

T_H9 immunodeficiency in patients with hyper-IgE syndrome



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In addition to increased serum IgE levels, patients with hyper-IgE syndrome (HIES) exhibit increased susceptibility to bacterial and fungal infection.¹ Initial studies identified HIES mutations within the transcription factor signal transducer and activator of transcription (STAT) 3.^{2,3} The majority of mutations identified thus far mapped to regions within the STAT3 DNA-binding domain, resulting in production of mutant proteins that acted as dominant negative or hypomorphic forms of the protein that repressed the transcriptional activity of intact STAT3 when coexpressed.³ Although patients with HIES have diminished differentiation of IL-17- and IL-22-producing subsets, the effect on all subsets has not been carefully documented, and Zhang et al⁴ provide a detailed study into the development of IL-9-producing T cells in patients with HIES.

T_H9 cells participate in a variety of immune responses involved in autoimmunity, allergic inflammation, tumor immunity, and pathogen immunity. The differentiation of both mouse and human T_H9 cells *in vitro* is directed by IL-4 and TGF- β signaling and is under the control of transcription factors that include PU.1, STAT5, interferon regulatory factor 4, and basic leucine zipper ATF-like transcription factor.⁵ STAT3 is a potent negative regulator of murine T_H9 development because Stat3-deficient cells had enhanced potential to produce IL-9, even under standard T_H2-polarizing conditions.^{5,6} In parallel, STAT3-inducing cytokines, such as IL-6, IL-21, and IL-10, were able to suppress T_H9 differentiation, with IL-6 being the most potent negative regulator of the T_H9 phenotype. These data were corroborated by Liao et al,⁷ who demonstrated IL-21, through induction of the transcriptional repressor B-cell lymphoma 6, also suppressed T_H9 differentiation. Together, these data indicate that STAT3 directs cells away from the T_H9 lineage commitment.

Although compelling, these data were at odds with what others had observed in human T_H9 cells. Wong et al⁸ demonstrated that T_H cells cultured under T_H9 conditions with IL-6, IL-10, or IL-21 had enhanced T_H9 differentiation,⁸ and IL-9-producing cells are

decreased in patients with HIES.⁹ These data suggested that although human and murine T_H9 cells require similar positive inducers for the development, the negative regulators can be substantially different.

Zhang et al⁴ directly address this very issue. Through the study of loss-of-function (LOF) mutations in CD4 T cells from patients with HIES and from studies using STAT3 inhibitors, they demonstrate a definitive requirement for functional STAT3 in the differentiation of human T_H9 cells. Interestingly, CD4 T cells from patients with STAT1 gain-of-function mutations exhibited the same loss of T_H9 differentiation, and in the absence of productive STAT3, there is reduced suppressor of cytokine signaling 3 expression that results in exaggerated phosphorylated STAT1 (pSTAT1) activation that suppresses the T_H9 program (Fig 1). How STAT1 mediates this negative effect remains unclear.

Another major difference between mouse studies and the studies in Zhang et al⁴ is the distinction between STAT3 deletion and an LOF mutation. Although LOF mutations dramatically alter STAT3 binding to target genes, the protein is still present and can interact with other STAT proteins and affect gene transcription indirectly.⁴ To address this possibility, Zhang et al⁴ demonstrated that naive CD4 T cells cultured under T_H9 conditions from mice bearing a STAT3 LOF mutation observed in patients with HIES exhibited the same enhanced capacity to differentiate into IL-9-producing cells, as observed in Stat3-deficient T_H cells.^{5,6} These data strongly suggest that differences observed in mouse and human studies are not due to the distinction between gene loss and hypomorphic alleles and are likely indicative of a more profound difference between mouse and human T_H cell biology or cytokine signaling.

Our previous work identified the STAT5 signaling pathway as a major target of STAT3-mediated suppression of T_H9 differentiation. Treatment of cells under T_H9 conditions with IL-6 reduced cell-surface expression of CD25 and the capacity of cells to produce IL-2, attenuated STAT5 activation, and resulted in reduced expression of STAT5 target genes, including *Ii9* (Fig 1). Conversely, Stat3 deficiency resulted in enhanced IL-2 production and pSTAT5 expression under T_H9 conditions.⁵ In STAT3 LOF cells under T_H9 conditions, pSTAT5 levels are not increased, and further stimulation of these cells with exogenous IL-2 resulted in dramatically reduced pSTAT5 activation compared with that in cells isolated from healthy control subjects. These data are likely a result of reduced capacity to respond to IL-2 signals and might explain this key difference in murine and human T_H9 cells.

The origins of IL-9-producing cells in human subjects and mice might also be fundamentally different. In human subjects IL-9 is often coproduced with IL-17, and the loss of IL-9-producing cells in patients with HIES comes primarily from the IL-17⁺ population.⁹ In human T_H cell cultures both T_H9- and T_H17-polarizing conditions give rise to equivalent IL-9-producing

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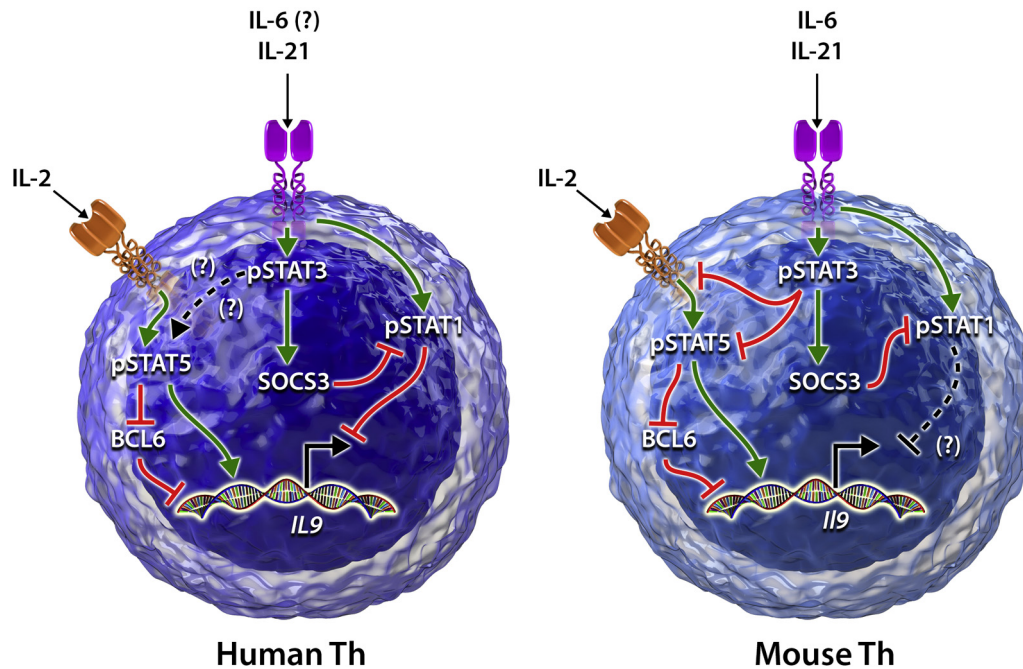


FIG 1. Effect of STAT3 in Th9 development. In developing human Th9 cells (*left*), activation of STAT3 induces SOCS3, which represses the inhibitory STAT1 pathway activation but does not seem to affect the IL-9-promoting effects of STAT5. In patients with HIES with STAT3 LOF mutations, control of the inhibitory pathway is lost. In contrast, in developing mouse Th9 cells (*right*), STAT3 activation inhibits STAT5 activation that is required for maximal IL-9 production. Mouse STAT3 activation can also interfere with STAT1 activation, although the inhibitory effects of STAT1 in mouse cells is less defined, and the loss of STAT5 activation is the dominant effect. BCL6, B-cell lymphoma 6; SOCS3, suppressor of cytokine signaling 3.

Th populations.¹⁰ However, in mice IL-9-producing cells are usually IL-17 negative *in vitro*.^{5,10} Because the polarizing conditions used in Zhang et al⁴ were a combination of Th9- and Th17-inducing cytokines (ie, with IL-1 β), it is unclear which type of Th9 cell is being induced in these cultures, and it might suggest that STAT3 would be necessary for a “Th17-like” variety of IL-9-producing cells.

Additionally, naive CD4 T cells isolated from patients with HIES were selected by expression of CD62L,⁴ which is a marker of both naive and memory CD4 T cells. Treatment of memory cells with TGF- β and IL-2 is sufficient to induce both IL-17 and IL-9 production,¹⁰ and it is possible that the phenotypes observed by Zheng et al⁴ are a combination of diminished differentiation of Th9 cells from naive cells and defects in restimulation of memory cells. Furthermore, CD62L-positive CD4 T cells isolated from patients with HIES might not be the same as cells isolated from healthy control subjects, and differences might reflect the pre-existing inflammatory situation observed in these patients.

Zhang et al⁴ provide critical information regarding Th9 differentiation in human subjects, which is amplified by inflammatory signals that are associated with allergic inflammation and autoimmune disease. Conversely, our data might explain why IL-9-producing Th cells have a low observed frequency in mice where STAT3-inducing inflammatory signals that are ubiquitous across multiple inflammatory diseases actually suppress and destabilize this cell population. Importantly, how IL-9-producing T cells contribute to human immunity and how they can

contribute to pathology in patients with HIES remains an outstanding question.

REFERENCES

1. Mogensen TH. STAT3 and the hyper-IgE syndrome: clinical presentation, genetic origin, pathogenesis, novel findings and remaining uncertainties. *JAKSTAT* 2013; 2:e23435.
2. Holland SM, DeLeo FR, Elloumi HZ, Hsu AP, Uzel G, Brodsky N, et al. STAT3 mutations in the hyper-IgE syndrome. *N Engl J Med* 2007;357:1608-19.
3. Minegishi Y, Saito M, Tsuchiya S, Tsuge I, Takada H, Hara T, et al. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. *Nature* 2007;448:1058-62.
4. Zhang Y, Siegel AM, Sun G, Dimaggio T, Freeman AF, Milner JD. Human TH9 differentiation is dependent on signal transducer and activator of transcription (STAT) 3 to restrain STAT1-mediated inhibition. *J Allergy Clin Immunol* 2019; 143:1108-18.
5. Olson MR, Verdan FF, Hufford MM, Dent AL, Kaplan MH. STAT3 impairs STAT5 activation in the development of IL-9-secreting T cells. *J Immunol* 2016;196: 3297-304.
6. Ulrich BJ, Verdan FF, McKenzie AN, Kaplan MH, Olson MR. STAT3 activation impairs the stability of Th9 cells. *J Immunol* 2017;198:2302-9.
7. 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;111:3508-13.
8. 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;88:624-31.
9. Becker KL, Rosler B, Wang X, Lachmandas E, Kamsteeg M, Jacobs CW, et al. Th2 and Th9 responses in patients with chronic mucocutaneous candidiasis and hyper-IgE syndrome. *Clin Exp Allergy* 2016;46:1564-74.
10. Beriou G, Bradshaw EM, Lozano E, Costantino CM, Hastings WD, Orban T, et al. TGF-beta induces IL-9 production from human Th17 cells. *J Immunol* 2010;185: 46-54.