

Editorial

Is 9 more than 2 also in allergic airway inflammation?

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Since the discovery of 2 functionally distinct CD4⁺ T-cell subpopulations about 30 years ago, called T_H1 and T_H2 cells, it became evident rather quickly that T_H2 cells, through release of their specific cytokines IL-4, IL-5, and IL-13, play the crucial role in development of allergic inflammatory disorders. This concept is still true today and has led to the development of a variety of novel therapeutic approaches interfering with mechanisms of T_H2 activation that have recently entered clinics or showed promising results in clinical studies.¹ However, a variety of further T_H cell subpopulations have been identified that exert specific functions in the regulation of diverse inflammatory conditions, such as regulatory T (Treg), T_H17, T_H22, T_H9, and follicular T helper cells. Moreover, it has been demonstrated that the majority of these subpopulations do not represent finally differentiated static conditions but rather show a certain degree of plasticity depending on environmental influences. Thus T-cell subpopulations create a complex network of functionally different cells that together mount a concerted response to an invading inflammatory stimulus. This also applies to allergic immune responses, in which it is well accepted that in addition to T_H2 cells, other T-cell subpopulations (eg, Treg, T_H1, T_H17, T_H9, and T_H22 cells) are involved in the development of allergy-specific inflammatory mechanisms.²

The contribution of IL-9 and T_H9 cells to the development of allergic immune reactions has come into focus over recent years.³ Already in 1998, Temann et al⁴ demonstrated that ectopic transgenic overexpression of IL-9 in the lungs of mice without any further (allergen) stimulation resulted in massive airway inflammation with many features of an allergic inflammatory response, such as local eosinophilia, increased mast cell numbers, goblet cells hyperplasia, mucus accumulation, and bronchial hyperresponsiveness. Although initially attributed to T_H2 cells, IL-9 production was shown to be associated with a variety of T_H cells and innate lymphocytes, among them T_H9 cells, which produce high levels of IL-9 in the absence of T_H2 (and other T_H subclasses) cytokine release. In fact, a variety of experimental conditions have demonstrated that T_H9 cells alone can induce allergic airway

inflammation in mice. For example, adoptive transfer of *in vitro*-differentiated allergen-specific T_H9 cells to T cell-deficient recombination-activating gene 2 (*Rag2*) knockout mice led to the development of typical features of allergic airway inflammation after allergen challenge. This pathology was reduced significantly when cells were transferred that lacked either of the transcription factors interferon regulatory factor (IRF) 4 or PU.1, which are both essentially involved in T_H9 cell differentiation.^{5,6} This critical role of T_H9 cells in development of allergic airway inflammation is further supported by a study published in this issue of the *Journal* in which Wang et al⁷ demonstrate that inhibition of T_H9 development, through interference with a novel inhibitory pathway of IL-9 promoter activation, leads to significant attenuation of the main features of allergic airway inflammation in mice.

Although the differentiation and activation of several established T_H cell subpopulations occur under tight control of specific and thus subpopulation-identifying transcription factors (T_H1 cells, T-box transcription factor [T-bet]; T_H2 cells, GATA-3; T_H17 cells, retinoic acid-related orphan receptor γ [ROR γ]; and Treg cells, forkhead box protein 3 [Foxp3]), no such master transcription factor has been identified thus far for the differentiation and activation of T_H9 cells. In contrast, a variety of transcription factors seem to be crucial to T_H9 development that all directly bind to the IL-9 promoter in these cells. *In vitro* T_H9 cells can be differentiated through T-cell receptor activation in the simultaneous presence of IL-4 and transforming growth factor- β (TGF- β). Accordingly, transcription factors involved in signaling of the respective receptors are involved in T_H9 development. As in T_H2 and T_H17 cells, interferon regulatory factor 4 (IRF4) and basic leucine zipper ATF-like transcription factor (BATF), both of which are downstream of T-cell receptor signals, are essential for T_H9 differentiation accompanied by signal transducer and activator of transcription 6 (STAT6) and GATA-3 mediating IL-4 signals and SMA- and MAD-related protein (SMAD)2/3 conducting TGF- β signaling. Moreover, TGF- β induces expression of the transcription factor PU.1, which is essential for T_H9 differentiation. *Vice versa*, IRF1 inhibits production of IL-9 by T_H9 cells and shapes T_H9 cells with an interferon/T_H1 gene signature.⁸

Adding to this already very complex situation, Wang et al⁷ describe a novel regulatory pathway of IL-9 expression and thus T_H9 activation involving 2 other transcription factors: wild-type p53-induced phosphatase 1 (Wip1) and c-Jun/c-Fos. The concept was based on the interesting observation that mice deficient in Wip1, a novel oncogene that has been shown to be involved in hematopoietic developmental pathways, show significantly reduced features of ovalbumin-induced allergic airway inflammation compared with wild-type mice, such as lower levels of allergen-specific IgE, IgG, and IgG₁; reduced expression of mucin genes (eg, Muc5ac), which is associated with less mucus production; reduced mast cell protease 1/mast

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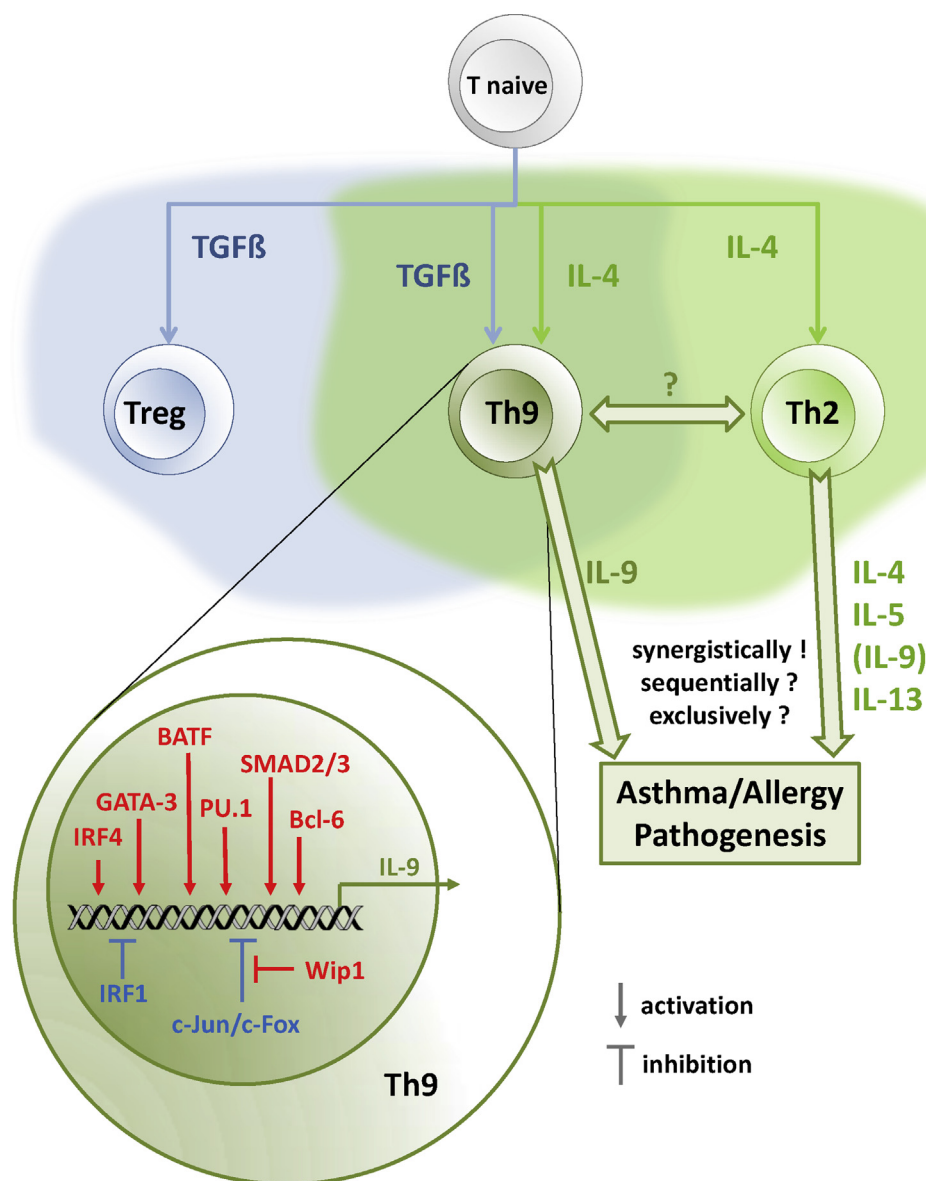


FIG 1. Induction and complex transcriptional regulation of T_H9 cell activation.

cell levels; significantly abolished airway inflammation (mainly because of reduced eosinophil infiltration); and improvement of airway hyperresponsiveness. Similar results were obtained by using pharmacologic Wip1 inhibition. Even though Wip1 has a functional role in thymic T-cell maturation, Wip1 deficiency had no influence on CD4 T-cell activation in general or on T_H1, T_H2, and T_H17 differentiation/development and not even on IL-9 production by T_H2 cells. However, in addition to some slight effects in promotion of inducible Treg cell development, lack of Wip1 resulted in a selective impairment of IL-9 expression in T_H9 cells. Subsequent in-depth analyses did not reveal any interaction with phosphorylation (ie, activation) of most of the transcription factors so far known to be involved in regulation of IL-9 promoter activity. Interestingly, c-Jun N-terminal kinase (JNK) and c-Jun, were significantly activated in Wip1 knockout conditions associated with poor T_H9 cell

development, which could be reverted by pharmacologic inhibition of JNK activity. Chromatin immunoprecipitation assays demonstrated that the c-Jun/c-Fos (activator protein 1, AP-1) complex directly binds to the IL-9 promoter and inhibits IL-9 transcription. Thus IL-9 expression/T_H9 activation is negatively regulated by c-Jun/c-Fos, which itself is tightly controlled by Wip1.⁷

The complex regulatory mechanisms contributing to the differentiation and activation of T_H9 cells, which involve a variety of transcription factors, as well as tightly controlled inhibitory pathways (Fig 1), indicate that this cell type maintains a high level of plasticity rather than representing a finally differentiated effector cell. This notion is further supported by the multiple functional roles of T_H9 cells in quite different pathophysiologic conditions, such as allergy and autoimmunity, parasite infection, leukemia development, and melanoma control.⁹

The new findings by Wang et al⁷ implicate new questions, such as the following: What mechanisms other than Wip1 might influence negative regulation of IL-9 expression by c-Jun/c-Fos? How is the expression/activity of Wip1 controlled during T_H9 cell differentiation processes? How is the balance established and maintained between T_H9-promoting (IRF4, PU.1, BATF, GATA-3, and Bcl-6) and inhibiting transcription factor (Wip1, AP-1, and IRF1) activities? Furthermore, why is the mechanism of inhibition of IL-9 transcription selective for T_H9 but does not occur in T_H2 cells that are also able to produce IL-9?

Additional aspects remain to be investigated regarding the role of T_H9 cells in the development of allergic airway inflammatory processes. Do T_H9 cells play a similar role in human asthma, as suggested by results from (mainly ovalbumin-driven) mouse models? Thus far, it has been demonstrated that atopic infants show a high predisposition to generation of IL-9-producing T cells.¹⁰ Is there a T_H9-driven endotype of human asthma? Alternatively, it might be speculated that T_H9-driven processes precede T_H2-mediated disease perpetuation and thus T_H9-mediated inflammation represents an (early) intermediate state in the development of T_H2-driven (chronic or sustained) asthma. Taken together, the increasing evidence for an important role of T_H9 cells in the development of allergic inflammatory processes significantly increases our understanding of the complexity of

the underlying mechanisms of disease in this space and will finally help us identify new concepts of prevention and therapy.

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