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MYELOID NEOPLASIA

Comment on Greiner et al, page 1006

Systemic mastocytosis: dying or survivin

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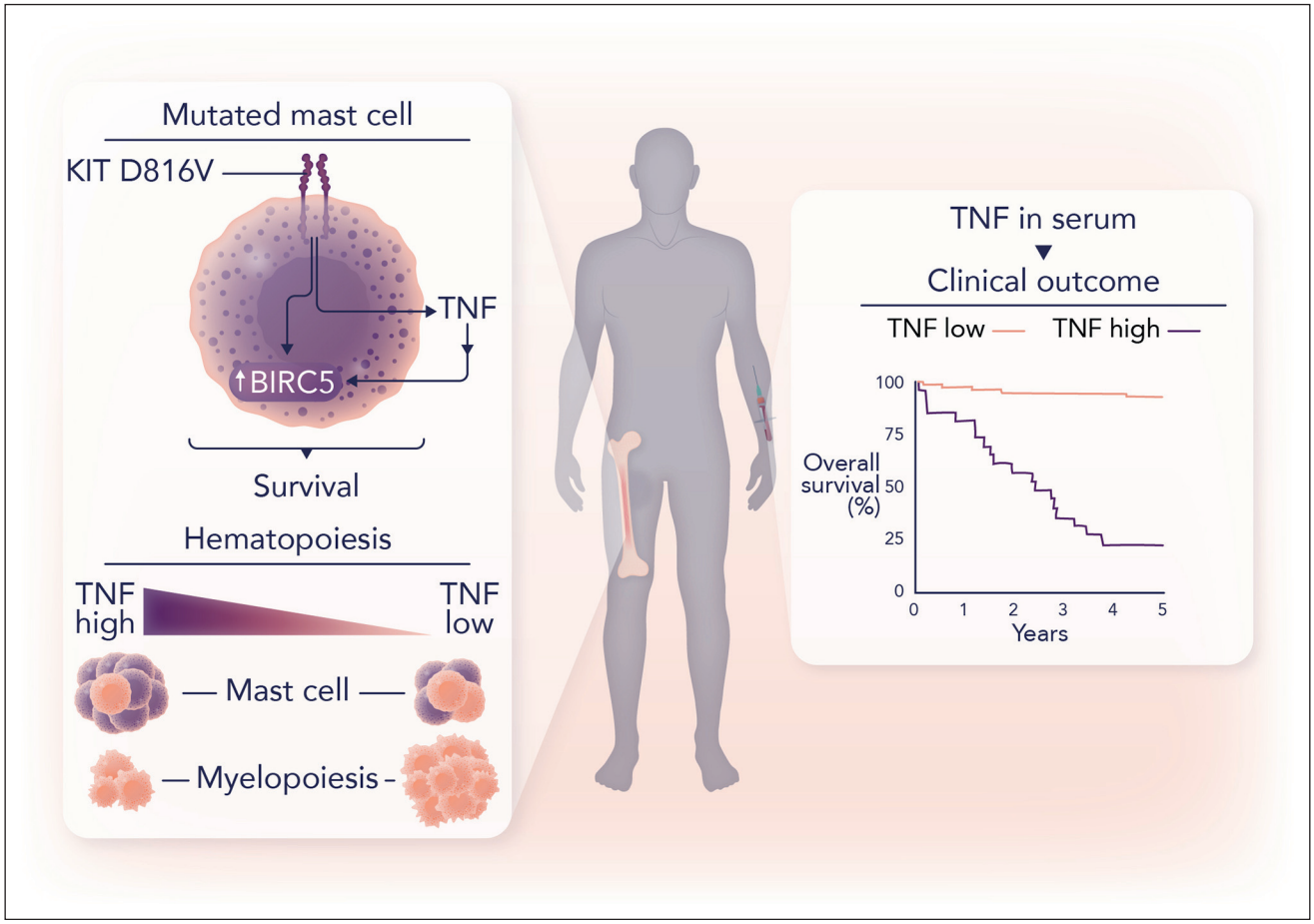
In this issue of *Blood*, Greiner et al[1] demonstrate that aberrant mast cells in systemic mastocytosis with the *KIT* D816V mutation release tumor necrosis factor (TNF), which gives the cells a growth advantage, compared with nonmutated myeloid cells, and worsens the clinical outcome for the patients (see figure).

Systemic mastocytosis is a heterogeneous mast cell disease, where most of the patients have a D816V mutation in KIT, the receptor for stem cell factor, causing receptor autoactivation. The majority of the patients have an indolent form of the disease, suffering from mast cell mediator symptoms from various organs, whereas advanced forms of systemic mastocytosis have a much worse prognosis.[2] The *KIT* D816V mutation affects many important functions in mast cells; besides growth advantages and improved survival, the mutant cells also exhibit increased migration and enhanced cytokine production and release. At least some of the cytokines that are elevated in systemic mastocytosis are driven by the *KIT* D816V mutation. An example is interleukin-6 (IL-6),[3] and the serum level of IL-6 is associated with risk of disease progression.[4] However, the insights into how the altered cytokine production affects disease have remained limited. A deregulated cytokine production could potentially affect the mast cells themselves, disturb hematopoiesis, and/or have systemic effects on other cell types and organs, leading to some of the symptoms related to systemic mastocytosis. Thus, deciphering the contribution of different cytokines to the pathophysiology of systemic mastocytosis can shed light on the disease mechanisms and has potential to identify new drug targets. Here, we discuss the results from Greiner et al and highlight how the neoplastic mast cells transform their microenvironment to promote their own dominance.

Greiner et al identified that patients with systemic mastocytosis display elevated serum levels of TNF. By comparing mast cells with and without the *KIT* D816V mutation, the study shows that the presence of the *KIT* D816V mutation results in dramatically increased TNF production. These results demonstrate that *KIT* D816V mutant mast cells constitute a possible source of the elevated TNF levels observed in the patients. More importantly, they showed that TNF can suppress myelopoiesis and the proliferation of mast cells without the *KIT* D816V mutation. By contrast, TNF has no effects on the proliferation of *KIT* D816V mutant mast cells. Thus, the neoplastic clone has the potential to promote its dominance over nonmutated mast cells through TNF production—an advantage beyond the cell-intrinsic effects of bypassing stem cell factor binding to stimulate cell proliferation and survival. Translated into the clinical setting, *KIT* D816V mutant mast cells are likely promoting a TNF-rich microenvironment in which only the neoplastic clone thrives and the growth of normal mast cells is suppressed. Over time, this would result in the complete takeover of mutant mast cells in the patients.

That elevated TNF levels promote a neoplastic clone has been reported in myeloproliferative neoplasms[5] and juvenile myelomonocytic leukemia.[6] In myeloproliferative neoplasms, the *JAK2* V617F mutation promotes TNF production while conferring resistance to the myelopoiesis-suppressing effects of TNF. In juvenile myelomonocytic leukemia, not only is the neoplastic monocyte clone resistant to the high TNF levels, but TNF also stimulates the neoplastic monocytes' growth. In Greiner et al, the tryptase levels (as proxy of mast cell burden) in systemic mastocytosis correlated weakly with the systemic TNF levels, which indicates that cells other than mature mast cells contribute to the TNF-rich microenvironment that promotes the neoplastic mast cell dominance. One possibility is that the acquisition of the *KIT* D816V mutation results in gained TNF-producing capacity also in a non–mast cell population and that these cells contribute to the TNF production in disease. The hematopoietic progenitor cell population expresses *KIT* and harbors the *KIT* D816V mutation in various patients with systemic mastocytosis[7] and could potentially contribute to the increased TNF levels and worse outcome. However, the lack of correlation between *KIT* D816V mutation burden and TNF levels is difficult to interpret in this context. An alternative scenario is that nonmutated monocytes gain potential to produce excessive amounts of TNF in the atypical disease setting,[8] thereby contributing to the mutated mast cell expansion and worse disease outcome.

The molecular mechanisms behind the *KIT* D816V mutant mast cells' resistance to TNF is possibly attributed to the *BIRC5* gene, coding for the antiapoptotic protein survivin. Mast cells that harbor the *KIT* D816V mutation upregulate survivin following TNF treatment, and the knockdown of *BIRC5* in the cells results in impaired cell proliferation. Together, the results hint that the *KIT* D816V mutation confers resistance to TNF via survivin. However, it is important to point out that



KIT D816V promotes clonal mast cell expansion and resistance to TNF. The *KIT* D816V mutation in mast cells induces secretion of TNF and upregulation of the *BIRC5* gene, promoting mast cell survival and a growth advantage compared with nonmutated cells. A microenvironment with high TNF levels suppresses myelopoiesis and selectively promotes clonal expansion of mast cells with the *KIT* D816V mutation. Purple cells indicate *KIT* D816V mutated cells, and orange cells indicate wild-type cells. Elevated serum TNF levels in patients with systemic mastocytosis are associated with worse overall survival (the graph is adapted from Figure 5C in the article by Greiner et al that begins on page 1006). Professional illustration by Somersault18:24.

the DepMap consortium classifies *BIRC5* as a common essential gene in (cancer) cell lines, that is, virtually all screened cell lines die or stop proliferating following *BIRC5* disruption.[9] Further experimental evidence is therefore warranted to verify whether the *KIT* D816V mutation establishes a survivin-mediated TNF resistance pathway.

Although the TNF-related mechanisms that promote neoplasia are not entirely new for the broader cancer research community, they represent a significant step toward understanding systemic mastocytosis on the cellular, molecular, and patient levels (see figure). Elevated levels of TNF in serum appear to be a commonality among patients with systemic mastocytosis, regardless of whether the disease is of indolent or advanced nature, when compared with healthy individuals. Interestingly, a subset of these patients displays higher TNF levels, coinciding with diminished survival. It is presently unclear whether and how TNF-targeting therapies would benefit these patients, but such an approach may present one of many tools to evaluate in the precision medicine era.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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Comment on Han et al, page 1018

Hemolysis impairs sickle cell erythropoiesis

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In this issue of *Blood*, Han et al[1] show that erythropoiesis is impaired in a sickle cell disease mouse model because of hemolysis-induced inflammation and interferon-α production that promotes erythroid CISH (cytokine-inducible SH2 [Src homology 2 domain]-containing protein) expression, a negative regulator of erythropoietin signaling. Although hemin treatment suppresses erythropoiesis, knockout of interferon-α receptor 1 improves the defective bone marrow erythropoiesis in sickle cell mice. The study by Han et al identifies an unexpected role for the heme–interferon-α–CISH axis in impaired erythropoiesis in sickle cell disease (see figure) that may be relevant for erythropoietin resistance and anemia of chronic disease.

Erythropoietin regulates bone marrow erythropoiesis for production of red blood cells that are responsible for oxygen transport from the lungs to the tissues. Mouse models for β-hemoglobinopathies, such as β-thalassemia[2] and sickle cell disease,[3] provide insight into the complex pathophysiology associated with the resultant anemia and erythropoietin-driven erythropoietic response. Causes of anemia include ineffective erythropoiesis and hemolysis. Ineffective erythropoiesis in β-thalassemia is associated with the imbalance of erythropoietin-stimulated expansion of early-stage erythroid progenitor cells and disrupted differentiation of late-stage erythroid precursors to circulating red blood cells.[4] Peripheral hemolysis in sickle cell anemia is largely a consequence of polymerization of deoxygenated sickle hemoglobin causing repeated sickle (SS) erythrocyte sickling and unsickling, increased erythrocyte fragility, and oxidative stress.[5,6] Associated intravascular hemolysis in sickle cell disease releases cell-free heme that contributes to decreased nitric oxide bioavailability and promotes a proinflammatory state, increasing inflammatory cytokines, such as interleukin-6, placental growth factor, and endothelin-1, and disease-associated vaso-occlusion, pulmonary hypertension, and organ damage.[5]

Endogenous erythropoietin was elevated by about ≥5-fold in both anemic β-thalassemia and sickle cell (SS) mouse models. Although β-thalassemia mice exhibited 2- to 3-fold increased bone marrow erythropoietic activity with increased erythroblasts and erythroid colony-forming ability, in SS mice, bone marrow erythropoietic activity was impaired and only modestly increased compared with AA control mice expressing normal adult human hemoglobin. Sorted SS erythroid progenitor cells showed decrease erythroid colony-forming ability and a decreased response to erythropoietin compared with AA control. Hemolysis in sickle cell disease increases cell-free heme and hemin injection into AA mice, used to model chronic intravascular hemolysis, resulted in reduced bone marrow erythropoiesis and erythroid progenitor cell response. Similarly, injection of red cell lysate into wild-type mice decreased bone marrow erythroid colony formation and erythroblast number, and erythropoietin stimulated Stat5 and extracellular signal-regulated kinase signaling in erythroid cells. However, hemin treatment did not affect cultures of erythropoietin-stimulated bone marrow erythroid cells or Stat5 phosphorylation, indicating that hemolysis and/or hemin-induced suppression of erythropoiesis in vivo in mice is via an indirect mechanism.

Increased hemolysis is associated with increased interferon-α, and plasma heme levels were shown to correlate with circulating interferon-α in patients with sickle cell disease.[7] Interferon-α levels were elevated in SS mice[7] and in AA mice with heme injection. Interferon-α treatment in AA mice decreased bone marrow erythropoiesis, increased erythroid cell expression of suppressor of cytokine signaling family member, *Cish*, and decreased erythropoietin response in erythroid progenitor cell cultures. *Cish* expression was also elevated in bone marrow erythroid cells from SS mice with increased endogenous interferon-α. Knockout of interferon-α receptor 1 attenuated the reduction in mouse bone marrow erythropoiesis with hemin treatment and increased bone marrow erythropoietic activity and erythropoietin-stimulated erythroid cell response and decreased erythroid cell *Cish* expression in SS mice. Deletion of interferon-α receptor 1 rescued the impaired bone marrow erythropoiesis in SS mice without changing peripheral blood red blood cell count, hemoglobin, hematocrit, and reticulocyte number. Analogous to mouse erythroid cell response, human erythroid cultures treated with interferon-α activated interferon signaling, inhibited cell growth and erythropoietin-stimulated STAT5 phosphorylation, and induced CISH expression.

This research provides a mechanistic understanding for the impaired erythropoietic response in sickle cell disease to endogenous erythropoietin and relates to the higher erythropoietin dose required for treatment of anemia in sickle cell disease compared with patients with chronic kidney disease. The heme–interferon-α–CISH axis is identified as a contributing factor to impaired erythropoietin-stimulated erythropoietic response in SS mice that may also relate to anemia of chronic disease and erythropoietin resistance, but will require further validation in humans. These results are especially timely with the increasing availability of hypoxia-inducible factor–