

## Increasing serum transferrin to reduce tissue iron overload due to ineffective erythropoiesis

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Transferrin (Tf) is the serum protein responsible for delivering iron to the erythron and peripheral tissues. Tf takes up iron derived from dietary absorption and from macrophage recycling and delivers it to utilizing cells that internalize the Tf-bound iron through transferrin receptor 1 (TfR1).<sup>1</sup> A second transferrin receptor exists, TfR2, which binds the iron-loaded Tf with a lower affinity than TfR1.<sup>2</sup> TfR2 has a regulatory function in the liver and in the erythroid compartment.<sup>3,4</sup> Under physiological conditions, almost all iron in the circulation is bound to Tf. However, in diseases resulting in iron overload, Tf is saturated with iron, and excess iron is present in the plasma as non-Tf-bound iron (NTBI). The exact route of uptake of NTBI remains unclear, but it is well established that NTBI is responsible for parenchymal cell iron overload.<sup>15</sup>

$\beta$ -thalassemia is a congenital anemia characterized by ineffective erythropoiesis, increased Tf saturation and tissue iron overload.<sup>6</sup> It represents a paradigmatic disorder of erythropoiesis characterized by anemia and dysfunction of iron homeostasis. In 2010, Li and co-authors reported that administration of exogenous apoTf to  $\beta$ -thalassemic mice ameliorated anemia. Specifically, apoTf injections normalized red blood cell survival and increased hemoglobin production and concomitantly decreased reticulocytosis, erythropoietin abundance and splenomegaly. Moreover, Tf treatment normalized plasma NTBI and increased hepcidin expression.<sup>7</sup>

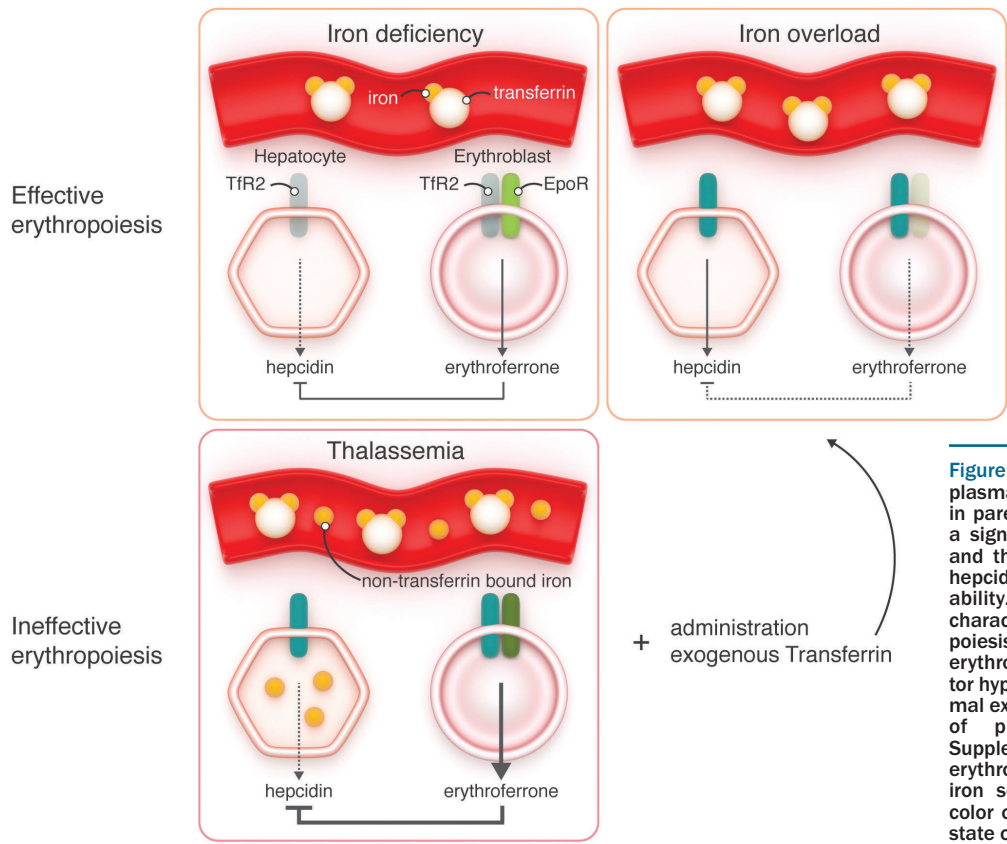
In this issue of *Haematologica*, Gelderman, Baek and co-authors extended those results and reported that apoTf may be used to reverse hemochromatosis in  $\beta$ -thalassemia.<sup>8</sup> In a different mouse model of  $\beta$ -thalassemia intermedia (*Hbb<sup>th3/4</sup>*), they confirmed that chronic apoTf administration normalizes anemia. Furthermore, they demonstrated normalization of tissue iron content in the liver, kidney and heart. ApoTf treatment was also found to attenuate transfusion-mediated increases in plasma NTBI and tissue iron. These therapeutic effects were associated with normalization of Tf saturation and suppressed plasma NTBI.

The positive effect of apoTf treatment on erythropoiesis is due to restriction of erythropoiesis resulting from reduced iron uptake. The expansion of the plasma Tf pool decreases the amount of diferric-Tf in favor of monoferric-Tf, which has a lower affinity for TfR1. This is expected to reduce iron availability for heme synthesis and consequently limit  $\alpha$ -globin aggregates and hemichrome formation thus ameliorating ineffective erythropoiesis. Similar results were obtained in  $\beta$ -thalassemic mice placed on a low-iron diet or engineered to overexpress hepcidin to limit iron availability.<sup>9</sup> Furthermore, administration of apoTf to hypotransferrinemic mice, which suffered from an iron-restricted anemia associated with tissue iron overload, corrected anemia by improving iron delivery to erythroid cells.<sup>10</sup> These results clearly indicate that the plasma Tf pool controls iron delivery to the erythron thus representing a limiting factor in regulating erythropoiesis in mice.

The second positive effect of the Tf therapy in  $\beta$ -thalassemic mice is a reduction of tissue iron accumulation. Tissue iron overload represents an important risk for  $\beta$ -thalassemic patients, especially for transfusion-dependent ones. In fact,

despite chelation therapy, myocardial disease remains the life-limiting complication of secondary iron overload in thalassaemic patients.<sup>6</sup> Reduction of tissue iron in apoTf-treated  $\beta$ -thalassaemic mice is achieved through two main mechanisms, reduction of NTBI and normalization of hepcidin level. Plasma NTBI increases when Tf is fully saturated and is responsible for iron accumulation in parenchymal tissues. Expansion of the plasma Tf pool reduces NTBI and consequently iron uptake by tissues. In this context, exogenous Tf represents a natural iron chelator. Concomitantly, Tf supplementation, by correcting anemia, normalizes hepcidin level. Hepcidin is the master regulator of iron metabolism. Hepcidin is a liver hormone and regulates iron absorption and iron recycling by controlling the plasma membrane expression of the iron exporter ferroportin. Hepcidin is regulated by iron, hypoxia, inflammation and erythropoiesis.<sup>11</sup> Interestingly, holoTf regulates hepcidin expression through TfR2 and the hemochromatosis gene *HFE*. HoloTf binds both TfR1 and TfR2, although it has a stronger affinity for TfR1. In a proposed model, HFE associates with TfR1 under low iron conditions and is displaced when TfR1 binds holoTf. As serum iron and holoTf concentrations rise, the ratio of TfR2 to TfR1 expression increases. Together, this leads to TfR2/holoTf membrane stabilization and induces HFE/TfR2 binding and hepcidin expression.<sup>12</sup> Nevertheless, in the context of stress erythropoiesis, such as in  $\beta$ -thalassaemia, hepcidin is suppressed despite high Tf saturation, due to the dominant effect of the erythroid regulator. The latter senses the erythropoietic requirement for iron and signals to the liver to reduce hepcidin expression. Recently, erythroferrone, a hormone produced by erythroblasts in response to erythropoietin has been demonstrated to be the erythroid regulator, at least in conditions of stress erythropoiesis.<sup>13</sup> When erythropoiesis is stimulated, erythroferrone is induced and this results in suppression of hepcidin expression and increased iron availability for the erythroid compartment. Data from Gelderman, Baek and co-authors support this role since they found that normalization of anemia due to apoTf treatment was associated with suppression of erythroferrone expression and an increase of hepcidin level that, as expected, resulted in reduced ferroportin in the gut.<sup>8</sup> It is likely that normalization of anemia reduces erythropoietin level and attenuates erythropoietin receptor signaling including erythroferrone expression. Interestingly, TfR2 was shown to act as an iron sensor on erythroid cells.<sup>14,15</sup> TfR2 is associated with erythropoietin receptor on erythroblasts and negatively regulates their sensitivity to erythropoietin. Moreover, it has been reported that holoTf stabilizes TfR2 at the plasma membrane while in conditions of iron deficiency (low Tf saturation) TfR2 is shed from the plasma membrane and a soluble form is released.<sup>16</sup> According to the model of Nai *et al.*, shedding of TfR2 from the plasma membrane of erythroid cells results in increased erythropoietin sensitivity, high erythroferrone and low hepcidin.<sup>14</sup>

Thus, the plasma Tf pool controls iron accumulation in tissues and iron delivery to the erythroid compartment and concomitantly regulates hepcidin expression through TfR2 (Figure 1). In iron deficiency, holoTf is reduced and this results in the



**Figure 1.** In physiological conditions, plasma Tf limits iron accumulation in parenchymal tissues and acts as a signal sensed by the hepatocyte and the erythroblast for regulating hepcidin expression and iron availability. In pathological conditions characterized by ineffective erythropoiesis, such as  $\beta$ -thalassemia, the erythropoietin/erythropoietin receptor hyperactivation causes an abnormal expansion of erythropoiesis and of plasma and tissue iron. Supplementation with Tf normalizes erythropoiesis and restores normal iron sensing. The intensity of the color of TfR2 or EpoR indicates the state of stabilization/activation. See text for details. Erfe: erythroferrone.

shedding of TfR2, which, on hepatocytes reduces TfR2/HFE signaling on hepcidin promoter and on erythroblasts promotes erythropoietin signaling and erythroferrone expression. In conditions of iron overload with normal (effective) erythropoiesis, holoTf is increased and this stabilizes TfR2 on hepatocytes, inducing hepcidin, and on erythroblasts, limiting erythropoietin sensitivity, maybe to avoid excessive red blood cell production. When erythropoiesis is ineffective, the regulatory role of Tf/TfR2 is overridden by the hyperactivation of erythropoietin signaling which suppresses hepcidin leading to abnormal expansion of the plasma iron pool. Supplementation with exogenous Tf reduces NTBI avoiding tissue iron loading, and normalizes anemia thus reducing erythropoietin signaling and restoring normal iron-Tf control of hepcidin expression (Figure 1).

Another interesting observation from the work of Gelderman, Baek and co-authors is the lack of effect of haptoglobin and hemopexin in reducing hemochromatosis in  $\beta$ -thalassemic mice.<sup>8</sup> Haptoglobin and hemopexin are plasma proteins that bind hemoglobin and heme, respectively, with high affinity. In hemolytic conditions, when hemoglobin and heme are released into the bloodstream, haptoglobin and hemopexin mediate hemoglobin and heme recovery by hepatocytes and macrophages, respectively, thus promoting heme iron recycling and preventing heme from having toxic effects.<sup>17</sup> Administration of hemopexin to a different mouse model of  $\beta$ -thalassemia ( $Hbb^{thi/thi}$ ) was shown to be effective in preserving vascular homeostasis by pro-

moting heme iron recovery by the liver.<sup>18</sup> Gelderman, Baek and co-authors showed that hemopexin as well as haptoglobin supplementation does not alter plasma iron levels or tissue iron loading.<sup>8</sup> This indicates that in the  $Hbb^{thi/+}$  mouse model the rate of intravascular hemolysis does not contribute significantly to hemochromatosis. Nevertheless, in pathological conditions associated with a higher rate of hemolysis, such as in sickle cell disease, or under a chronic transfusion regimen, the supplementation of hemoglobin and heme scavengers might be beneficial.<sup>19</sup>

In conclusion, it is time to speculate that the modulation of plasma protein pools represents a valuable therapeutic approach to control iron delivery to bone marrow and parenchymal tissues. Increasing serum Tf, with serum purified or recombinant protein, may be useful not only in  $\beta$ -thalassemia but in all pathological conditions characterized by ineffective erythropoiesis.

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## Jekyll and Hyde: the role of heme oxygenase-1 in erythroid biology

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There is currently a great deal of excitement regarding the role of stem cell niches that regulate hematopoietic stem cell self-renewal and differentiation. It should be noted, however, that the original description of a hematopoietic niche actually occurred in 1958 when the French hematologist, Marcel Bessis, described erythroblastic islands.<sup>1</sup> The island was characterized by developing erythroblasts surrounding a central macrophage and, based on careful structural observations, Bessis and colleagues made a number of interesting inferences about the role of central macrophages in erythropoiesis. It was suggested that the macrophage functions as a “nurse” cell, providing iron to developing erythroblasts for heme synthesis and, furthermore, that the extruded nuclei produced at the end of erythroid differentiation are phagocytized by these central macrophages.<sup>2</sup> These concepts proved prescient as they have been supported by a number of recent findings which have shown that the macrophage-erythroblast interactions mediated by a large number of adhesion molecules are essential for the highly regulated process of erythroblast proliferation and survival which is necessary for the production of two million reticulocytes per second.<sup>3-6</sup> In this context the recent findings that *in vivo* depletion of erythroblastic island macrophages blocks erythroblast proliferation and maturation fully validates the central role of macrophages in regulating erythropoiesis.<sup>7</sup>

Apart from playing an important role in the genesis of red blood cells within the bone marrow, macrophages of the reticulocyte endothelial system in general and spleen in particular play a critical role in quality control by removing senescent and damaged red cells from the circulation.<sup>8-10</sup> Thus different macrophage subsets play a dual

role in both the production of red cells and in the elimination of senescent normal red cells and pathological red cells. This important symbiotic interrelationship between erythroid and macrophage biology is receiving increasing attention in hematology research since the findings of the studies have direct relevance to our understanding of both normal and disordered erythropoiesis.

In this issue of *Haematologica*, Fraser and colleagues describe exciting new findings regarding a key role for heme-oxygenase-1 in both regulating erythroid differentiation and in mediating clearance of circulating red cells through its effect on macrophages.<sup>11</sup> The work of Fraser *et al.* documents that heme-oxygenase-1 deficiency adversely affects steady-state erythropoiesis in murine bone marrow due to a diminished ability of erythroblasts to form erythroblastic islands. The reduction in erythroblastic islands was the result of decreased numbers of the subtype of bone marrow macrophages involved in island formation. These observations reinforce the concept of an essential requirement of a specific subset of macrophages for the formation of bone marrow erythroblastic islands and that island formation is necessary to sustain normal bone marrow erythropoiesis. Interestingly, the decreased erythropoiesis in the bone marrow led to increased erythropoiesis in the spleen, a common compensatory response in the murine system in which the spleen is the major erythropoietic organ that responds to stress erythropoiesis.

While heme-oxygenase-1 deficiency had a negative effect on bone marrow erythropoiesis, it had a positive effect on red cell life span in circulation as a result of compromised ability of the macrophages of the reticuloendothelial system to remove senescent red cells. It thus