inside blood commentary

22 JANUARY 2015 | VOLUME 125, NUMBER 4

IMMUNOBIOLOGY

Comment on Milner et al, page 591, and Haapaniemi et al, page 639

STAT3: too much may be worse than not enough!

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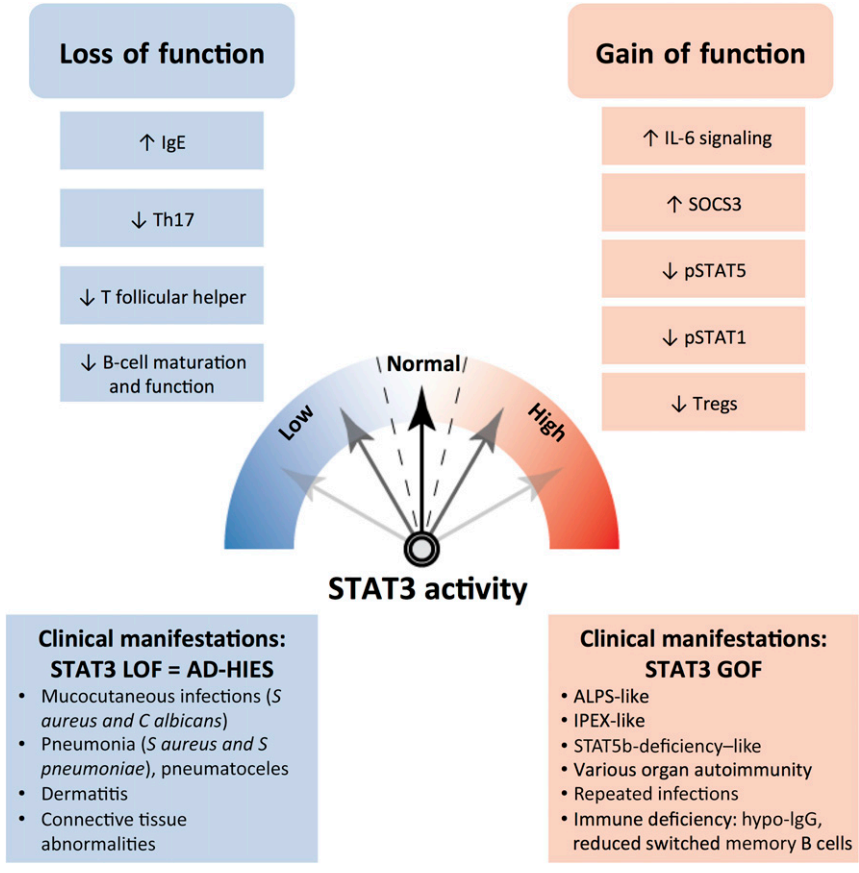
In this issue of *Blood*, Milner et al[1] and Haapaniemi et al[2] report that *STAT3* gain-of-function (GOF) mutations are responsible for multiorgan autoimmunity, autoimmune cytopenia, lymphoproliferation, and immune deficiency.



Both LOF and GOF *STAT3* mutations have significant clinical consequences. Whereas STAT3 LOF is responsible for autosomal-dominant hyper–immunoglobulin E syndrome (AD-HIES) with increased susceptibility to infection via reduced Th17 and B-cell function, STAT3 GOF is responsible for both immune dysregulation and immune deficiency. The clinical phenotype of STAT3 GOF-induced immune dysregulation is very diverse and can be seen as a mix of IPEX-like, ALPS-like, and STAT5b-deficiency–like symptoms. IgE, immunoglobulin E; IgG, immunoglobulin G.

Recently, Flanagan et al identified germline heterozygous *STAT3* GOF mutations in 5 patients with early-onset multiorgan autoimmune diseases, including type 1 diabetes, and growth retardation.[3] The 2 studies of this issue extend the phenotype of signal transducer and activator of transcription 3 (STAT3) hyperactivity and provide some insights into the underlying mechanistic consequences. Milner et al describe 13 new cases (in 10 families) and Haapaniemi et al 3 cases (1 new and 2 already reported by Flanagan et al), altogether resulting in a cohort of 19 patients reported so far. The *STAT3* GOF-associated phenotype is very diverse, and therefore its definition is equally complex. In lay terms, pick up some symptoms among immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX)-like (early-onset autoimmunity with diabetes and enteropathy), autoimmune lymphoproliferative syndrome (ALPS)-like (autoimmune cytopenia and lymphoproliferation), and STAT5b-deficiency–like (short stature, autoimmunity, interstitial pneumoniae) diseases, add a flavor of immune deficiency, and mix! Among the 19 cases, the more frequent manifestations include autoimmune cytopenia (n = 14), lymphadenopathy (n = 11), lymphoproliferation (n = 10), short stature (n = 12), recurrent infections (n = 11), intestinal manifestations (n = 9), and cutaneous manifestations (n = 9). In contrast to the first description of the disease by Flanagan et al,[3] in which early-onset type 1 diabetes was prominent (4/5), diabetes is now reported in "only" 6/19 patients. The immune deficiency consists mainly of hypogammaglobulinemia, with all patients of Haapaniemi's study having decreased levels of switched memory B cells, natural killer cells, and plasmacytoid dendritic cells.

Contrary to Flanagan and Haapaniemi's studies, which reported only de novo mutations, Milner et al reported some inherited cases. The data pointed to incomplete penetrance, as some

family members carrying a *STAT3* mutation were asymptomatic or had a much less severe phenotype.

Both studies clearly show that STAT3 GOF is likely due to an intrinsic increase of transcriptional activity and that unlike STAT1 GOF,[4] there is no spontaneous or cytokine-induced STAT3 hyperphosphorylation. Thereby, there is no relatively simple functional test to screen STAT3 hyperactivity. Milner's study shows delayed dephosphorylation in 1 patient, but this will have to be confirmed in more cases. Therefore, STAT3 sequencing remains today the best strategy for diagnostic purposes. The absence of genotype-phenotype correlation and the fact that STAT3 hyperactivity, measured by luciferase reporter assay, does not correlate with the severity of the phenotype, argue in favor of a modulation of the clinical phenotype by environmental or other genetic factors.

STAT3 is a transcription factor involved in cell proliferation, inflammation, differentiation, and survival.[5] STAT3, activated by interleukin-6 (IL-6), plays a critical role in Th17 differentiation. STAT3 loss-of-function (LOF) mutations are responsible for autosomal-dominant hyper–immunoglobulin E syndrome in which increased susceptibility to infection is linked to reduced TH17 function[6] and abnormal B-cell function that is likely due to the role of STAT3 in follicular helper T-cell differentiation[7] and interleukin-21 signaling during B-cell maturation and activation.[8] Subsequently, one could predict that *STAT3* GOF mutations lead to autoimmunity via increased Th17 function and B-cell proliferation. However, things are not that simple! First, paradoxically, all patients from Haapaniemi's cohort had reduced numbers of Th17 cells, whereas 1 out of 7 patients in Milner's cohort had increased levels of Th17 cells. More studies are required to understand this discrepancy associated with Th17 numbers among patients. Second, both LOF and GOF of STAT3 result in immune deficiency, albeit GOF is associated with immune dysregulation. This is reminiscent of STAT1 dysregulation, in which both LOF and GOF induce an immune deficiency while GOF also induces an immune dysregulation.[4] Many patients with STAT3 GOF have hypogammaglobulinemia, suggesting that both "not enough" and "too much" STAT3 impair B-cell function.

To further understand the consequences of STAT3 hyperactivity, Milner et al describe an increase of suppressor of cytokine signaling 3 (SOCS3), a major downstream target of STAT3 and negative regulator of STAT3 signaling.[9] SOCS3 has been shown to also inhibit other STAT proteins,[9] and their data strongly suggest a decrease of both STAT5 and STAT1 phosphorylation (see figure). STAT5 is important for regulatory T cell (Treg) differentiation and function, and in both studies, patients have low Tregs (with reduced Treg function in Haapaniemi et al). These Treg abnormalities likely play a major role in autoimmunity, although autoimmunity is also observed in patients with normal Tregs in both cohorts. The partial decrease of STAT1 phosphorylation likely participates in the immune deficiency.[10]

A broad spectrum of solid and hematologic cancers is associated with somatic STAT3 activation.[11] Therefore, one would have expected that germline *STAT3* GOF mutations would increase the risk of cancer. However, only 1 patient presented with large granular lymphocytic leukemia in Haapaniemi's study, and 1 parent presented with Hodgkin lymphoma in Milner's study. As most of the patients studied are still young, one cannot exclude the possibility that they might develop cancer later on. Because *NRAS* or *KRAS* somatic mutations can induce hematologic malignancy[12] or ALPS-like phenotype with minimally increased $CD3^+CD4^-CD8^-/TCR\alpha\beta$ cells,[13] one can speculate that similarly, somatic *STAT3* GOF mutation could be responsible for ALPS-like disease.

Milner's study is also interesting for its therapeutic aspects. Blocking IL-6 activation with tocilizumab in one patient resulted in a dramatic improvement of arthritis and reduction of Th17 cells to normal values. Could IL-6 blockade be efficient in lymphoproliferation and other autoimmune processes? Two patients have been treated by hematopoietic cell transplantation (HCT). Although one patient died, the other was cured of autoimmune symptoms and improved growth. This is a proof of concept that HCT has the potential to improve the outcome in these patients. Larger cohorts are required to further validate these therapeutic approaches. STAT3 itself might be an interesting therapeutic avenue. New molecules that specifically inhibit STAT3 are in development, and it will be interesting to test them, although such an approach will certainly require fine-tuning.

The diversity of the phenotype described in these studies will make *STAT3* GOF a logical hypothesis for many patients. Future research will have to better delineate the pathophysiology, explain the broad diversity of phenotype, and better define the therapeutic strategy.

Conflict-of-interest-disclosure: The author declares no competing financial interests. ■

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