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CLINICAL TRIALS AND OBSERVATIONS

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When sugar isn't sweet: neutropenia in GSD-Ib

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In this issue of *Blood*, Wortmann et al describe successful treatment of neutropenia and neutrophil dysfunction in a cohort of patients with glycogen storage disease type Ib (GSD-Ib) using empagliflozin, an inhibitor of the sodium glucose transport protein 2 (SGLT2).[1]

Off-label use of a diabetes medication for treatment of a disorder associated with hypoglycemia and aberrant endogenous glucose production initially would seem risky and foolhardy. In this study, however, the authors convincingly demonstrate amelioration of neutropenia, improved neutrophil function, and normalization of neutrophil chemotaxis in this population with congenital neutropenia without causing significant untoward effects.

The association between GSD-Ib and congenital neutropenia was recognized in 1980, and life-threatening infections frequently occurred in this population before the introduction of therapy using granulocyte colony-stimulating factor (G-CSF).[2,3] Treatment with G-CSF has markedly improved the prognosis for people with GSD-Ib, but recent concerns about complications associated with use of the hematopoietic growth factor have arisen. Not only is G-CSF therapy associated with severe splenic complications, including splenic rupture and infarction, but there is increasing evidence that chronic use in this population can predispose patients to myelodysplasia and acute myeloid leukemia.[4,5] Notably, Li et al[6] recently demonstrated shortening of telomere length in patients with GSD-Ib who are treated with continuing G-CSF therapy. Telomere shortening has been associated with chromosomal instability and possible proliferation of abnormal hematopoietic cells leading to the development of the hematologic malignancies.

Although G-CSF–induced stimulation of white blood cell production has been the primary treatment for neutropenia for the last 30 years, the therapy is not directed at the cause of the pathology. Several studies convincingly demonstrated that white blood cell maturation is normal in GSD-Ib, but survival of the neutrophil progenitors is impacted because of premature apoptosis.[7,8] Accumulation of a toxic metabolite was hypothesized, and 1,5-anhydroglucitol-6-phosphate (1,5AG6P) was identified by Veiga-da-Cunha et al[9] in 2019 as the potential cause of neutrophil apoptosis in the GSD-Ib population and also in those with congenital neutropenia resulting from glucose-6-phosphatase 3 (G6PC3) deficiency. Subsequent studies demonstrated that 1,5AG6P shares excretion patterns similar to those of its structural analog glucose, and use of an SGLT2 inhibitor in the murine models of the diseases resulted in amelioration of the neutropenia.

In this first-in-human trial of an SGLT2 inhibitor, Wortmann et al have shown that increased urinary excretion of 1,5AG6P results in improved neutrophil survival and function in a small cohort of people with GSD-Ib. Better neutrophil function also resulted in fewer infections, and the patients experienced marked improvement in their associated inflammatory bowel disease. Although the significance of the improvement should not be minimized, it is critical to note that neutropenia was not cured with the intervention. The studies were also performed in only 4 individuals who phenotypically were more severe than most GSD-Ib patients. SGLT2 inhibitors have not been approved for use in the pediatric population, and their use has been associated with hypoglycemia and genitourinary infections.[10] Therefore, more studies are strongly recommended before this therapy is widely adopted, as was noted by the authors. This is particularly important in very young children who may be at higher risk for infections because of the chronic exposure to glycosuria in the setting of diaper use. Continuous glucose monitoring is also recommended to better assess the risk of hypoglycemia, and data should be captured as part of a larger clinical trial or international registry.

Inhibition of SGLT2 offers the potential of minimizing or discontinuing G-CSF therapy in the GSD-Ib population. This study also demonstrates the importance of understanding the pathophysiology of diseases instead of simply treating the phenotype. In light of the toxicity associated with using G-CSF treatment in the GSD-Ib population, this study is the most significant advancement in this field in decades, and the importance should not be underestimated because of the small study population.

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HEMATOPOIESIS AND STEM CELLS

Comment on Di Giandomenico et al, page 1044

Megakaryocytes tame erythropoiesis with TGFβ1

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In this issue of *Blood*, Di Giandomenico et al have used a megakaryocyte (MK)-specific *tgfb1* knockout strategy to identify an unsuspected physiological role of MKs in regulating steady-state erythropoiesis by restraining progenitor cell and erythroblast (ERB) production.[1]

Transforming growth factor β1 (TGFβ1) is the major member of a superfamily of cytokines that is encoded by 33 genes involved in cell survival, proliferation, and differentiation. They are classified in 4 main families: BMP, GDF, activins, and TGFβ. They have multifunctional roles in most cellular systems through binding to 2 types of cell-surface serine-threonine kinase receptors, known as type I and type II receptors, that lead to the activation of transcription factors of the SMAD family. They behave as regulators of inflammatory and reparative responses and are involved in several diseases.[2]

Using in vitro approaches, it has been shown that the TGFβ superfamily plays an important role in the regulation of hematopoiesis at different cellular levels, including hematopoietic stem cell (HSC) cell cycle, cell fate, and differentiation, more particularly for the erythropoietic and megakaryocytic lineages. The in vivo role of TGFβ1 for hematopoiesis has been difficult to assign because mice constitutively lacking *Tgfb1* died early after birth from an inflammatory disease. However, by this approach it was still possible to show that TGFβ1 plays a central role in the function of HSC.[3]

In the bone marrow, MKs are the main cellular sources of TGFβ1, which is also synthesized by several immune cells, macrophages, and bone marrow stromal cells. The major role of the MK-derived TGFβ1 on HSC quiescence has been demonstrated by diphtheria toxin–induced MK ablation. This ablation leads to a 74% decrease in TGFβ1 protein associated with induction of HSC proliferation. However, MK-derived CXCL4 (PF4) also controls HSC quiescence. Therefore, Zhao et al could directly demonstrate the role of MK-derived β1 by a specific *tgfb1* ablation in MK.[4] Di Giandomenico et al, using the same so-called *tgfb1*$^{\Delta Mk/\Delta Mk}$ model, have extended this approach to erythropoiesis.

Suppression of TGF-β1 production by MKs expands erythroid progenitors and ERBs. However, ERBs undergo apoptosis, and no excess of red blood cells (RBCs), or other blood cells, was observed. Similarly, blockade of TGFβ1 activity with an antibody amplified the early erythroid progenitor pool resulting in apoptotic ERBs. In both models, supplementation by erythropoietin (EPO) triggered RBC production by rescuing apoptotic ERBs. These results are in agreement with data showing that TGFβ1 inhibits early progenitor proliferation and decreases the number of differentiation mitosis to accelerate terminal erythroid differentiation through different Smad2/3-Smad4 or -TIF-1γ signalings.[5,6]

Interestingly, the present work supports the hypothesis that terminal erythroid differentiation is mainly regulated by apoptosis. It has been hypothesized that erythroid precursors exhibit differential sensitivity toward EPO. In a steady state, low EPO levels support only the most EPO-sensitive progenitors for terminal differentiation, whereas the others undergo apoptosis. During erythropoietic stress, increased EPO levels induce a massive rapid terminal differentiation from the entire compartment of erythroid progenitors (see figure). Thus, TGFβ1 restrains this EPO-responsive erythroid compartment, preventing an EPO response in case of erythropoietic stress. This important role of TGFβ1 in the regulation of erythropoiesis is a bit surprising, as recent studies have underscored the role of other members of the TGFβ superfamily. Indeed, ActRIIA/B ligand traps are able to increase RBC production both in a steady stage and in several diseases, such as myelodysplasia or β-thalassemia.[7] This effect is mediated through binding of GDF11 and probably other members of the activin/GDF family that are cleared, however, not by TGFβ1 that does not bind ActRIIA and B. These opposite results may suggest that TGFβ1 and Activin/GDF11 play different complementary roles in erythropoiesis.

This study raises several questions related to MK-derived TGFβ1:

1. TGFβ1 is stored in α-granules of MKs, and its secretion may be related to 2 mechanisms: either a leaky storage or a continuous α-granule content release without the activation process as required for platelets. It is not known