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Significance of Soluble Transferrin Receptor

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Abstract

Iron transport in the plasma is carried out by transferrin which donates iron to cells through its interaction with a specific membrane receptor, the transferrin receptor (TfR). A soluble form of the TfR (sTfR) has been identified in animal and human serum. Serum sTfR levels average 5,000±1,000 ng/ml in normal subjects. The most important determinant of sTfR levels appears to be total erythropoietic activity which can cause variations up to 8 times below and up to 20 times above average normal values. This is of great interest in the investigation of the pathophysiology of anemia and the monitoring of the erythropoietic response to various forms of therapy, in particular to rHuEpo. Iron status also influences sTfR levels and this may be useful in detecting functional iron deficiency. The placenta is not an important source of sTfR. With the exception of CLL and possibly hepatocellular carcinoma, sTfR levels are not elevated in patients with malignancies not involving the erythron. Soluble TfR is a truncated monomer of tissue receptor, lacking its first 100 amino acids, which circulates in the form of a complex of transferrin and its receptor. A small amount of serum TfR is in the form of an intact dimer circulating in exocytic vesicles called exosomes. The erythroblasts are the main source of serum sTfR. We conclude that soluble TfR represents a valuable quantitative assay of marrow erythropoietic activity.

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Introduction

Iron transport in the plasma is carried out by transferrin which donates iron to cells through its interaction with a specific membrane receptor. The transferrin receptor (TfR) is a 760 amino acid glycoprotein of approximately 95 kD (1,2). The functional receptor is composed of two such monomers, linked by two disulfide bridges to form a molecule of 190 kD (Figure 44.1). Each monomer has an N-terminal cytoplasmic domain of 61 amino acids, a short trans-membrane segment of 28 amino acids, and an extracellular segment of 671 residues. After binding of transferrin to its receptor, the complex is internalized in an endocytic vesicle or endosome. After iron is released to the cytoplasm by acidification of the endosome, the TfR is recycled to the cell membrane and apotransferrin to the plasma. A soluble form of the TfR is present in serum and we review here its significance.

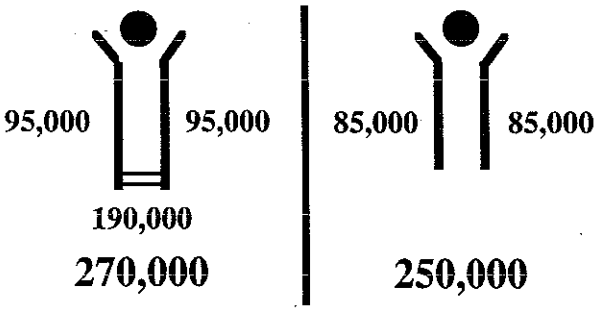

Figure 44.1 Schematic representation of the TfR. The left panel represents cellular TfR, composed of two monomers (MW 95,000 each) linked by two disulfide bridges, and capable of binding one transferrin molecule (Closed circle, MW 80,000), to give a total MW of 270,000 for the complex. The right panel represents sTfR, probably a complex of Tf and two truncated monomers (MW 85,000 each), to give a total MW of 250,000.

Clinical measurement of sTfR

Soluble TfR in normal serum

Fuccello was the first to detect material in human serum which reacted with the monoclonal anti-human TfR antibody OKT9 (3). Kohgo (4) and Beguin (5) were then the first to measure sTfR quantitatively in human and rat serum, respectively. A number of quantitative assays, including radiolabeled Fab fragment precipitation assay (6), as well as radioimmunoassays (4,7–9) and enzyme-linked

immunosorbent assays (3,5,10–14) using polyclonal or monoclonal antibodies, have now been set up to measure sTfR in biological fluids, such as solubilized cells, culture supernatant or serum. The performance of these assays (sensitivity, reproducibility, standardization, convenience) is highly variable. In a group of 165 normal human subjects, receptor levels averaged 5,000±1,100 ng/ml (M±SD), and no difference was observed with sex or age in the 18–78 year age range, although few elderly persons were included (15,16). These values are considerably higher than those initially reported by Kohgo, but this may relate to his use of low-affinity monoclonal antibodies (4) and problems with standardization (11) because similar levels were reported by others (11). No reference values are available in children. Levels in normal rat serum were quite similar (5) but varied with sex (higher in females) and age (lower in older animals), reflecting the erythropoietic response to changes in growth rate (17).

Soluble TfR and erythropoiesis

Erythropoietic activity depends on the number of erythroid precursors involved in proliferation and differentiation (18). The level of erythropoietic activity has been found to be the most important determinant of sTfR levels. Decreased sTfR levels are found in situations characterized by erythroid hypoplasia (Figure 44.2), such as hypertransfusion (5), chronic renal failure (10,13,19), severe aplastic anemia (10,11,20,21), or after intensive chemotherapy (10,11,22). Increased sTfR levels are seen in situations of hemolysis or stimulated ineffective erythropoiesis (Figure 44.3), such as immune hemolytic anemia (5,10,20,21), hereditary spherocytosis (10), sickle cell anemia (11), thalassemia (10), megaloblastic anemia (23), or secondary polycythemia (21). Soluble TfR levels range from a minimum of about 700 ng/ml – which would represent the contribution of nonerythroid tissues to serum levels – when erythropoiesis is totally suppressed, to about 100,000 ng/ml in a severely anemic thalassemia patient (10,15).

The plasma iron turnover (PIT), in which the daily transport of iron through the plasma is calculated from the plasma iron and the radioiron disappearance curve after intravenous injection of a tracer dose, has been extensively used as a quantitative measure of red cell production (24,25). A simplified approach to the ferrokinetic evaluation of erythroid marrow activity has been proposed by Cazzola (26,27) and the erythron transferrin uptake (ETU) is now established as the best available method for the quantitation of erythropoiesis (27,28). The dependence of sTfR levels on the activity of the erythron is further demonstrated by the strong correlation observed between serum receptor and ferrokinetic measurements of erythropoiesis. This has first been shown in rats with normal, suppressed, or stimulated erythropoiesis (5). This has also been confirmed in humans in whom the relationship between mean erythron transferrin uptake (ETU) and mean sTfR level in groups of subjects with a variety of diagnoses is very close to the line of proportionality (10,16,29).

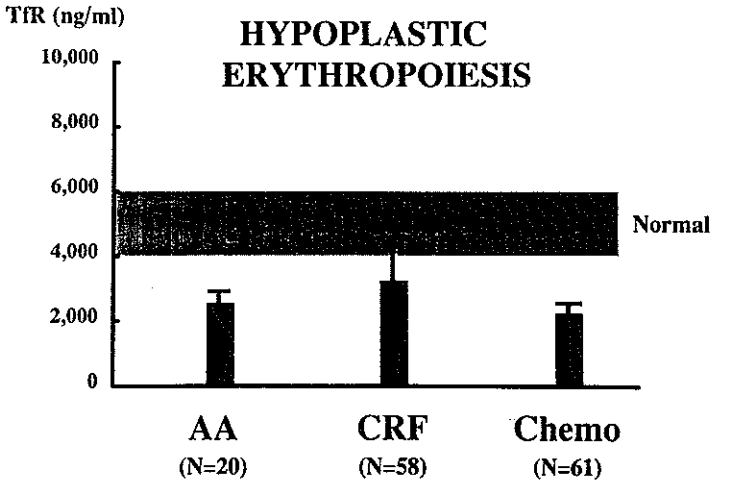

Figure 44.2 Average ±1SD sTfR levels in groups of patients with hypoplastic erythropoiesis, including severe aplastic anemia (AA), chronic renal failure (CRF), or after intensive chemotherapy (Chemo). The gray zone represents M±SD levels in normal subjects.

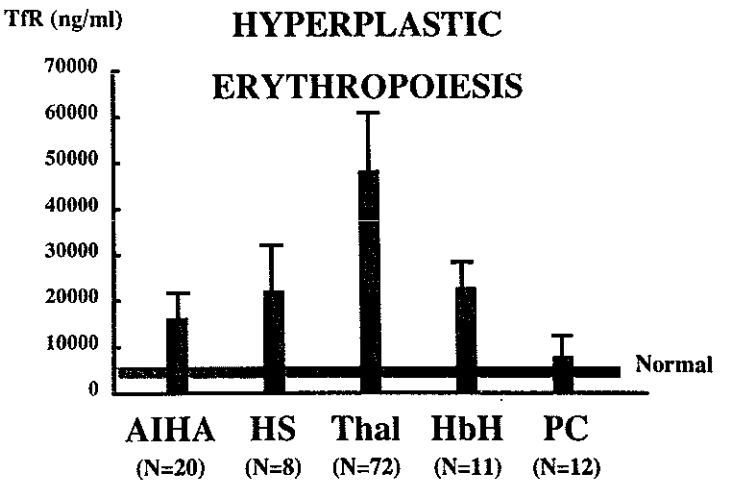

Figure 44.3 Average ±1SD sTfR levels in groups of patients with hyperplastic erythropoiesis, including autoimmune hemolytic anemia (AIHA), hereditary spherocytosis (HS), β-thalassemia major (Thal), HbH disease (HbH), or polycythemia (PC).

Soluble TfR may be used as a monitor of the erythropoietic response to various form of therapy, including bone marrow transplantation (22), the treatment of pure red cell aplasia with cyclosporin (30), the correction of megaloblastic anemia with cobalamin (23) or iron deficiency anemia with iron (20), or the removal of excess iron by phlebotomy (12). It is of particular interest in assessing response to recombinant human erythropoietin (rHuEpo) in patients with chronic renal failure (13,19,31), hematologic malignancies (32,33), rheumatoid arthritis (14) or genetic hemochromatosis (13), in premature infants (34), as well as in normal subjects (13,35). The quantitative determination of the erythropoietic effect of rHuEpo early in the course of treatment helps predict the later hematologic response (31,32). Early recognition of a low probability of hematologic response could help identify and correct specific causes of treatment failure or suggest rapid dose escalation, in order to hasten clinical improvement and avoid prolonged ineffective use of an expensive medication. However, this early prediction will not be valid in patients in whom rHuEpo essentially stimulates ineffective erythropoiesis (32,36).

Soluble TfR and iron status

TfR expression on cells is related to iron availability, with iron deprivation inducing and iron excess suppressing TfR synthesis (37,38). This explains the reciprocal changes between cellular iron load and sTfR release observed in cell culture systems (39). This also influences sTfR levels in serum. Altering serum iron concentration in normal or non-deficient rats has no immediate effect on sTfR levels (5). However, chronic iron overload reduces sTfR levels by about 20% in rats (5) as well as in hemochromatotic patients (10,40), although therapeutic phlebotomy may induce values up to twice basal levels (15). Soluble TfR levels in severely anemic iron-deficient rats increase 6-fold over normal values, in proportion to the increase documented for erythron membrane receptors (5,15,41). As compared to normal individuals, levels are marginally increased by 20% in non-anemic iron-deficient subjects but more dramatically so in patients with iron deficiency anemia (10,11,20).

In normal volunteers undergoing graded phlebotomy, sTfR did not change much during the phase of storage iron depletion, but increased significantly when marrow functional iron deficiency and anemia developed (9,42) and this may be useful to indicate when phlebotomy should be discontinued in an hemochromatotic subject (12). Part of these modifications may be caused by parallel changes in serum erythropoietin levels (9,12,15). Iron supplementation returns sTfR to normal values (9,20) but this may be preceded by some transient elevation of sTfR values (20). As sTfR levels are not increased in the anemia of chronic disorders (44,45), during acute infection (44), or in chronic liver disorders (44,46), they may help distinguish these clinical problems from iron deficiency. Actually, sTfR may decrease temporarily during acute inflammation (5).

Soluble TfR and the placenta

The placenta is particularly rich in TfR involved in the maternofetal transfer of iron (47) and increased sTfR levels have been observed in the third trimester of pregnancy (48). However, larger studies have demonstrated that sTfR levels were normal in late pregnancy and therefore that the placenta was an unlikely source of sTfR in the mother (49,50). Actually, sTfR levels are decreased in the first two trimesters, normalize in the first part of the 3rd trimester, and are slightly increased in late pregnancy and the early postpartum (49). These changes parallel those in Epo production and may explain known alterations in the red cell mass throughout pregnancy (49,51,52). However, depletion of iron stores also produces a moderate elevation of sTfR over levels observed in non-iron deficient pregnancy (49,50).

Soluble TfR and cancer

Increased expression of transferrin receptors has been documented on the surface of malignant tumors as compared to their normal counterparts (53-55). Soluble TfR levels are elevated in patients with myelofibrosis, myelodysplastic syndromes, and myeloproliferative disorders, but are essentially within normal range in chronic myelogenous leukemia or essential thrombocythemia, which is in keeping with our understanding of erythropoiesis in these disorders (10,56). In patients with polycythemia vera, sTfR levels may be considerably elevated (10,21). Levels in acute leukemia have been found to be increased (57) but this has been contradicted by other studies (10,56). Patients with lymphoid malignancies, including lymphoma, hairy cell leukemia, and multiple myeloma, have been found to have normal sTfR values (10,56,58). However, levels may be considerably elevated in chronic lymphocytic leukemia (56,59). Levels are also normal in patients with breast carcinoma (60) or other solid tumors (10), with the possible exception of those with hepatocellular carcinoma (46).

Summary

Serum sTfR levels average 5,000±1,000 ng/ml in normal subjects. The most important determinant of sTfR levels appears to be total erythropoietic activity which can cause variations up to 8 times below and up to 20 times above average normal values. Iron status also influences sTfR levels and this may be useful to detect functional iron deficiency, but these changes may not be entirely independent from erythropoietin stimulation. The placenta is not an important source of sTfR release to the maternal circulation. With the exception of CLL and possibly hepatocellular carcinoma, sTfR levels are not elevated in patients with malignancies not involving the erythron.

Origin and molecular form of serum soluble TfR

Exosomes

More receptors are seen on CFU-E than on BFU-E, and their number increases to about 300,000 on early normoblasts and up to 800,000 on intermediate normoblasts, before declining to about 100,000 on reticulocytes and none on mature red cells (1,61–64). During maturation of reticulocytes to red blood cells, a number of membrane functions are retained, while others, including the TfR, are selectively lost (65–67).

A population of vesicles (named exosomes), 40–100 nm in diameter, has been retrieved after ultracentrifugation of plasma from reticulocyte-rich blood of various mammalian and ovarian species, including man, rat, sheep, pig, rabbit, dogs, cats, and embryonic chickens (65,68–70). These exosomes appeared to originate from multivesicular bodies in maturing reticulocytes (65,68,69,71,72) and were shown to contain intact TfR (68,69,72–75) as well as various plasma membrane functions, including the clathrin-uncoating ATPase/heat shock protein (67,68,76,77). Metabolic activity and ATP are essential for exosome formation (69,74,75,77). Exosome formation was shown to be a major route of externalization of obsolete proteins during maturation of sheep reticulocytes (69,70).

It may be speculated that membrane proteins targeted for externalization undergo subtle denaturing changes which lead to binding to the clathrin-uncoating ATPase, segregation to a class of endosomes where fusion and budding occurs, forming multivesicular bodies which can fuse with plasma membrane and release buds, or exosomes, into the circulation (65).

Origin and molecular form of soluble TfR

Total binding to circulating Tf was demonstrated by the complete disappearance of sTfR from serum treated with anti-Tf antibodies (5,10). Separation of human plasma by PAGE electrophoresis or gel filtration demonstrated a single peak of immunoreactivity with an apparent MW slightly smaller than that of purified placenta TfR (5,10). Kogho found that serum sTfR was in the form of nicked dimers of 55 kD, with a MW of 110 kD in non-reducing conditions and of 46 and 23 KD in reducing conditions (21). However, purification of sTfR by affinity chromatography yielded a single peak of TfR with a MW of 85 kD together with a 75 kD peak representing Tf under reducing as well as non-reducing conditions (78,79). The purified placental TfR had a MW of 190 and 95 kD, respectively in the same conditions. Analysis of the amino-terminal amino acid sequence of sTfR revealed that residues 1–19 were identical to residues 101–119 of tissue TfR. Thus, sTfR appears to be in a truncated form, lacking the cytoplasmic and transmembrane domains (residues 1–100) of intact

TfR (78). Using domain-specific antibodies, it has been shown that less than 1% of serum TfR is intact receptor consistent with an exosomal origin, whereas virtually all is in the form of a truncated extracellular domain. This percentage is however increased to 3.8% in patients with sickle cell anemia (80).

Human erythroleukemia K562 cells release TfR in vesicular (30%) and soluble (70%) forms. On SDS-PAGE electrophoresis, the soluble receptor had a MW of 85 kD, lower than the 95 kD for the monomer of cellular TfR. The first 19 residues of sTfR were identical to residues 101–119 of cellular TfR demonstrating that, like serum sTfR, it is a truncated form of the intact receptor (81). Solubilized cell membranes contained a 105 kD receptor consistent with truncation of one extracellular domain monomer and exosomes contained a 25 kD fragment consistent with a dimeric remnant of intact receptor (82). The human promyelocytic cell line HL60 has been shown to release exclusively soluble truncated fragments of 80 kD, which are distinctly smaller than the 190 kD dimer or 94 kD monomer of cellular TfR (70,83). Hepatoma cell lines have also been shown to release TfR, mostly in a vesicular form, in an energy-dependent process (46). Pancreatic, colorectal, gastric cancer cell lines also release soluble TfR fragments (84).

In vitro incubation of rat reticulocytes was followed by the release of TfR in vesicular (65 %) as well as soluble (35 %) forms, which both had an apparent MW of 190 kD and 95 kD under non-reducing and reducing conditions, respectively, similar to the cellular receptor (85).

In vitro incubation of sheep reticulocytes has been shown to release TfR both in a vesicular (75 %) and a soluble (25 %) form (70). The soluble form has a MW of 160 kD under non-reducing conditions and 80 kD under reducing conditions, in contrast to 190 and 94 kD, respectively, for cellular as well as vesicular TfR. The dimer may be due to the presence of an additional disulfide bridge in sheep TfR or if its cysteine residues are downstream of the cleavage site. Trypsin digestion of reticulocytes or of exosomes produced an 80 kD soluble monomer and left a 17 kD fragment detected by MoAb directed against the cytoplasmic domain. This fragment was detected in untreated exosomes but not in untreated cell membranes, suggesting that the soluble TfR originated from proteolytic cleavage in the exosomes but not at the cell membrane.

Factors affecting the release of soluble TfR

Rat (85) or sheep (69,70,77,86) reticulocytes, as well as the human erythroleukemia K562 (39,84,87), promyelocytic HL60 (70,83,88), hepatoma (46), gastric or colorectal or pancreatic cancer (84) cell lines release TfR in the culture medium in a time-dependent and energy-dependent fashion. Incubation at 4° and the presence of metabolic inhibitors produce substantial decreases in the amount of TfR shed (46,69,70,75,77,83,84). The addition of protease inhibitors to the culture medium has led to conflicting results, from no effect in

sheep reticulocytes (70) or K562 cells (87), to complete suppression of TfR shedding by HL60 cells (83). Treatment by trypsin increased the production of a truncated soluble TfR by sheep (70) or rat (79) reticulocytes. Redistribution of surface TfR to intracellular locations caused a reduction of TfR release by HL60 cells (88), whereas the opposite was observed with K562 cells (87). Redistribution of intracellular TfR to the cell surface by insulin increases serum levels of sTfR and soluble IGF-II receptors (89,90). Attempts to alkalinize the endocytic vesicles produced discordant effects of increasing (87) or decreasing (75) the release of TfR. Inhibitors of protein synthesis decreased TfR release by HL60 cells (88). Manipulating the iron status of K562 or HL60 cells produced reciprocal changes between cellular ferritin content on the one hand, and cellular and soluble TfR on the other (39,88). The presence of homologous diferric Tf has been shown to increase (84) or decrease (87) the release of TfR by K562 cells. TfR in the supernatant was a remarkably constant 5 % of the cellular TfR over a wide range of cellular iron (39). The only exception (3 %) was observed after the inclusion of human diferric Tf which might protect bound TfR against shedding or cause redistribution of TfR.

Summary

In summary, it is now established that serum TfR is a soluble truncated monomer of tissue receptor, lacking its first 100 amino acids, which circulates in the form of a complex of transferrin and its receptor. A possible conformation is two receptor monomers (85 kD) binding to one transferrin molecule (80 kD) to give a total MW of around 250 kD (Figure 44.1). A small amount of serum TfR (although this may vary with the patient's diagnosis) is in the form of an intact dimer circulating in exocytic vesicles called exosomes.

The origin of the serum sTfR is not clear. It may originate directly from the integral cell membrane, either at the cell surface or in endocytic vesicles, or indirectly after exosomes are produced, in which the TfR is selectively segregated together with other plasma functions. The discrepancies observed in the *in vitro* investigation of TfR release may relate to differences in the cells and species studied, in the use of quantitative or semi-quantitative techniques, and in the nature of TfR antibodies.

An excellent correlation between cellular TfR and soluble TfR has been demonstrated both *in vivo* by ferrokinetic studies and *in vitro* by incubations of tumor cell lines. Since more than 80 % of tissue receptors are in the erythroid marrow and since the highest sTfR levels are observed in thalassemic patients in whom reticulocytes counts are quite low, the erythroblast compartment, not reticulocytes, should be the main source of sTfR.

It is quite possible that several mechanisms for TfR shedding are operating simultaneously or sequentially, if not in a single cell, at least in different cells. Therefore one can imagine that direct proteolytic cleavage of the extracellular

domain of TfR occurs in growing cells, such as erythroblasts or leukemic cell lines, whereas exosome formation occurs in maturing cells, such as reticulocytes.

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