being defined and likely vary depending on the disease process. Activated platelets were described in 2007 to be a potent stimulator of NETs.\(^7\) The potential mechanisms by which platelets induce NETs include the release of thromboxane A\(_2\) and \(\beta\)-defensin-1.\(^8\) Etzalín et al have extended these mechanisms to include P-selectin/PSGL-1. In a series of experiments using isolated cells from mice, the authors convincingly demonstrate that thrombin-activated platelets can trigger NET formation, and this process can be inhibited by blocking either P-selectin or PSGL-1. Activated platelets from P-selectin null mice were unable to trigger NETs, which implies that the platelet releasate alone is insufficient. Cellular contact was demonstrated between platelets and NET-releasing cells, but it was also apparent that soluble P-selectin promoted NET formation. Using neutrophils obtained from mice that overproduce soluble P-selectin, these neutrophils (with bound P-selectin) had exaggerated agonist-induced NET formation, which suggests a priming effect by P-selectin. The authors conclude that interrupting P-selectin/PSGL-1 interactions is a therapeutic target in NET-forming diseases.

Several questions emerge from these important findings. First, what are the intracellular events downstream of PSGL-1 that drive histone citrullination,\(^9\) chromatin decondensation, mixing of the nuclear and cytoplasmic compartments, and the ultimate release of NETs? These intracellular events are still poorly understood. Second, what is the nature of the priming effect by P-selectin? NET formation has been classically associated with a reactive oxygen species (ROS)-dependent process, although ROS-independent NET formation is also recognized.\(^1\) Indeed, P-selectin–dependent NET formation was shown by the authors to be ROS dependent.\(^1\) Therefore, soluble P-selectin may prime the neutrophil NADPH oxidase for NET formation. Finally, how do we explain the significant (although minor) fraction of circulating neutrophils in healthy humans that exist as heterotypic aggregates with platelets? What is the function of these aggregates relative to NET formation? Perhaps neutrophils circulating as heterotypic aggregates represent a “primed” population that, in the setting of “activating” signals, can rapidly release NETs. A “2-event” model may emerge, with P-selectin being critical to the priming effect.

Ultimately, the big question that remains in the field of NET biology is the relative importance of NETs during in vivo host defense vs their potential for tissue injury. The answers will come from continuing to define the basic biology of NETs, including the relevant triggers, the intracellular events that culminate in the expulsion of the chromatin lattice, and the mechanisms by which NETs provoke cellular injury. The reported findings are a conceptual advancement, and position events on the neutrophil surface as a target of intervention to prevent or treat NET-associated diseases.

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**Iron and erythropoietic porphyrias**

Elisabeth I. Minder and Jasmin Barman-Aksözen

In this issue of *Blood*, Egan et al describe an experiment of nature, whereby gastrointestinal blood loss and related iron deprivation were followed by symptom improvement in a case of congenital erythropoietic porphyria (CEP).\(^1\) They then show that prolonged iron deprivation by a chelator, deferasirox, reduces porphyrin overproduction and ineffective erythropoiesis and ameliorates symptoms in both the case and her affected sibling. The authors complement their clinical observation with in vitro investigations showing that erythropoietic precursor cells originating from the CEP cases have an improved survival in an iron-restricted environment.

**P**orphyrrias are mainly inherited disorders of heme biosynthesis and can be divided into hepatic and erythropoietic forms depending on the tissue of porphyrin overproduction. A major difference between hepatic and erythropoietic heme synthesis is that 2 different genes encode the enzyme of the first and rate-limiting step. In hepatic heme synthesis, it is aminolevulinate-synthase 1, a hepatic and ubiquitous enzyme, and in erythropoiesis heme synthesis, it is aminolevulinate-synthase 2 (ALAS2), which is limited to erythropoiesis. In the liver, the pathway is regulated by the end product heme via a negative feedback mechanism, whereas in erythropoiesis, the regulation is mainly controlled by iron. As shown in the figure, ALAS2 mRNA contains an iron responsive element (IRE) in the 5’ untranslated region. During low iron
function mutations in erythropoietic porphyria related to gain-of-X-linked dominant protoporphyria, an IRP2.3 of IRP2. The level of iron likely is available. The level of iron likely is sensed by a protein called "F-box and leucine-rich repeat protein 5." IRE in the 5′ end of ALAS2 is unbound, and translation of ALAS2 protein proceeds. This leads to increased activity of this enzyme, controlling the rate of erythropoietic heme synthesis, and an increased overflow of porphyrin intermediaries such as uroporphyrin I in CEP. In CEP, the bottleneck of heme synthesis is the activity of the enzyme uroporphyrinogen III synthase (UROS), whereas in other erythropoietic porphyrias, other enzymes represent the bottlenecks, such as uroporphyrinogen decarboxylase in hepatoerythropoietic porphyria or ferrochelatase in erythropoietic protoporphyria. As shown by Barman-Aksözen et al, decreased heme synthesis increases ALAS2 activity by a yet-unknown mechanism. (Right) In an iron-deprived situation, IRP2 is active, binds to the IRE, and blocks ALAS2 translation. As less porphyrin intermediaries are synthesized, their overflow at the bottleneck of UROS is diminished, which reduces symptoms and improves survival of erythrocyte precursor cells in bone marrow. Similar effects of iron deprivation can be expected in other erythropoietic porphyrias. Professional illustration by Luk Cox, Somersault18:24.

This figure illustrates the hypothesis of Egan et al1 and may be applied analogously to other erythropoietic porphyrias. (Left) A normal to increased iron availability is shown. Thereby, IRP2 is ubiquinated and destroyed, likely by an action of the iron-sensing protein "F-box and leucine-rich repeat protein 5." IRE in the 5′ end of ALAS2 is unbound, and translation of ALAS2 protein proceeds. This leads to increased activity of this enzyme, controlling the rate of erythropoietic heme synthesis, and an increased overflow of porphyrin intermediaries such as uroporphyrin I in CEP. In CEP, the bottleneck of heme synthesis is the activity of the enzyme uroporphyrinogen III synthase (UROS), whereas in other erythropoietic porphyrias, other enzymes represent the bottlenecks, such as uroporphyrinogen decarboxylase in hepatoerythropoietic porphyria or ferrochelatase in erythropoietic protoporphyria. As shown by Barman-Aksözen et al,6 decreased heme synthesis increases ALAS2 activity by a yet-unknown mechanism. (Right) In an iron-deprived situation, IRP2 is active, binds to the IRE, and blocks ALAS2 translation. As less porphyrin intermediaries are synthesized, their overflow at the bottleneck of UROS is diminished, which reduces symptoms and improves survival of erythrocyte precursor cells in bone marrow. Similar effects of iron deprivation can be expected in other erythropoietic porphyrias. Professional illustration by Luk Cox, Somersault18:24.

ALAS2 became more into focus as an important factor in the erythropoietic porphyrias when Whatley et al4 described X-linked dominant protoporphyria, an erythropoietic porphyria related to gain-of-function mutations in ALAS2, in 2008. Next, To-Figueras et al5 described another gain-of-function mutation in ALAS2 causing a CEP phenotype of increased severity. Recently, our group detected that ALAS2 expression is increased in erythropoietic protoporphyria and that diminished iron availability in those patients is not only associated with a decreased hemoglobin concentration but also with a diminished blood concentration of the relevant protoporphyrin.6 The publication of Egan et al now underlines the importance of ALAS2 activity in the phenotype of CEP, because they show that iron restriction indeed reduced the amount of ALAS2 protein in CEP erythroid bone marrow cells.

Can the conclusions of the authors be generalized that iron restriction may also benefit other CEP patients and patients with other types of erythropoietic porphyrias? CEP is a rare, devastating disorder with a prevalence of 1 per 0.5 to 1.0 million. The severity of CEP varies greatly, whereby the extent of metabolic disturbance, environmental factors (light exposure), and age affect the phenotype. As the rarity of disease has prevented any therapeutic trials, treatment reports have been limited to a single or a few cases. The published options can be separated in those aimed at reducing the metabolic disturbances, preventing the phototoxic skin damage or correcting the sequelae of the disease as outlined by a comprehensive, but retrospective, study of 29 CEP patients.7 Besides strict light avoidance, reflectant sunscreen, β-carotene, or narrow-band UV phototherapy was used to prevent phototoxic damage. Charcoal or cholestyramine was given orally to try to interrupt enterohepatic recirculation of porphyrins, although such an enterohepatic recirculation has never been shown for the water-soluble porphyrin intermediates excessively produced in CEP. The efficacy of all those treatments was ambiguous.7

The different attempts to manipulate erythropoietic heme biosynthesis included hematopoietic stem cell or bone marrow transplantation (BMT) that may be curative. Katugamola et al7 list 16 published cases with a mean observation period of 2.3 years and a mortality rate of 19%. All 13 survivors of this series were apparently well. However, when all 6 cases alive were analyzed in another series,7 serious acute complications were found in all of them. The long-term outcomes included no BMT-related complications in only 3 of 5 patients, progression of CEP in 1 patient, and chronic graft-versus-host reaction in 1 patient. The observation period after BMT in the last patient was only 82 days.

Other means to reduce excess porphyrins include suppression of heme biosynthesis by hypertransfusion or hydroxyurea to ameliorate the hemolytic anemia by splenectomy or to activate the erythropoiesis by erythropoietin.7 The effect of splenectomy was disappointing.7 Only a single case report with short-term hydroxyurea treatment has been published.9 Hypertransfusion in CEP patients inevitably leads to iron overload, and these patients require chelation therapy.7 Some of them received erythropoietin, which apparently induced some improvement of skin symptoms for a limited period.7 This short
summary emphasizes that available treatment options in CEP are far from satisfactory; therefore, new approaches as suggested by Egan et al are indeed welcome. Considering their data, hypertransfusion in CEP needs reevaluation, as it may induce a vicious circle of iron overload, overactivity of ALAS2, augmented porphyrin excess, and ineffective erythropoiesis. In symptomatic anemia, a combination of iron chelation with erythropoietin could be attempted, as 2 of 3 CEP patients, and 1 patient with hepatoporphyrin, reported improved wound healing.7,10 The symptoms of 1 of the 3 CEP patients on erythropoietin deteriorated after we added iron supplements, an observation also supporting the hypothesis of Egan et al1 (E.I.M., unpublished data, 2004).

Other erythropoietic porphyrias could profit from iron chelation as well. In the rare complication of liver failure in erythropoietic protoporphyria, where only liver transplantation is life saving, an attempt to reduce ALAS2 overactivity, excess protoporphyrin production, and intrahepatic protoporphyrin accumulation by iron chelation can be considered based on data from Barman-Aksözen et al.6

However, caution is needed, as iron chelators have been evaluated for iron overload only; interruption of their application is recommended when ferritin falls to <500 μg/L (deferiprone, deferasirox) or 1000 μg/L (deferoxamin), and the long-term adverse effects of iatrogenic iron deficiency induced by chelators remains unknown. Treating CEP and other erythropoietic porphyrias with iron chelation could be as treacherous as sailing between Scylla and Charybdis.

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Elisabeth I. Minder and Jasmin Barman-Aksözen