Review

Circulating transferrin receptors and assessment of iron status

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A soluble form of the transferrin receptor is uniformly detectable in human sera. Biochemical studies have shown that this soluble material consists of the extracellular domain of the intact receptor with a truncation site between arginine (100) and leucine (101). Cell biologic models have demonstrated that the serum receptor is produced by proteolytic cleavage at the surface of the exosome within the multivesicular endosome. The cleavage is produced by a membrane-associated serine protease at a site distinct from that produced by trypsin.
Clinical studies have demonstrated that the serum transferrin receptor concentration correlates with the number of red cell precursors in the bone marrow in various hematologic disorders and with the deficit in functional iron in patients with iron deficiency. The latter finding has led to a significant refinement in the assessment of iron status. By employing the serum ferritin determination as a measure of body iron stores and the serum transferrin receptor as an index of tissue iron deficiency, it is possible to evaluate the entire spectrum of body iron status. Measurement of the serum transferrin receptor has been particularly useful in detecting iron deficiency in pregnancy and in distinguishing iron deficiency anemia from the anemia of chronic disease. (J. Nutr. Biochem. 5:322–330, 1994.)

Keywords: transferrin receptor; soluble form; serum form

Introduction

Most receptors for protein hormones and growth factors are expressed on the cell surface membrane, the exception being the receptors for steroid, thyroid, and retinoid hormones, which reside in the cell nucleus or cytoplasm. The classic view of receptor-protein interactions has been of a stationary membrane receptor that binds to a soluble protein hormone or growth factor. In recent years, it has become apparent that many protein receptors also exist in soluble form in biological solutions. Soluble forms or genetic material encoding for them have been identified for many receptors including insulin,[1] epidermal growth factor,[2] interleukin-1 (IL-1),[3] IL-2,[4] IL-4,[5] IL-5,[6] IL-6,[7] IL-7,[8] granulocyte colony stimulating factor (G-CSF),[9] granulocyte macrophage CSF,[10] leukemia inhibitory factor (LIF),[11] growth hormone,[12,13] prolactin,[14] ciliary neurotropic factor,[15] tumor necrosis factor,[16] nerve growth factor,[17] and erythropoietin.[18] The physiologic significance of these soluble forms is unclear, although several functions have been suggested. In the case of growth hormone binding protein, the soluble receptor functions as a carrier by protecting ligand from degradation until it can be delivered to the tissue-bound receptor. On the other hand, if the soluble receptor has an affinity for its ligand comparable to the tissue form of the receptor, it may serve as an endogenous growth factor or hormone antagonist.[19] A third physiologic role for soluble receptor has been identified for the ciliary neurotropic factor in which the soluble-ligand binding receptor subunit of a multicomponent receptor may confer ligand sensitivity to tissues not normally expressing the binding moiety.[15] It is also possible that certain soluble receptors simply represent one mechanism of protein degradation or, in some instances, a flaw in the genetic machinery of a cell.

The receptor for transferrin was one of the first receptors for which a soluble form was demonstrated. The transferrin receptor falls into a different functional category than most membrane receptors in that it facilitates the entry of an essential cell nutrient rather than mediating the effects of a hormone or growth factor. While the function of the soluble form of the transferrin receptor remains as obscure as for most other soluble receptors, its origin, mechanism of pro-

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duction, and biochemical identity have now been established together with its important nutritional and clinical applications. This rapidly accumulating knowledge of the soluble transferrin receptor forms the basis of the present review.

Cellular transferrin receptor

All growing and metabolizing cells require iron. The versatile chemistry of iron and the noxious reactions it may promote have necessitated the development of transport and storage pathways that maintain the metal in an available but harmless form. There are several pathways by which iron gains entry to cells. The most sophisticated of these transport systems is the manner by which iron is ferried from its extracellular carrier, transferrin, to the cytosol. In this highly specific pathway, which accounts for the major influx of cellular iron, diferric transferrin binds to its receptor on the cell surface and is then internalized by endocytosis. Not only does the transferrin receptor facilitate the transport of transferrin-bound iron from the extracellular environment to the intracellular endocytic vesicle, but it also impedes the release of iron from transferrin at the cell surface and thereby protects the membrane from peroxidative injury. Within the acidic environment of the endocytic vesicle, the transferrin receptor has the intrinsic property of promoting iron release from its carrier protein.[20] These various properties of the transferrin receptor ensure the safe and efficient delivery of iron to the endocytic vesicle and facilitate the onward movement of iron to intracellular transport and storage pathways.

Molecular organization

The transferrin receptor is a transmembrane glycoprotein comprising identical 95kDa monomers linked by a pair of disulfide bridges. Each monomer consists of 760 amino acids organized into an amino terminal cytoplasmic domain of 61 amino acids, a membrane-spanning region of 28 amino acids and a carboxy terminal extracellular domain of 671 amino acids.[21,22] The cytoplasmic domain is required for appropriate intracellular trafficking,[23] and the peptide sequence of tyrosine-threonine-arginine-phenylalanine in this region has been identified as a consensus signal for endocytosis via coated pits.[24] N-linked glycosylation sites have been identified at amino acid residues 251, 317, and 727, while threonine at amino acid position 104 has been identified as the only *O*-linked glycosylation site.[25,26]

The cellular expression of the transferrin receptor has been the subject of intensive investigation in recent years.[27] The most important regulatory factor is the iron status of the cell. This control appears to be largely posttranscriptionally mediated by the iron response element (IRE) in the mRNA for the transferrin receptor, which in turn interacts with a binding protein for the IRE. When the cellular content of iron is adequate, this protein has predominantly aconitase enzyme activity, whereas its RNA coating activity prevails when there is a relative deficiency of intracellular iron. Coated RNA is protected from degradation. These differing functions of the same protein appear to be modulated by changes in cellular levels of nitric oxide, which in turn respond to differences in cellular iron concentration.[27] There is a second controlling influence on transferrin receptor expression that appears to be transcriptionally determined in relation to the proliferative status of the cell[28]; this mechanism accounts for variations in receptor expression at different stages of the cell cycle.

Intracellular pathways

The endocytic pathway is shared by several receptors but has been best studied in relation to the endocytosis of receptors bound to diferric transferrin. In this process, receptors bound to ligand are concentrated in clathrin-coated pits on the cell surface. The pit deepens and invaginates to form an endocytic vesicle, which then undergoes progressive, energy-dependent protonation. In the reduced pH environment that results, iron dissociates from its carrier transferrin and is transported across the vesicle membrane by a poorly understood mechanism.[29,30] The apotransferrin that remains in the endocytic vesicle has a high affinity for its receptor in this acidic milieu and thereby escapes degradation. On return to the physiologic pH at the cell surface, this affinity is lost, and the ligand is released from its receptor to replenish its iron-binding sites.[31,32]

There appear to be two pathways of intracellular trafficking of the endocytosed transferrin receptor (*Figure 1*). In the major pathway, the transferrin-receptor complex within endocytic vesicles is rapidly recycled to the cell surface via a paragolgi apparatus route. In addition, there is an alternative, minor pathway for the endocytosed transferrin-receptor complex that has been defined in studies employing sequential imaging of gold labeled transferrin after endocytosis[33] along with electron microscopic and immunochemical evaluation.[34–39] This is a degradation pathway in which the endocytic vesicle membrane forms multiple internal blebs resulting in a larger membrane-bound structure termed the multivesicular endosome. Within this structure are multiple 50 nm microvesicles with receptors on their surface that have been named "exosomes." The contents of the multivesicular

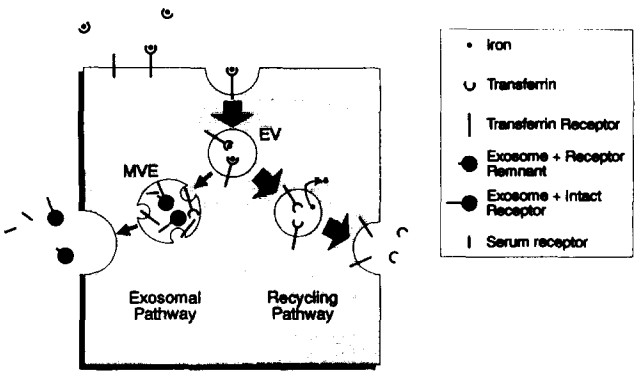

Figure 1 Schematic representation of the two well-recognized transcellular pathways, followed by endocytosed transferrin receptor. EV, endocytic vesicle; MVE, multivesicular endosome. Receptor truncation resulting from proteolysis within the extracellular domain is shown as occurring at the exosome surface within the multivesicular endosome. This is consistent with our current data showing most complete proteolysis at the exosome surface. The possibility that some clipping of the receptor occurs at the cell surface has not been fully excluded.

endosomes are eventually expelled by the process of exocytosis in which these small vesicular structures are released into the extracellular fluid. This process is reported to account for only a portion of transferrin receptor loss by the cell as compared with almost 100% of that for other surface proteins.[39,40] Most of these studies of transferrin receptor have been performed with reticulocytes because of their high content of transferrin receptor and not surprisingly have led to the suggestion that transferrin receptor loss is an erythroid maturation phenomenon, the loss being primarily in intact form bound to exosomes. Other workers, however, have established that receptors are lost not only as a terminal maturation event but in most cells by a continuous process of receptor synthesis and catabolism.[41]

Serum transferrin receptor

Elucidation of the exosome pathway of transferrin receptor release from reticulocytes prompted a search for transferrin receptors in the circulation using commercially available monoclonal antibodies to the cell surface receptor. The presence of immunoreactive receptor material in the circulation was established[42–45] and interpreted in several reports as reflecting the exosome form of the receptor.[43,44,46–48] The presence of a serum form of the transferrin receptor was confirmed by other groups using assay systems based on monoclonal[49] and polyclonal[47] antibodies developed against purified receptor. While studies of the exosome pathway prompted the search for serum receptors, it is highly unlikely that a receptor bound to a particulate structure such as a vesicle would be detected in an enzyme-linked immunoabsorbent assay (ELISA). In the light of such reservations it became important to further evaluate the serum transferrin receptor by isolating it and determining its biochemical composition.

Biochemical characterization

The isolation of ample quantities of transferrin receptor from sera known to contain large amounts of receptor was accomplished by using monoclonal antibody affinity columns. Electrophoretic, immunologic, glycoprotein and amino acid sequence analysis were used to examine the proteins eluted from the column.[50] The serum transferrin receptor was found to have a molecular mass of 85 kDa under reducing and non-reducing conditions as compared with values of 190 kDa and 95 kDa, respectively, for the intact receptor. The 85 kDa protein reacted with monoclonal and polyclonal antibodies developed to intact receptor and was shown to be a glycoprotein based on concanavalin-A staining and a change in migration after treatment with N-glycanase. Electrophoresis, Western blot analysis, and ELISA measurements demonstrated that the purified protein from serum is a complex of transferrin with its receptor in which the non-covalent linkage is disrupted in the presence of sodium dodecyl sulfate (SDS).

As determined by amino acid sequence analysis, the serum form of the transferrin receptor is an abbreviated extracellular domain of intact receptor arising as a result of a truncation between arginine (100) and leucine (101). The amino terminal sequence of the serum receptor is compared with the sequence of the intact receptor between residues 95 and 122 as follows:

Serum receptor *L A G* X *E S P V* X *E E P G E D F P A A* ***

Intact receptor *** L T E C E R *L A G T E S P V R E E P G E D F P A A* ***

X indicates non-identified residues

The threonine at position 104 has recently been shown to be the only site of *O*-linked glycosylation in the receptor molecule,[25,26] and this may explain failure to identify this residue during sequencing.

Polyclonal antibodies to specific peptide sequences in the extracellular and cytoplasmic domains on either side of the biologically relevant truncation site have been useful in further clarifying the nature of the circulating form of the transferrin receptor.[51] The sequences employed are shown below:

31 G D N S H V E M K L A V D E E E N A D N N T K A N V T K P P

61 R **C S G S I C Y G T I A V I Y F F L I G F M I G Y L G Y C** K

91 G V E P L T E C E R ↑ L A G T E S *P V R E E P G E D F P A A R*

121 R L Y W D D L K R K L S E K L D S T D F T ⇑ S T I K L L N E N

small letter, 40 to 54, cytoplasmic peptide; italics, 107 to 120, extracellular domain peptide; bold, membrane spanning region; ↑, truncation site[50]; ⇑, putative trypsin cleavage site.[52]

These sequences were selected on the basis of their strategic location, hydrophilicity, proline richness, and paucity of intra-sequence lysines. The cytoplasmic domain antibody reacts with purified intact receptor but not with purified serum receptor, whereas the extracellular domain antibody reacts with both intact and serum receptor.

The development of competitive binding ELISA's using the above reagents provides a means of quantifying the concentration of extracellular and/or cytoplasmic transferrin receptor domains. When sera that had been ultracentrifuged to remove any microvesicles were assayed, the soluble immunoreactive material contained only the extracellular domain. When the microvesicles were isolated by ultracentrifugation and solubilized in detergent, the content of transferrin receptor accounted for <3% of the serum concentration. Moreover, the content of the cytoplasmic domain was significantly higher than the content of the extracellular domain.[51] These results indicate that serum receptor consists almost entirely of truncated extracellular domain with only a trivial amount of exosomal material.

In vitro models of soluble receptor production

In vitro cell culture studies have provided important confirmation of the results obtained from analysis of receptor material in human serum. With the initial system employing K562 erythroleukemia cells, soluble receptors can be measured in the culture supernatant using the monoclonal antibody ELISA for serum receptor.[53] The concentration varies directly with the number of cells in culture and increases progressively with time in stationary phase cultures. The concentration at any time correlates directly with the cell

receptor number, which in turn varies inversely with cellular iron content as measured by the cytosolic concentration of L-rich ferritin. Soluble receptor isolated from the supernatant and analyzed in the same manner as the serum receptor is identical to the 85 kDa glycoprotein isolated from serum with the same truncation site between arginine (100) and leucine (101).[54] However, contrary to the findings in serum, the solubilized pellet obtained by ultracentrifugation of culture supernatant contains a higher proportion of the exosomal form of intact receptor, approximately 20% of the total receptor released to the supernatant. Similar findings can be obtained using the HL60 promyelocytic cell line. The supernatant receptor is qualitatively the same as in the K562 system, but the HL60 cells release the 85 kDa soluble receptor at 2 to 3 times the rate. Exosome receptor in this system accounts for only about 5% of non cell-associated receptor.[55,56]

The findings obtained with these two in vitro systems therefore closely mimic the in vivo results in that soluble receptor is the dominant form of receptor released from cells, and the quantity bears a direct relationship to the content of cellular receptor (see below). Only a small amount of intact receptor is released bound to microvesicles, and it is not clear whether this is significant in vivo because normal human serum contains negligible amounts of particulate receptor. The possibility that any or most of released microvesicles undergo rapid in vivo clearance remains to be evaluated.

Production of soluble transferrin receptor

The two predominant mechanisms of soluble receptor production that have been described for other protein receptors are proteolysis and alternate mRNA splicing. The latter mechanism, which is especially common in the hemopoietin receptor superfamily, involves premature termination in reading of the RNA template prior to the sequences encoding the transmembrane portion of intact receptor. However, in the case of the transferrin receptor, the carboxyterminal ending of the extracellular domain coupled with the biochemical characteristics of the soluble form strongly favor proteolysis as the primary mechanism for generating the circulating form of the receptor. If proteolysis is involved, one can predict fragments of the receptor that should result based on the structure of the circulating form. Because the truncation site for the serum transferrin receptor occurs between arginine (100) and leucine (101), whereas the cysteine residues for the disulfide linkages of the intact dimeric protein are located at positions 98 and 89, cleavage of both extracellular arms of the intact receptor (190 kDa) would produce two soluble monomers (85 kDa each) and a membrane bound dimeric remnant (20 kDa) as follows:

$$\text{TfR} \rightarrow 2\ \text{sTfR} + \text{rTfR}$$

where TfR = intact transferrin receptor, sTfR = soluble form, and rTfR = remnant. However, if only one extracellular domain is cleaved, then a mixed fragment (srTfR) with a molecular mass of 105 kDa and one sTfR of 85 kDa would result. Both the rTfR and srTfR should be associated with the membrane of either the intact cell or exosomal vesicle, whereas the sTfR should exist only in solution. The domain-specific immunological reagents described previously provide a means of identifying these theoretical proteolytic fragments of transferrin receptor *(Table 1)* and have indeed confirmed their existence in the following studies.

When cultured cells and supernatant are ultracentrifuged and the particulate matter solubilized, the rTfR is detected by Western blotting with the cytoplasmic domain antibody, while the srTfR remnant is identified by its reaction with both peptide antibodies.[57] However, this combined particulate fraction contains both whole cell and vesicle membranes. Exosomes extracted from the culture supernatant contain both the rTfR and srTfR remnants, whereas cell membranes isolated from the supernatant exosomes contain only a small amount of the 105 kDa srTfR.[57] These findings strongly suggest that the serum receptor is produced by proteolytic cleavage at the surface of the exosome. Furthermore, when exosomes are incubated in culture supernatant, additional soluble sTfR is not produced,[58] suggesting that proteolysis at the exosome surface occurs prior to exocytosis and presumably within the multivesicular body. It should be noted that no evidence was obtained in these series of investigations for cleavage at the putative trypsin site that has been identified by other workers.[52] It can be argued that the demonstration of maximal receptor proteolysis at the exosome surface merely reflects an active clearing mechanism for the rTfR in the whole cell, but this is unlikely in the light of recent studies showing that trypsin cleavage of receptor at the surface of intact cells does not modify the clearance rate of receptor remnants as compared with intact cells.[59] The serum transferrin receptor, therefore, appears to be produced by proteolytic cleavage predominantly at the exosome surface within the multivesicular body.

Clues about the nature of the proteolytic enzyme involved in the production of the serum transferrin receptor can be obtained by incubation of intact purified receptor with various cellular fractions including supernatant, fractionated membranes, and cytosol. When the post-incubation samples are examined with the extracellular domain antibodies to detect sTfR, culture supernatant and cytosol lack the relevant proteolytic activity. However, the surface membrane fraction, confirmed by the greatest enrichment of 5′ nucleotidase activity, does contain proteolytic activity capable of producing the serum form of receptor. Using various protease inhibitors, this activity appears to be mediated by a serine protease.[60] Because both the limiting membrane of the multivesicular body and the exosome membrane are surface-derived, this subcellular location would provide optimal receptor-membrane protease interaction.

An important remaining question relates to the determinants of proteolysis of the transferrin receptor. It was proposed in earlier reports that production of soluble receptor was increased by transferrin,[44] but this may have been due

Table 1 Predicted fragments of intact transferrin following proteolysis

Fragment	Location	Molecular Mass (kDa)	Peptide antibody reactivity	
			Extracellular	Cytoplasmic
TfR	membrane	190	+	+
sTfR	extracellular	85	+	−
rTfR	membrane	20	−	+
srTfR	membrane	105	+	+

to the use of transferrin preparations that are often contaminated with serum receptor. In later studies employing transferrin purified by affinity chromatography with antireceptor antibodies, added transferrin was shown to cause a modest decrease in serum receptor production.[61] Recent studies of the effect of *O*-linked carbohydrate on transferrin receptor function[62] have suggested another possible determinant of proteolytic cleavage of intact receptor. In these studies cDNA encoding the human receptor was modified by site-directed mutagenesis to substitute threonine (104) with aspartic acid. Transferrin-resistant variant Chinese hamster ovary cells,[63] which do not express transferrin receptor, were stably transfected with either wild type or mutant cDNA. The mutant cells produced much higher amounts of soluble transferrin receptor compared with the wild type, and amino acid sequence analysis demonstrated that the truncation site was the same as for the serum receptor. Whether the *O*-linked glycosylation of threonine 104[25,26] protects the arginine (100) leucine (101) site against proteolysis or modifies intracellular trafficking of receptor away from the multivesicular body is not known. Another possibility, however, may be that the substituted threonine may merely serve as a model for any receptor defect detected by the cell. A schematic representation of the production of the serum transferrin receptor is shown in *Figure 2*. In this the *O*-linked glycosylation is shown at position 104 and the biologically relevant truncation site between arginine (100) and leucine (101) just distal to the disulfide linkage between cysteine (98).

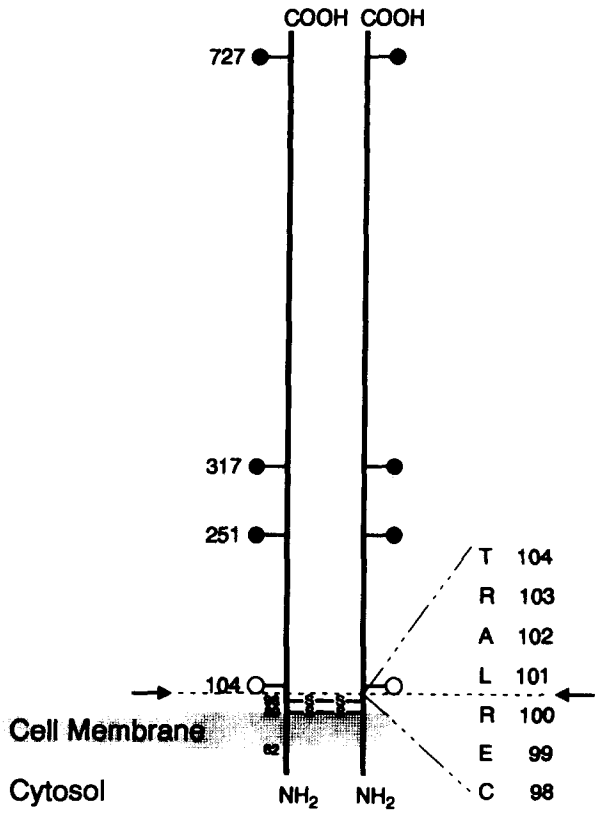

Figure 2 Schematic representation of the origins of the serum transferrin receptor. The intact transferrin receptor is composed of two disulfide-linked monomers, each containing 760 amino acid residues. The disulfide linkages are located at amino acid residues 89 and 98. The N-terminal cytoplasmic domain of the polypeptide consists of 61 amino acids and the transmembrane portion has 28 amino acid residues between 62 and 89. The remaining 671 amino acids are extracellular. The circulating form of the receptor is truncated between amino acid residue 100 (arginine) and 101 (leucine). Solid circles indicate N-linked glycosylation sites. The open circles indicate *O*-linked glycosylation sites.

Clinical applications of the serum transferrin receptor

Clinical assay methodology

In the first clinical reports of serum transferrin receptor, a two-site immunoradiometric assay (IRMA) was used that employed two commercially available monoclonal antibodies, OKT9 and B3/25, developed against intact transferrin receptor on the cell surface.[43] Normal values of 251 ± 94 μg/L in males and 256 ± 99 μg/L in females were reported. In a subsequent report using an ELISA with monoclonal antibodies against purified transferrin receptor from human placenta, a 20-fold higher value of 5.63 ± 1.42 mg/L was observed in normal subjects. This 20-fold discrepancy between assays was shown to result from a difference in the sensitivity in the original IRMA between free receptor that was used as the standard and the fully saturated receptor present in serum.[49] An ELISA employing polyclonal antibodies raised to the transferrin-receptor complex and subsequently adsorbed with transferrin has yielded values of 8279 ± 1261 μg/L whole blood.[47] This latter assay is standardized against the receptor-transferrin complex assuming that 69% of the protein in the complex is receptor. Thus, at least some of the marked disparities in normal values reported with existing assays are related to the nature of the standard, whereas other differences presumably arise from variations in the reactivity of the immunological reagents. Programs for interlaboratory standardization of serum transferrin receptor measurement will be important in resolving these disparities.

Clinical studies of serum transferrin receptor

The initial reports by Kohgo et al.[43,45] indicated marked differences in the concentration of serum transferrin receptor in various hematological disorders. The relative changes reported subsequently in different clinical disorders have been remarkably consistent in light of the differences in assay technique and the reported normal values. Because more than two-thirds of the transferrin-bound iron outflow from plasma in normal subjects is directed to erythroid precursors in the bone marrow for hemoglobin synthesis, the serum receptor concentration in patients with no erythroid bone marrow indicates the contribution of nonerythroid sources to the circulating receptor. Using our monoclonal ELISA, we observed a decline in serum receptor of 50 to 60% in patients with aplastic anemia and in those given ablative chemotherapy in preparation for bone marrow transplantation, similar to the relative decreases reported by others.[43,45,47,64] These findings indicate that erythroid precursors are the major source of the serum receptor. Other body tissues contribute no more than one-third of the circulating

protein. The basal contribution from nonerythroid cells significantly limits the clinical utility of serum transferrin receptor measurements in the detection of hypoproliferative anemias.

Measurements of serum receptor are of far greater importance when the concentration is increased above the upper cutoff level of 8.5 mg/L with our monoclonal ELISA. Not unexpectedly, the most dramatic elevations occur in patients with an increase in the number of red cell precursors in the bone marrow. The latter is referred to as total erythropoiesis and can be accurately quantified by measuring the rate of transferrin-bound plasma radioiron disappearance and subsequent incorporation into newly formed red blood cells. Using these ferrokinetic measurements, an excellent correlation has been demonstrated in patients with a wide spectrum of hematological disorders between the concentration of serum receptor and total erythroid precursor mass. The highest elevations of serum transferrin receptor occur in patients with thalassemia major (10-fold) who are known to have maximal expansion of the erythroid marrow. Increases of 5 to 6 times normal are typically seen in hemolytic anemias such as autoimmune hemolytic anemia, hereditary spherocytosis, and sickle cell disease.[43,45,47,49,65] Thus, when the results of ferrokinetic measurements of erythropoiesis were compared with serum transferrin receptor levels in 148 patients with a wide spectrum of erythropoietic disorders, a remarkably high correlation of 0.86 was obtained.[47] The ability to substitute costly and tedious ferrokinetic measurements of erythroid precursor mass with a simple assay of circulating transferrin receptor is of enormous value and will eventually secure a permanent place for this new measurement in the clinical laboratory.

Assessment of iron status

The one important exception to the rule that the serum receptor is determined by the size of the erythroid mass is in patients with iron deficiency who do not normally expand their red cell marrow. In our initial description of the serum receptor assay, we observed a mean value of 18.8 +/− 11.4 mg/L in 19 patients with overt iron deficiency anemia, or a relative increase from normal of about three times. This elevation in iron deficiency reflects the upregulation of receptor synthesis and parallels the rise in supernatant soluble receptor concentration that is seen when iron supply in the culture media of K562 cells is curtailed. In view of the major role that is evolving for measurements of the serum receptor in the nutritional assessment of iron status, the published information on this important application of serum receptor measurements will be reviewed.

When evaluating body iron status, it is helpful to consider total body iron as residing in two major compartments of functional and storage iron. The large compartment of functional body iron is contained in circulating hemoglobin, muscle myoglobin, and various tissue enzymes. It is the depletion of this functional iron that results in the clinical manifestations and liabilities associated with iron deficiency.[66] The only physiological role of the second smaller and more variable compartment of storage iron is to replenish losses from the functional compartment. When assessing body iron status, the identification of storage iron is important because if present, iron deficiency can be confidently excluded.

An important question relating to the elevation in serum receptor levels in patients with iron deficiency is at what stage in the evolution of iron deficiency does the concentration become abnormal? This was examined by performing serial phlebotomies in 14 normal subjects to obtain a broad spectrum of iron status.[67] The amount of iron remaining in the storage and functional compartments could be calculated at each weekly blood drawing. The concentration of serum ferritin accurately portrayed the level of storage iron up to the point of complete exhaustion of iron reserves but showed little further change with increasing deficiency in functional iron. On the other hand, the concentration of serum transferrin receptor was not affected by declining iron stores but showed a linear rise with progressive depletion of functional iron. Comparison of these changes with those observed with other iron indices commonly used to detect early tissue iron deficiency such as erythrocyte protoporphyrin, mean red cell volume, and red cell size heterogeneity indicated that the serum transferrin receptor was most sensitive and reliable. Moreover, most of the other laboratory indices of iron-deficient erythropoiesis did not fall into the abnormal range at the point at which the baseline hemoglobin concentration had fallen by more than 20 g/L, the end point of the phlebotomy program. The serum transferrin receptor appeared to rise at an earlier stage of tissue iron deficiency although it also remained within the normal range in some of the volunteers.

One of the important findings in this phlebotomy study was the possibility that body iron status can be portrayed by a single laboratory index calculated as the ratio of the serum receptor to ferritin concentration. Changes in this ratio are determined predominantly by the serum ferritin during iron storage depletion and by the serum transferrin receptor during increasing deficiency in functional iron. In fact, when body iron status was plotted against the logarithm of the receptor to ferritin ratio, a linear relationship was observed over the entire spectrum of induced changes in body iron. This observation is perhaps not unexpected in view of the close reciprocal control of ferritin and transferrin receptor synthesis at the cellular level by the IRE. While much more information in various epidemiological and clinical trials will be needed before the usefulness of the receptor/ferritin ratio for estimating body iron is established, it offers many potential advantages over the numerous and more cumbersome laboratory measurements of body iron that are currently employed in population studies. The serum ferritin and transferrin receptor measurements require only a few microliters of serum that can be obtained readily by capillary sampling. Moreover, both serum proteins utilize the same ELISA with the exception of the immunological reagents.

Detection of iron deficiency during pregnancy

One of the vexing problems in assessing nutritional iron status is the identification of iron deficiency in pregnant women. This has assumed greater importance in recent years in view of the mounting evidence that iron-deficiency anemia predisposes one to premature delivery and low birth weight.[68,69] The difficulty in detecting iron deficiency arises

because many of the laboratory indices of iron status are significantly influenced by gestation. Despite an actual increase in red cell mass, the plasma volume increases even more, resulting in a dilutional anemia that is unrelated to iron status. Serum ferritin determinations, which are valuable in identifying iron deficiency anemia in other settings, are of little help in pregnant women because of the rapid decline that occurs with the mobilization of stores for the fetus and the expansion of the mother's red cell mass. Other laboratory indices such as the erythrocyte protoporphyrin or mean red cell volume change too slowly to be useful in detecting the early onset of iron deficiency that typically develops during the latter half of pregnancy.

Our initial experience with measurements of the serum transferrin receptor indicates that it will circumvent the difficulties with existing laboratory measurements. From a study of 176 women in their third trimester of pregnancy, the frequency distribution of receptor levels in those with residual iron stores as defined by a normal serum ferritin was similar to that observed in nonpregnant controls, indicating that the high content of transferrin receptor in the placenta does not contribute significantly to the serum concentration in the mother.[70] Elevation of the serum transferrin receptor above 8.5 mg/L was limited to women with depleted iron stores as determined by a reduced serum ferritin concentration. Iron-deficiency anemia was seen only infrequently in this study because the majority were taking iron supplements regularly. However, in 13 women with unequivocal iron-deficiency anemia as defined by multiple laboratory criteria, 11 had serum transferrin receptor levels in the diagnostic range for iron deficiency. In a recent field trial of the efficacy of iron supplementation in pregnancy in Jamaica, the serum transferrin receptor was especially helpful in characterizing the response to oral iron.[71] As in our phlebotomy study, the receptor to ferritin ratio appeared to be the best single index of overall iron status of the sampled population of Jamaican women.

Clinical assessment of iron status

The recognition of iron deficiency anemia in a clinical setting is more difficult than defining the iron status of healthy individuals or a normal population because of the frequency with which diseases that influence iron measurements are seen clinically, particularly in hospitalized patients. The most difficult distinction is between iron-deficiency anemia and the anemias associated with chronic infection, inflammation, or malignancy, which are referred to collectively as the anemia of chronic disease (ACD). Because these disorders impair the release of iron from stores, they induce the same alterations in circulating red blood cells that occur in iron deficiency. The distinction is further obscured by the fact that the serum ferritin is elevated above the low diagnostic range for iron deficiency by inflammatory disorders. Consequently, there is no single laboratory measurement that separates iron-deficiency anemia from ACD.

Our initial experience suggests that measurements of serum transferrin receptor will be useful in distinguishing these two common causes of anemia.[72] In 17 patients with iron deficiency anemia, the mean serum receptor concentration was 13.9 ± 4.6 mg/L, and all but two patients had abnormal values. In 41 patients with the ACD, however, the mean receptor concentration was 5.65 ± 1.91 mg/L; similar to the mean of 5.36 ± 0.82 mg/L in 17 normal controls. Slight elevations of serum receptor were observed in only four of the ACD patients. If these findings are confirmed in other patient populations, the cost and discomfort of bone marrow examinations, which are commonly used to identify iron deficiency in hospitalized patients, could be circumvented in many instances.

The ability of serum receptor measurements to identify true iron deficiency will also be important in the epidemiological assessment of populations living in developing countries where the prevalence of nutritional anemia is highest. As in many clinical situations, however, there are several potential causes of anemia in lower socioeconomic segments of a population that may be confused with nutritional anemia. If the anemia is due to chronic inflammation or infection, a normal serum receptor level will assist in distinguishing the anemia from iron deficiency. Disorders or deficiencies other than iron deficiency may elevate the serum receptor concentration. Megaloblastic anemia due to vitamin B12 or folic acid deficiency is associated with an expanded red cell mass with ineffective erythropoiesis and elevated receptor levels.[73] The hemolysis associated with malaria infection might also increase the receptor levels, and in regions such as Southeast Asia where thalassemia affects a large proportion of the population, increases in receptor concentration unrelated to iron deficiency are likely to occur. Some of these disorders may be distinguished by the use of the receptor to ferritin ratio. Nevertheless, more information is needed to define the role of receptor measurements in these complex field settings.

Conclusion

The serum transferrin receptor is a soluble form of intact receptor, produced by proteolytic cleavage, that can be detected by sensitive immunological methods. Its concentration provides a direct measure of total tissue receptor expression and consequently measures the size of the erythroid precursor mass and the severity of tissue iron deficiency. This important new laboratory tool represents a significant advance in the assessment of nutritional iron status and the evaluation of the anemic patient.

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