BRIEF REVIEW

Transferrin: Physiologic Behavior and Clinical Implications

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THIS REVIEW attempts to summarize current concepts of the role of transferrin in iron transport and related clinical disorders.

THE NEED FOR AN IRON TRANSPORT SYSTEM

The chronicle of iron metabolism began when primitive organisms first employed iron as a necessary part of their energy-generating system. The reasons for choosing iron for this task may have been its chemical properties as a catalyst and its relative availability. At that time, there was little oxygen in the atmosphere, and abundant iron was present in the reduced ferrous form. With the transition to an aerobic environment, iron was oxidized to the ferric form and soluble iron became scarce. Some bacteria and plants coped with the diminished iron supply by synthesizing low molecular weight chelators that “mined” the ferric iron in their surroundings and transported it back to, or even across, the cell membrane for internal use. In similar fashion, mammals have developed a mechanism to augment iron uptake from food; in this instance, it is a high molecular weight mucosal transferrin that chelates iron within the intestinal lumen and shuttles it into the mucosal cells of the small intestine. Inside the body, once again, iron required some mechanism for its effective distribution, as virtually no free iron could exist in the body’s milieu.

The internal iron transport system of most mammals consists of a circulating β-globulin, which ferries iron between sites on iron-donating tissues and membrane receptors designed to procure iron for the cell. Tissues differ by two orders of magnitude in their iron requirement, and it is the difference in receptor number that adjusts iron uptake to the individual tissue’s need. The consequence of a breakdown in this dispersal system is seen in the rare genetic disorder, atransferrinemia. Affected individuals have a severe iron deficiency anemia due to the inability of the erythron to take up iron by any alternate mechanism, and, at the same time, these individuals show an excess of storage iron in other tissues.

BEHAVIOR OF TRANSFERRIN

Specific transport proteins existed in primitive organisms long before iron requirements were increased by the invention of hemoglobin to fulfill the needs of oxygen transport. For example, the pyura, an anephric invertebrate, has a transferrin with a molecular weight (mol wt) of about 40,000 that contains one binding site for iron. Today’s mammalian transferrin, consisting of two globular domains of a single polypeptide chain with a molecular weight of 79,500, may be the product of a gene duplication of the smaller invertebrate protein. It bears two chemically and spectroscopically distinct iron binding sites that are located in the N- and C-terminal halves of the molecule (Fig 1). One of the remarkable features of iron binding by transferrin under physiologic conditions is that the metal is bound specifically only when bicarbonate is bound concomitantly. The function of the anion is probably to lock the iron into place in the molecule by serving as a bridging ligand between the iron and the protein. Under physiologic conditions, the apparent stability constants for the two binding sites are in the range of $10^{19}$ to $10^{20}$ mol/L. Transferrin contains 6% carbohydrate in two biantennary glycan chains. Although the subject of much speculation, the function of the carbohydrate remains unknown.

The past two decades have seen a heightened inter-
The increase in plasma iron turnover observed with increasing transferrin saturation is largely the result of the greater capacity of diferric transferrin to deliver iron to tissues. The implication of this iron-unloading behavior is that plasma iron delivery to tissues increases as a function of increasing transferrin saturation, quite apart from any change in receptor number (Fig 2). By correcting for changes in iron turnover related to predictable variations in the amount of monoferric and diferric transferrin, it is possible to define more precisely erythroid marrow function.

TRANSFERRIN RECEPTORS

Just as transferrin is an ancient device, so it would appear are transferrin receptors. Indeed, invertebrates, such as the dungeness crab and pyura, can bridge the evolutionary gap, with their transferrins complexing with mammalian reticulocyte receptors and delivering iron to be synthesized into hemoglobin.

Recently, more precise methods for separating apo-ferric, monoferric, and diferric transferrin have made it possible to characterize the behavior of transferrin in detail. The loading of the two binding sites of transferrin in vitro and in vivo was shown to occur at random, one atom of iron at a time. The delivery of iron was nonhomogeneous, but not as originally suspected. The two monoferric transferrins showed no difference in the rate of iron delivery or in its tissue distribution. The explanation of the original Fletcher-Huehns phenomenon was a preference of tissue receptors for the more spheroidal diferric transferrin. In humans, there was a 3.5-fold advantage of diferric as compared with monoferric transferrin.

The cellular uptake of iron begins with the binding of the transferrin-iron complex to a specific receptor. The richest sources of transferrin receptors for biochemical characterization have been hemoglobin-synthesizing reticulocytes and the placental trophoblast. Certain monoclonal antibodies have greatly aided in the isolation of receptors from other cell types, particularly those with a much lower receptor density. Results in various species indicate that the receptor is a disulfide-linked transmembrane protein with a molecular weight of about 180,000 (Fig 3). It consists of two monomers of 90,000. These monomers contain two types of glycan chains at the cell surface side. Within the cell membrane, and perhaps at the cytoplasmic side of the cell, is a covalently bound fatty acid. The transferrin receptor is frequently isolated in a phosphorylated form, the phosphate being bound to the hydroxyl group of a serine residue on the 5,000 mol wt "tail" of the molecule. One transferrin molecule binds to each subunit of the receptor. When the receptor is treated with trypsin, a fragment of 70,000...
mol wt is released. This fragment is still able to bind transferrin and retains the antigenic binding sites for the monoclonal antibodies OKT9 and B 3/25. Although the monoclonal antibody OKT9 binds specifically only to the human transferrin receptor, transferrin itself binds efficiently across species and phylogenetic barriers.

It was some 15 years ago that Morgan first suggested the intracellular migration of the transferrin—iron receptor complex,20 and evidence for this phenomenon now seems to be conclusive.4 A group of such complexes produce an invagination of the cell membrane, leading to the formation of a vacuole within the cell’s cytoplasm. In some manner, perhaps related to a fall in pH, iron is released. Meanwhile, the apotransferrin, which is tightly bound to the receptor at a pH of about 5, returns within the vacuole to the cell membrane. There the receptor is reincorporated into the membrane, while the apotransferrin molecule is freed to resume its iron transport activity.

Disturbances in the cellular transport of iron have been described. For example, the Belgrade rat, while having a normal transferrin, a normal receptor number, and normal internalization, lacks the ability to release sufficient iron from the intracellular transferrin—receptor complex.21 Shahidi et al reported an interesting family with an excessive iron supply, but a severe block in iron uptake by the erythron.22 Whether this represents a deficit in erythroid membrane receptors or an impaired intracellular iron release has not been determined.

At present, very little is known of the nature of the sites on the reticuloendothelial (RE) cell, hepatocyte, and intestinal mucosa, which donate iron to transferrin. It may be assumed that some membrane mechanism must exist, since direct contact between transferrin and membrane is required. It has also been suggested that a plasma ferrous oxidase, such as ceruloplasmin, may facilitate the transfer of iron to transferrin, as has been demonstrated in animals.23

REGULATION OF IRON PROCUREMENT

In the adult human, some three fourths of plasma iron turnover is concerned with erythropoiesis.24 It is reasonable, therefore, that the erythron regulates iron procurement. This relationship can be demonstrated in the experimental animal by carrying out an exchange transfusion with reticulocytes, thereby doubling the receptors for transferrin within a few minutes.25 The plasma iron turnover is immediately increased as more iron is mobilized from the RE cells and intestinal mucosa. Of particular interest was the observation that the plasma iron did not change significantly. This indicated that the increased iron procurement was not mediated by an increased number of open iron binding sites on transferrin molecules. It seemed, rather, that the act of supplying an increased amount of iron in some manner “activated” the transferrin procurement system.

Although the receptor mass sets iron requirements and determines procurement, the adequacy of response and the site of procurement depends on the available iron supply of the individual donor tissues.30 The first line of iron supply is the RE cell. Any increase in iron absorption implies an inadequate iron supply from the RE cell. In iron deficiency, any increase in iron supply must come from the gut, since body tissues are already depleted of storage iron. Another situation that may also evoke an absorptive response is when the iron requirements are increased to such an extent that the procurement—delivery system is no longer able to keep up (relative iron deficiency). In such a setting, plasma iron supply will be normal or even increased, yet red cells will show evidence of iron deficiency.27,28

EXCESSIVE IRON PROCUREMENT

There are three conditions described as causing increased iron stores through increased absorption. The first is iron overload due to excessive oral iron intake. This is uncommon, and the difficulty in producing any significant overload attests to the effectiveness of the mucosal barrier. Only the South African black has been able to overcome the block consistently.29 It is speculated that the alcoholic fermentation products, acting as chelators for iron, are responsible for the increased iron absorption. Even so, intestinal biopsy shows heavy submucosal deposits of iron, suggesting that a kind of RE blockade still exists.

Quite different is the situation in the erythropoietic overload states, where the receptor mass is markedly expanded. Similar to the effect of the reticulocyte transfusion, there is an increased iron release from RE cells and gut.30,31 With time, the body iron load increases to the point that the plasma iron level becomes elevated, permitting red cell production to occur at the maximal rate. Increased absorption can be shut off if erythropoiesis is suppressed by transfusion, demonstrating the regulatory effect of the erythroid marrow.32

Why then does iron overload occur so predictably in thalassemia and so rarely in hemolytic anemia? Two differences between these two conditions can be suggested. First, the rate of erythropoiesis in thalassemia major is higher than that seen in hemolytic anemia. The importance of this is implied in the presence of iron overload in occasional patients with hemolytic anemia who have erythropoietic rates eight times basal or more. Secondly, in thalassemia, there is considerable uptake of hemoglobin-haptoglobin by the liver. Such iron may be less readily mobilized than is the RE
iron, which is so efficiently processed in hemolytic anemias.

The most common form of iron overload in the temperate zone is the HLA-related disorder, known in its homozygous symptomatic form as idiopathic hemochromatosis. Although the nature of this genetic disorder is not known, the similarity of its pathophysiology with that of the erythropoietic overload states is impressive. Iron absorption is increased, as is iron release from RE cells. The excess iron is stored in the hepatocyte. It would appear that here, too, procurement is activated, but it is a result of a genetic abnormality in the procurement system itself rather than the consequence of an expanded marrow mass.

SUMMARY

The transferrin iron transport system, along with its procurement sites and delivery receptors, provides a highly effective means of satisfying internal iron requirements. Iron uptake by individual tissues is determined by their receptor number, by the relative amounts of monoferric and diferric transferrin in circulation, and by the amount of available iron in donor tissues. Although the modus operandi of this system under basal conditions has been characterized, its exquisite regulation remains an enigma. In some manner, the procurement of iron is determined by iron requirements. What seems to be an inappropriate behavior of the absorptive mechanism in thalassemia and certain other erythroid overload states may actually be life-saving in the absence of transfusion, since it results in higher levels of plasma iron and thereby higher levels of erythropoiesis. The definition of the regulatory mechanism in such conditions may well lead to an understanding of the molecular defect in idiopathic hemochromatosis.

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