Similarities between the Transferrin Receptor Proteins on Human Reticulocytes and Human Placenta*

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The transferrin receptor of the human reticulocyte was isolated by two different immunofluorescence procedures. These included indirect immunoprecipitation with a transferrin/anti-transferrin complex and direct immunoprecipitation with antisera to purified transferrin receptor from placenta. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the receptor isolated from reticulocytes reveals a polypeptide at Mr = 94,000 identical in molecular weight with that of the placenta. A radioimmunoassay using purified 125I-labeled transferrin receptor from placenta and antiserum to transferrin receptor fails to distinguish any immunological differences between the reticulocyte and placental forms of the protein. In addition, proteolytic digests of both of these polypeptides with Staphylococcus aureus protease show identical proteolytic patterns, indicating similar sequences.

Binding of transferrin to a membrane-bound transferrin receptor is the initial step in the mechanism of cellular incorporation of iron. This step has been studied extensively in reticulocytes and trophoblast cells, as the transferrin receptor protein has been identified, isolated, and characterized in both trophoblast cells (1-7) and in reticulocytes (8-23). The receptor has also been identified in growing cells (24-30). Transferrin appears to be necessary for the growth of cells in culture (24-30). Dividing cells have a continuing requirement for incorporation of iron into newly synthesized mitochondrial and cytoplasmic proteins. With the exception of the placenta and reticulocyte, nonproliferating tissues appear to have 10-20 fold less transferrin binding (31, 32). This has led to the postulate that the receptor is a good marker of proliferating cells, including neoplastic cells (32, 35, 36).

Previous studies in this laboratory have identified and characterized the properties of the cell surface transferrin receptor from the placenta (1) and from cells in culture (28). A subunit Mr = 94,000 has been reported for the receptor from human trophoblast and human cells in culture (28, 29). Studies of the receptor from human placenta (49) and from a human trophoblast cell culture have shown it is a dimer (7).

In this report, we have investigated whether the receptors in the trophoblast, a differentiated embryonic cell, and in the reticulocyte, a terminally differentiated adult cell, are the same protein. The transferrin receptor from the human reticulocyte is compared with respect to its molecular weight, immunogenicity, and polypeptide structure to the corresponding protein isolated from the placenta.

MATERIALS AND METHODS

Preparation of Placental Membranes—Fresh placenta from uncomplicated term pregnancies were obtained within 30 min of delivery, processed immediately to prepare trophoblast membranes following the method of Schiller et al. (37) and stored frozen at -20°C. Prior to use, they were iodinated with lactoperoxidase (Sigma) and solubilized with Triton X-100 as described previously (1).

Preparation and Iodination of Red Blood Cell Membranes—Blood samples were obtained from a normal subject (one of the authors) to provide the erythrocyte membrane preparation and from control and anemic patients. To provide the reticulocyte-rich membrane preparations. The normal subject was determined to have a reticulocyte count of less than 0.5% by methylene blue staining (38) and the anemic patients ranged from 4-60 reticulocyte counts. Membranes were prepared by the method of Van Vunakis et al. (39). They were resuspended in phosphate-buffered saline, 125I-labeled, and solubilized in an identical manner with that of the trophoblastic membranes. Protein concentrations in the presence of Triton X-100 were determined by the method of Warburg and Smith (40) using bovine serum albumin (Sigma) as a standard.

SDS-Polyacrylamide Gel Electrophoresis—Preparation of samples for electrophoresis and visualization has been described previously (1). Molecular weight standards included Escherichia coli β-galactosidase (Sigma) Mr = 125,000, human transferrin (Sigma) Mr = 81,000, rabbit muscle phosphorylase B (Boehringer Mannheim) Mr = 94,000 and chicken ovalbumin (Sigma) Mr = 43,000.

Indirect Immunoprecipitation of Solubilized Membranes with a Preformed Transferrin/Transferrin Complex—Human transferrin (0.2 mg) was incubated with sufficient goat anti-human transferrin serum to form an immunoprecipitate, allowed to incubate 16 h at 4°C, centrifuged 1 min (11,000 g) at 22°C, and washed with and suspended in phosphate-buffered saline containing 1 mg/ml ovalbumin. The preformed immunoprecipitate was then added to the solubilized membranes (1 ml), the pH was adjusted to 5 with 1 M NaOH, incubation overnight at 4°C, the samples were washed four times with 50 mM sodium phosphate, 150 mM NaCl, pH 5.0, 1% Triton X-100, and stored frozen at -20°C. Prior to use, the precipitate was accomplished by incubation with 0.1 M Tris-HCl, pH 8.0, 0.1% Triton X-100.

Direct Immunoprecipitation of Solubilized Membranes with Transferrin Receptor Antibody and Staphylococcus aureus—Antibodies to the transferrin receptor were generated in a goat using purified transferrin receptor isolated from human placenta by the method of Seligman et al. (44). Anti-transferrin receptor activity was absorbed out of an IgG preparation of the antiserum by using Sepharose-linked transferrin. This antiserum has been characterized previously (41). Solubilized samples of 125I-labeled membranes were preabsorbed 1 h at 4°C with normal goat serum-coated formaldehyde-fixed S. aureus. After centrifugation, the supernatant was immunoprecipitated with anti-transferrin receptor antibody and S. aureus. Following incubation overnight at 4°C, the samples were washed four times with phosphate-buffered saline, 1 mg/ml ovalbumin, 0.1% Triton X-100, and counted.

The abbreviations used are: SDS, sodium dodecyl sulfate; RIA, radioimmunoassay.

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1. Previous studies in this laboratory have identified the molecular mass of the placental peptide as being 90,000 daltons. The differences in reported molecular weights stem from revised estimates of the molecular weight markers used. In the past, the molecular weight of human transferrin was assumed to be 76,000 and that of rabbit muscle phosphorylase B 90,000. They are revised in this paper to 81,000 and 94,000, respectively. The former is based on a publication of the amino acid sequence and carbohydrate content of transferrin (44) and the latter on more commonly accepted values for phosphorylase B.

The abbreviations used are: SDS, sodium dodecyl sulfate; RIA, radioimmunoassay.
RESULTS

Identification of a Transferrin Receptor in Human Reticulocytes—The results of the indirect immunoprecipitation of the transferrin receptor from a solubilized membrane preparation using the preformed transferrin/anti-transferrin antibody complex are shown in Fig. 1. It is the same method used previously to identify transferrin binding proteins. In these experiments, the membranes were prepared, iodinated with \(^{125}\)I, and solubilized with Triton X-100. This solubilized homogenate was then incubated with a preformed immunoprecipitate of human transferrin and anti-human transferrin serum. The transferrin in the complex bound to the transferrin receptor and the other solubilized components washed away. This immune complex was then extracted at pH 8 with 0.1 M Tris-HCl, 1% Triton X-100 and was reduced, denatured, and subjected to SDS-polyacrylamide gel electrophoresis. An autoradiogram of the gel (Fig. 1A) shows a single 94,000-dalton peptide that is indistinguishable in electrophoretic mobility from the purified placental transferrin binding protein. The receptor can be visualized directly with Coomassie blue (Fig. 1B). Also visible in Fig. 1A are transferrin (M, = 81,000) and IgG (M, = 55,000 and 23,000) of the immune complex. No transferrin binding can be detected on \(^{125}\)I-labeled membranes from blood containing less than 0.5% reticulocytes using this method.

Immunochemical Similarities between the Transferrin Receptor from Reticulocytes and from Placentae—An RIA of the transferrin receptor in reticulocyte-rich solubilized red cell membrane preparations gives a binding curve similar to that obtained with purified transferrin receptor from placenta, indicating antigenic homology with the placental receptor (Fig. 2).

Direct immunoprecipitations of placental and reticulocyte-rich membranes with antiserum to the receptor also show polypeptides at 94,000 daltons corresponding to the band of the purified receptor standard (Fig. 3). An additional less intense band at 81,000 daltons is also observed in both preparations. This is endogenous transferrin that is iodinated and co-precipitated with the antibody (1). Since the antiserum to the receptor has been absorbed against transferrin and shows no anti-transferrin activity (41), the co-precipitation with the receptor most likely results from its being bound to the receptor. This iodinated transferrin band is not seen in the indirect immunoprecipitation procedure because the \(^{125}\)I-labeled transferrin presumably exchanges with the large excess of unlabeled transferrin used in the preformed immunoprecipitate.

Mature erythrocytes no longer bind transferrin (45). They also do not have any detectable levels of transferrin receptor by RIA (41) or by immunoprecipitation (Fig. 3, Lane 3). Both the erythrocyte and the reticulocyte preparations show an additional faint band of lower molecular weight (43,000–54,000). This peptide is not apparent when the preformed transferrin/anti-transferrin complex is used for immunoprecipitation. The protein either does not bind transferrin as avidly or is released with pH 5 washing.

Proteolytic Digestion of the Receptor Proteins—In order to determine whether the transferrin binding proteins isolated from reticulocytes and placentae have the same molecular structure, we subjected them to limited proteolysis and examined the peptide fragmentation patterns according to the
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The sample then was loaded onto the SDS-8% polyacrylamide gel. The single 94,000-dalton peptide was cut out of the polyacrylamide gel and reloaded onto another gel where it was digested and the peptides separated by electrophoresis. Fig. 4 indicates that the 94,000-dalton proteolytic peptide patterns of the receptor from placenta and reticulocytes are indistinguishable. In contrast, the peptide pattern of 125I-labeled transferrin is different.

DISCUSSION

Transferrin receptors have become of major interest recently, as they have been identified as being markers of cell proliferation (32, 46–48). In addition, several mouse monoclonal antibodies generated against T-cell-type human leukemic cells (antibody OKT9) (48) and K562, a human myeloid/erythroid cell line (antibody B3/25) (47) have been identified as being against the transferrin receptor. Thus, it is of particular interest to see if the transferrin receptor is the same for all human tissues or differs depending on the physiological purpose of iron in each particular cell.

The results of this investigation show that the transferrin receptor of the human reticulocyte and of the trophoblast of the human placenta are similar with regard to several structural parameters.

We have utilized a xenogenic antiserum developed in a goat directed against a purified transferrin receptor from placenta. Both this antiserum and the transferrin-anti-transferrin immune complex bind a 94,000-dalton protein from solubilized placenta and reticulocytes. The antiserum against the transferrin receptor has allowed us to specifically assess the immunological similarity or differences of a transferrin receptor by RIA from the two diverse tissues. Although the functions of the placenta and reticulocytes are widely divergent, they both have high iron requirements, the former for transport of iron through the syncytiotrophoblast to the fetal circulation and the latter for accumulation within the cell and incorporation into hemoglobin. The initial step, i.e., the binding of transferrin to the cell surface, appears to be accomplished by the same protein. Immunologically, they appear to be identical. The antiserum generated against the purified receptor is capable of precipitating a peptide from solubilized red blood cell membranes with an identical subunit molecular weight to the placental subunit of the receptor. The curves obtained for each in the RIA are superimposable, indicating antigenic homology. In addition, peptide maps generated by a series of protease digestions of purified 125I-labeled material show indistinguishable patterns. Since iodination took place before the membranes were solubilized, this method of analysis only labels surface tyrosines in each membrane preparation. Together with the immunoprecipitation and RIA results the data suggest that protein sequence and glycosylation pattern of the 94,000-dalton subunits are the same in these tissues.

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