen-induced collagen deposition was reduced to baseline levels. Of note, the combined anti-activin A and anti-TGF- β antibody treatment did not affect TH2 cytokine production or the number of infiltrating inflammatory cells (eg, eosinophils).

Collectively, this study reveals a novel pathway for the regulation of TH9 cell development *in vivo* and for the contribution of this cell to allergic airway inflammation by showing that (1) allergic patients have increased TH9 cell numbers; (2) intranasal allergen challenge to mice leads to rapid TH9 differentiation and proliferation, resulting in the specific recruitment and activation of mast cells; (3) activin A replicates the function of TGF- β 1 in driving *in vitro* generation of TH9 cells; (4) *in vivo* blockade of activin A and TGF- β results in decreased airway hyperreactivity and remodeling; and (5) adoptive transfer of TH9 cells results in increased airway pathology. Nonetheless, some caution needs to be taken in the interpretation of the results obtained from this study, and follow-up experiments will be required. For example, the current data do not definitely demonstrate that TH9 cells mediate airway pathology and remodeling through IL-9. It will be interesting to conduct a similar set of adoptive transfer experiments using IL-9^{-/-} TH9 cells. Furthermore, the authors conclude that TH9-induced pathology is mast cell mediated. Although this assumption is likely to be correct, the contribution of mast cells in murine experimental asthma models is dependent on the method of sensitization (eg, route of sensitization, dose of allergen, and presence of adjuvant). Thus it would be interesting to examine TH9-driven mast cell-mediated disease in mast cell-deficient mice.

In summary, this study provides exciting new data demonstrating the potential importance of IL-9 and TH9 cells in the regulation of allergic airway inflammation that might be significant to our understanding of the disease process in patients with allergic diseases.

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