Th9 immunodeficiency in Hyper IgE syndrome patients

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In addition to elevated serum IgE, 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 3 (STAT3) (2, 3). The majority of mutations identified so 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 co-expressed (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 al (this issue)(4) provide a detailed study into the development of IL-9-producing T cells in HIES patients.

Th9 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 Th9 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, IRF4 and BATF (5). STAT3 is a potent negative regulator of murine Th9 development as Stat3-deficient cells had enhanced potential to produce IL-9, even under standard Th2 polarizing conditions (5, 6). In parallel, STAT3-inducing cytokines like IL-6, IL-21 and IL-10 were able to suppress Th9 differentiation, with IL-6 being the most potent negative regulator of the Th9 phenotype. These data were corroborated by Liao et al (7) that demonstrated IL-21, through induction of the transcriptional repressor BCL6 also suppressed Th9 differentiation. Together, these data indicated STAT3 directs cells away from Th9 lineage commitment. Although compelling, these data were at odds with what others had

observed in human Th9 cells. Wong et al demonstrated that Th cells cultured under Th9 conditions with IL-6, IL-10 or IL-21 enhanced Th9 differentiation (8) and IL-9-producing cells are decreased in HIES patients (9). These data suggested that although human and murine Th9 cells require similar positive inducers for the development, the negative regulators may be substantially different.

Zhang et al (4) directly address this very issue. Through the study of LOF mutations in CD4 T cells from HIES patients, and from studies using STAT3 inhibitors, they demonstrate a definitive requirement for functional STAT3 in the differentiation of human Th9 cells. Interestingly, CD4 T cells from patients with STAT1 gain of function (GOF) mutations exhibited the same loss of Th9 differentiation and in the absence of productive STAT3 there is reduced SOCS3 expression that results in exaggerated pSTAT1 activation that suppresses the Th9 program (Figure 1). How STAT1 mediates this negative effect remains unclear.

Another major difference between mouse studies and the studies in Zhang et al (4) is the difference between STAT3 deletion and a LOF mutation. While LOF mutations dramatically alter STAT3 binding to target genes, the protein is still present and may interact with other STAT proteins and effect gene transcription indirectly (3). To address this possibility, Zhang et al (4) demonstrated that naïve CD4 T cells cultured under Th9 conditions from mice bearing a STAT3 LOF mutation observed in HIES patients exhibited the same enhanced capacity to differentiate into IL-9-producing cells as observed in *Stat3*-deficient Th cells (5, 6). These data strongly suggest 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 Th cell biology or cytokine signaling.

Our previous work identified the STAT5 signaling pathway as a major target of STAT3-mediated suppression of Th9 differentiation. Treatment of cells under Th9 conditions with IL-6 reduced cell surface expression of CD25, the capacity of cells to produce IL-2, attenuated STAT5 activation, and resulted in reduced expression of STAT5 target genes including *Il9* (Figure 1). Conversely, *Stat3*-deficiency resulted in enhanced IL-2 production and pSTAT5 under Th9 conditions (5). In STAT3 LOF cells under Th9 conditions, pSTAT5 is not elevated and further stimulation of these cells with exogenous IL-2 resulted in dramatically reduced pSTAT5 activation as compared to cells isolated from healthy controls. These data are likely a result of reduced capacity to respond to IL-2 signals and may explain this key difference in murine and human Th9 cells.

The origins of IL-9-producing cells in humans and mice may also be fundamentally different. In humans, IL-9 is often co-produced with IL-17 and the loss of IL-9 -producing cells in HIES patients come primarily from the IL-17+ population (9). In human Th cell cultures both Th9 and Th17 polarizing conditions give rise to equivalent IL-9-producing Th populations (10). In mice however, IL-9-producing cells are usually IL-17-negative in vitro (5, 11). As the polarizing conditions used in Zhang et al to polarize were a combination of Th9 and Th17-inducing cytokines (i.e. with IL-1β), 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 cell. Additionally, “naïve” CD4 T cells isolated from HIES patients were selected by expression of CD62L (4), which is a marker of both naïve 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 al (4) are a combination of diminished differentiation of Th9 cells from naïve cells and defects in re-stimulation of memory cells. Further, CD62L+ CD4 T cells isolated from HIES individuals may not be the same as cells isolated from healthy controls and differences may reflect the pre-existing inflammatory situation observed in these patients.

Zhang et al (4) provide critical information regarding Th9 differentiation in humans, which is amplified by inflammatory signals that are associated with allergic inflammation and autoimmune disease. Conversely, our data may 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 might contribute to the pathology in HIES patients remains an outstanding question.

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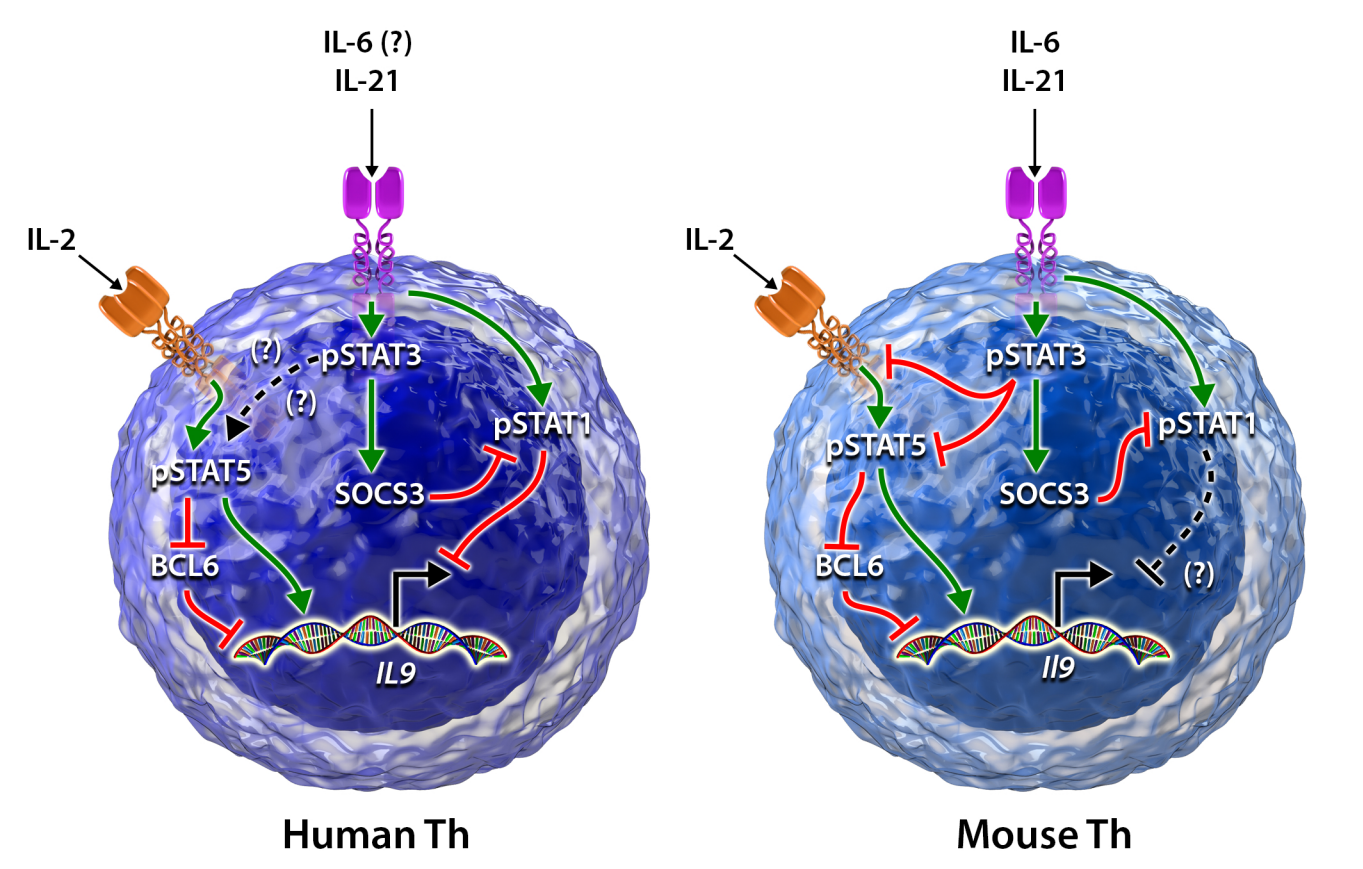
Figure Legend

Impact of STAT3 in Th9 development. In developing human Th9 cells (left), activation of STAT3 induces SOCS3 that represses the inhibitory STAT1 pathway activation but does not seem to affect the IL-9-promoting effects of STAT5. In HIES patients 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 might 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.


IL-6 (?)
IL-21
IL-2
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pSTAT3
pSTAT5
pSTAT1
SOCS3
BCL6
IL9
Human Th
IL-6
IL-21
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