CONCISE REPORT

Serum Transferrin Receptor as a New Index of Erythropoiesis

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Serum transferrin receptors were measured by a sandwich radioimmunoassay procedure in patients with iron deficiency anemia, autoimmune hemolytic anemia and aplastic anemia. The mean circulating transferrin receptor concentration of normal subjects and patients with iron deficiency anemia, autoimmune hemolytic anemia and aplastic anemia are 253 ± 82 ng/mL, 730 ± 391 ng/mL, 1,426 ± 1,079 ng/mL, and 182 ± 39 ng/mL, respectively. The values for those with iron deficiency anemia and autoimmune hemolytic anemia were significantly higher than that of normal controls and the values for those with aplastic anemia were lower than that of normal controls. After iron supplementation in iron deficiency anemia, the serum transferrin receptor values increased twofold over those of pretreatment values. This increase parallels an increase in peripheral reticulocytes. Therefore, the number of circulating transferrin receptors in anemic patients may reflect the level of bone marrow erythropoiesis and is a potentially useful new index for red cell production.

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TRANSFERRIN receptor is a transmembrane glycoprotein that is expressed primarily on the surface of erythroid cells and delivers iron during proliferation and hemoglobin synthesis.¹² The receptor binds serum dfferent transferrin and is endocytosed and transferred to acidosomes, in which inorganic iron is released into the cytosol and the remaining ligand and receptor is recycled back to the cell surface.³ During this unique recycling process, transferrin receptor was found to be externalized as a form of multivesicular endosomes.⁴ This externalization of transferrin receptors from the cell surface was first reported by Pan and Johnstone⁵ using cultured sheep reticulocytes in vitro. The released receptors may potentially circulate in the serum. Recently, we established a quantitative method for measuring immunoreactive transferrin receptors by using two different monoclonal antibodies, OKT9 and B3/25⁶ and found that immunoreactive transferrin receptor was detected at concentrations between 150 and 250 ng/mL. However, the clinical and pathophysiological significance of this circulating transferrin receptor is still obscure. In the present report, we evaluate the usefulness of circulating transferrin receptor as an index of erythropoiesis by measuring its serum level in patients with iron deficiency anemia, autoimmune hemolytic anemia, and aplastic anemia.

MATERIALS AND METHODS

Serum samples. Venous blood samples were taken from 38 normal adult males (age 18 to 72), 54 normal adult females (age 15 to 78), 41 patients with iron deficiency anemia, five patients with autoimmune hemolytic anemia, and five patients with aplastic anemia. The samples were collected before any treatments were started. Serial blood sampling was performed in ten patients with iron deficiency anemia and five patients with autoimmune hemolytic anemia for consecutive determination of serum transferrin receptor values. All serum samples were stored at −20°C until use.

Sandwich radioimmunoassay. Quantification of immunoreactive serum transferrin receptors by a sandwich radioimmunoassay was performed as previously described.⁴ Two monoclonal antibodies against the transferrin receptor, OKT9 and B3/25 were purchased from Ortho Diagnostic (Raritan, NJ) and Hybritech Inc (San Diego), respectively. Flat-bottomed polystyrene plates (Dynatech Lab, Alexandria, VA) were inoculated with 50 μL of OKT9 (10 μg/mL) overnight at 4°C. They were then washed and incubated for one hour at room temperature with 100 μL of phosphate buffered saline containing 1% bovine serum albumin (BSA, Sigma Chemical Co, St Louis) to prevent nonspecific binding. To each well in the plates, 40 μL of purified standard receptor (4 to 2,000 ng/mL) or serum samples was added. The standard transferrin receptors were obtained from human term placenta, which we reported previously.³ The plates were then incubated another 18 hours at 4°C, washed, and overlayed with 40 μL of ¹²⁵I-labeled B3/25 (5 × 10⁶ cpm/μg/0.5 mL). Additional incubation for 12 hours at 4°C, the plates were washed and the radioactivity of each well was determined. All samples were performed in triplicate. Radioiodination of proteins was carried out by the lactoperoxidase and glucose oxidase method using Enzymobeads (Bio-Rad, Richmond, VA).³

Assay for reticulocyte counts, hemoglobin concentration, serum iron, total iron binding capacity, and serum ferritin. Peripheral reticulocyte counts in 1,000 RBCs were determined by the method of Pappenheim.⁶ Hemoglobin was measured by cyanomethemoglobin method.⁹ Serum iron and total iron binding capacity (TIBC) were determined by the method of International Committee for Standardization in Haematology.¹⁰¹¹ Serum ferritin was quantitated by SPAC ferritin kit (Daichi Radioisotope Lab, Tokyo).¹¹

Statistical analysis. Student’s t test was used to assess the statistical significance of the results shown in the designated tables.

RESULTS

Serum transferrin receptor levels in normal subjects and patients with various anemias are shown in Table 1. The mean values for healthy males and females were 251 ± 94 ng/mL (mean ± 1 SD) (n = 38) and 256 ± 99 ng/mL (n = 54), respectively. No significant differences because of sex or age were observed. The mean values for nontreated patients with iron deficiency anemia and autoimmune hemolytic anemia were 730 ± 391 ng/mL and 1,426 ± 1,079 ng/mL, respectively, both of which were significantly higher than

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those of normal subjects. On the other hand, the mean concentration for patients with aplastic anemia was 182 ± 39 ng/mL, which was significantly lower than that of normal subjects. During the iron supplementation in patients with iron deficiency anemia, the concentration increased up to 1,016 ± 326 ng/mL, while the absolute reticulocyte count increased from 2.42 ± 0.97 × 10⁴/μL to 7.30 ± 4.09 × 10⁴/μL. Therefore, it seemed that the increase of serum transferrin receptor correlated well with the increase in reticulocytes. After the hemoglobin values were normalized by successful treatment with iron supplementation, the mean value returned to the normal range (350 ± 147 ng/mL) in association with the decreased reticulocyte counts (5.16 ± 1.61 × 10⁴/μL). In patients with autoimmune hemolytic anemia, the significant decrease of serum transferrin receptor was observed after effective therapy of prednisolone. The absolute reticulocyte counts before and after steroid therapy were 36.1 ± 6.4 × 10⁴/μL and 15.7 ± 5.8 × 10⁴/μL, respectively, indicating that the decline of transferrin receptor in autoimmune hemolytic anemia paralleled the decrease of reticulocyte counts and the improvement of hemoglobin concentration.

Table 1 demonstrates the correlation between serum transferrin receptor values and other hematological parameters such as reticulocyte counts, hemoglobin concentration, TIBC, serum iron, and serum ferritin in patients with iron deficiency anemia and autoimmune hemolytic anemia. There was a good correlation between serum transferrin receptor and reticulocyte counts in both conditions, suggesting that the increased serum transferrin receptor values may reflect the increase of reticulocytes and bone marrow erythroid cells. In patients with iron deficiency anemia, significant positive or negative correlations were observed between serum transferrin receptor and other markers such as TIBC, serum iron, and serum ferritin concentration, whereas no other correlations were observed in autoimmune hemolytic anemia.

The serial change of serum transferrin receptor values in a patient with iron deficiency anemia supplemented with intravenous (IV) iron administration and in a patient with autoimmune hemolytic anemia treated with prednisolone is shown in Fig. 1. Just after the initiation of iron supplementation, a transient increase of serum transferrin receptors was obtained, and thereafter the values increased again in association with increased reticulocyte counts and returned to normal levels with the improvement of anemia (Fig 1A). In a patient with hemolytic anemia, the serum transferrin receptor level also seems to parallel reticulocyte counts (Fig 1B).

**DISCUSSION**

We previously reported that immunoreactive transferrin receptor could be detected in human serum by a sandwich radioimmunoassay technique, although no clinical significance was elucidated. In the present report, we measured the receptor concentration in various anemias and obtained the higher values in iron deficiency anemia and autoimmune

<table>
<thead>
<tr>
<th><strong>Parameters</strong></th>
<th><strong>Iron Deficiency Anemia</strong></th>
<th><strong>Autoimmune Hemolytic Anemia</strong></th>
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<tbody>
<tr>
<td></td>
<td>No. of Cases (n)</td>
<td>Correlation Coefficient (r)*</td>
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<td>Reticulocyte counts</td>
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<td>Hemoglobin concentration</td>
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<tr>
<td>Serum ferritin</td>
<td>10</td>
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*Correlation between serum transferrin receptor and various parameters.
†Statistically significant linear regression.
SERUM TRANSFERRIN RECEPTOR AND ERYTHROCYTES

Fig 1. Changes of serum transferrin receptor (TR), hemoglobin concentration (Hgb) and peripheral reticulocyte counts (Retic). (A) A patient with iron deficiency anemia during iron supplementation. (B) A patient with autoimmune hemolytic anemia during treatment with prednisolone. TR, Hgb, Retic.

hemolytic anemia and lower values in aplastic anemia (Table 1). These values paralleled the peripheral reticulocyte counts (Table 2), suggesting that the serum transferrin receptors may reflect the turnover of transferrin receptors in erythrocyte progenitor cells in the bone marrow.

Since Jandl and Katz first reported the presence of transferrin receptors on the reticulocyte surface, erythrocyte progenitor cells have been considered the main source of transferrin receptors in the body as well as syntrophoblasts of placenta and malignant tissues. The overall dynamics of transferrin receptors has been extensively studied using the erythroleukemia cell line, K562, and the expression of transferrin receptors is thought to be regulated by the equilibrium between its recycling and synthesis. Receptor mRNA synthesis is increased with the addition of iron deficient serum and iron chelating agents in vitro. Therefore, it is likely that the reticulocytes in iron deficiency anemia may possess more receptors than usual.

Good correlations between serum transferrin receptor value and markers such as TIBC, serum iron, and serum ferritin were present only in iron deficiency anemia but not in autoimmune hemolytic anemia (Table 2). This result may explain that there is a double effect in iron deficiency anemia, ie, iron deficiency and erythropoiesis. Actually, in autoimmune hemolytic anemia, which could exclude the effect of iron deficiency, there is no correlation with TIBC, serum iron or serum ferritin.

According to Pan and Johnstone, the receptors of reticulocytes were shed and released into the medium. We also found the same phenomenon in K562 cells. The shedding of transferrin receptor was inhibited by metabolic inhibitors such as cytochalasin B, colchicine, and sodium azide. The presence of ligand, on the other hand, increased this externalization. These results suggest that this externalization of receptor is a ligand-dependent and energy-dependent process. Therefore the measurement of shed circulating receptors in the serum seems to be a practical and efficient way for estimating erythrocyte transferrin receptor turnover. The lower levels in aplastic anemia reflect decreased erythropoiesis in the bone marrow. Interestingly, the biphasic increase of serum transferrin receptor levels was obtained in the clinical course of iron deficiency anemia during which IV iron was supplemented (Fig 1A). The transient increase was observed just after iron supplementation and the second increase was observed two to three days before the increase in reticulocytes. The first increase, just after iron supplementation, may be explained by the transient increase of receptor shedding according to the increase of serum diferric transferrin, which facilitates the internalization and recycling-shedding process of the receptors. On the other hand, the second increase paralleled the reticulocyte increase may reflect the increase of erythron, namely the accelerated division and proliferation of erythroid precursor cells by incorporation of iron into the cells. It is noteworthy that
serum transferrin receptor appeared two to three days before the increase in reticulocytes. In patients with autoimmune hemolytic anemia, the serum transferrin receptor level also seems to parallel reticulocyte counts (Fig 1B) and the serial determination was useful for monitoring the activity of the bone marrow erythroid mass. Therefore, the measurement of serum transferrin receptor is a simple and rapid method for estimation of erythropoiesis, formerly estimated by reticulocyte counts or complicated ferrokinetic measurements such as plasma iron turnover (PIT) and percent red cell use (%RCU). In conclusion, the measurement of circulating transferrin receptor in patients with various anemias may be a useful new index for estimating the bone marrow erythropoiesis.

REFERENCES

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Y Kohgo, Y Niitsu, H Kondo, J Kato, N Tsushima, K Sasaki, M Hirayama, T Numata, T Nishisato and I Urushizaki