Characterization of Transferrin Receptor Released by K562 Erythroleukemia Cells

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Abstract. **A soluble form of transferrin receptor has been detected in human serum and has been shown recently to be a truncated form of the intact membrane bound receptor. Mechanisms governing the release of transferrin receptor by cells are poorly understood and could be better defined by tissue culture. The present investigation was undertaken to characterize the transferrin receptor released by K562 erythroleukemic cells. In contrast with maturing sheep reticulocytes, which have been shown to release transferrin receptor in small vesicles termed exosomes, we demonstrated, with a monoclonal enzyme-linked immunoassay, that less than 30% of the transferrin receptor released by K562 cells in log phase growth was in a particulate form. The relative amounts of soluble and particulate receptor released to the supernatant did not change significantly during 48 hr of incubation. Soluble receptor was purified by immunoaffinity chromatography. On polyacrylamide gel electrophoresis, its mobility was the same (85 kDa) as that of the truncated monomeric form recently identified in human serum. Further evidence that serum and soluble receptors released by K562 cells are identical was provided by amino acid sequence analysis, which demonstrated that 16 of the first 19 residues of the N-terminal sequence of soluble K562 receptor are homologous with the serum receptor. The remaining three were not identifiable. K562 cells provide a useful *in vitro* model for studying the production of membrane-bound and soluble forms of released transferrin receptor.** [P.S.E.B.M. 1991, Vol 197]

The transferrin receptor is a transmembrane protein which plays a fundamental role in the acquisition of iron by all body tissues (1, 2). Clinical interest in this receptor has increased after evidence was found that a soluble form of the protein can invariably be detected in human serum using sensitive immunoassay techniques (3–7). The concentration of serum receptor increases sharply when erythropoiesis is enhanced or when tissue iron supply is curtailed. Studies in both animals and humans suggest that the concentration of circulating receptor reflects the total body mass of this protein (7, 8).

The serum receptor has recently been identified as a truncated form of the receptor monomer (9). *In vitro* studies of sheep erythrocytes have shown that the transferrin receptor is released in small vesicles (10–14), but the relationship of these particles to serum receptor is unclear. Because of the potential advantage of an *in vitro* model for studying the release of transferrin receptor, the present investigation was undertaken to characterize immunoreactive transferrin receptor released by K562 cells. We observed that only a relatively small proportion of the released receptor is in particulate form and that the soluble receptor produced by K562 cells is a truncated form of intact receptor identical to that in human serum.

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Methods and Materials

Tissue Culture. K562 erythroleukemic cells were maintained in log phase growth in RPMI 1640 medium enriched with glutamine, pyruvate, and 10% fetal calf serum (Hazelton Research Products, Denver, CO). A tissue culture antibiotic (penicillin and streptomycin) and an antifungal (amphotericin) preparation (Gibco, Grand Island, NY) was added to the medium. In studies assessing the distribution of transferrin receptor in various cellular and supernatant fractions, 5-ml aliquots

were removed from culture flasks 24 hr into the culture period, during which log phase growth was maintained in a 5% CO_2 environment at 37°C. Viability of the cultures as assessed by trypan blue exclusion invariably exceeded 97.5%.

Following 24 hr of incubation, the 5-ml tissue culture aliquots were mixed gently for 2 min to obtain an even cell suspension, and 40 μl were removed for measurement of cell number using an electronic particle counter (ZBI Coulter Electronics, Hialeah, FL). Each tube was then centrifuged twice for 10 min at 8,000*g* and the supernatant was decanted. The remaining pellet was suspended in 1 ml of Hanks' buffered salt solution containing 0.001 *M* Tris (HBSS-Tris) and 1% teric (polyoxyethylene 9 lauryl ether; Sigma Chemical Co., St. Louis, MO) (pH 7.4) at 4°C. After thorough mixing overnight at 4°C, the solubilized pellets were twice sonicated with 30 sec of ultrasound (Vibracell Sonics and Materials, Inc., Danbury, CT) and centrifuged at 4°C at 15,000*g* for 15 min. The decanted supernatant was stored at −20°C.

A portion of the supernatant recovered after 24-hr incubation was diluted 1/1 with HBSS-Tris and stored (S_1). Another aliquot of the original supernatant was diluted 1/1 with HBSS-Tris and centrifuged at 100,000*g* for 90 min. The supernatant was recovered for later assay (S_2) and the putative pellet was resuspended in HBSS-Tris containing 1% teric, agitated overnight, sonicated, centrifuged at 15,000*g* for 15 min, and saved for assay. A final portion of the original supernatant was diluted 1/1 with HBSS-Tris containing 1% teric, agitated overnight, and sonicated. The supernatant, obtained by centrifugation at 15,000*g* for 15 min, was stored at −20°C (S_3). All samples for immunoassay were stored at −20°C until the day of assay. All assay values were calculated back to the original 5-ml aliquot.

To determine the change in these released receptor fractions over time, 15 ml of cells in log phase culture were centrifuged and the cell pellet was suspended in 50 ml of fresh complete medium. After 4 hr, 8 hr, 24 hr, and 48 hr in culture, 5-ml aliquots were removed and processed as outlined above.

Isolation of Soluble Transferrin Receptor. A 50-ml cell suspension was incubated in tissue flasks in 5% CO_2 at 37°C. Every 48 hr, 35 ml were removed and an equivalent volume of medium was added. Each volume of harvested suspension was centrifuged immediately at 4°C for 15 min at 1,000*g*. The supernatants were pooled in a storage container containing 0.5 m*M* phenylmethylsulfonyl fluoride and stored at −20°C. The pooled supernatants were thawed and concentrated 20 times in an Amicon pressure concentrator using an exclusion membrane of molecular mass cut-off of 30 kDa. The concentrated supernatant was diluted with an equal volume of equilibrium buffer containing phosphate-buffered saline (PBS) (pH 7.4), 0.5 m*M* diisopropyl fluorophosphate, and 0.02% sodium azide, and then centrifuged at 10,000*g* for 20 min at 4°C using a Beckman J21C centrifuge.

Soluble receptor from the supernatant of K562 cells was purified by affinity chromatography using monoclonal antibodies D3A12 and A4A6 developed for the measurement of soluble transferrin receptor in human serum (6). The monoclonal antibodies were coupled to cyanogen bromide activated Sepharose 4B according to the manufacturer's instructions. The supernatant was mixed with the derivitized Sepharose 4B resin at an approximate antibody to transferrin receptor ratio of 1.5. The mixture was placed on an end-over-end rotator overnight at 4°C and then centrifuged at 1500*g* for 30 min. The pellet was resuspended in equilibrium buffer, poured into a column, and washed with the same buffer until A_{280} approached zero. The receptor was eluted by incubating the affinity resin with 0.5 ml of 0.2 *M* diethanolamine (pH 11) for 10 min prior to elution (9). The eluent was collected into 0.125 ml of 0.5 *M* Tris (pH 7.5).

Table I. Transferrin Receptor Content of the Various Fractions Isolated from K562 Cultures by Centrifugation

Culture	Cell receptor content (pg/cell)	Supernatant				
		Untreated (S_1) (pg/cell)	Soluble (S_2)[a] (pg/cell)	Total (S_3)[b] (pg/cell)	Soluble (S_2) / Total (S_3)	Pellet[c] (pg/cell)
1	0.350	0.012	0.011	0.018	0.61	0.003 (15)
2	0.386	0.015	0.014	0.022	0.62	0.004 (18)
3	0.363	0.013	0.011	0.017	0.67	0.003 (20)
4	0.372	0.018	0.015	0.027	0.68	0.005 (22)
5	0.376	0.017	0.017	0.022	0.78	0.004 (19)
6	0.374	0.018	0.019	0.023	0.84	0.004 (17)
Mean ± 2SE	0.370 ± 0.011	0.015 ± 0.002	0.015 ± 0.002	0.021 ± 0.002	0.70 ± 0.07	0.004 (18) ± 0.001 (2)

[a] Concentration after centrifugation at 100,000*g*.
[b] Measured after addition of 1% teric.
[c] Values obtained after adding 1% teric to the pellet after centrifugation at 100,000*g*. Values in parentheses are expressed as percentage of the total.

Table II. Time-Dependent Changes in the Transferrin Receptor Content of the Various Fractions Isolated from K562 Cultures by Centrifugation and Solubilization

Culture duration (hr)	Cell number ($\times 10^6$)	Cell receptor content (pg/cell)	Supernatant				
			Untreated (S_1) (pg/cell)	Soluble (S_2)[a] (pg/cell)	Total (S_3)[b] (pg/cell)	Soluble (S_2) / Total (S_3)	Pellet[c] (pg/cell)
4	1.792 ± 0.076[d]	0.273 ± 0.043	0.005 ± 0.001	0.004 ± 0.001	0.005 ± 0.001	0.79 ± 0.25	—
8	1.942 ± 0.139	0.353 ± 0.055	0.007 ± 0.002	0.006 ± 0.002	0.009 ± 0.003	0.67 ± 0.10	0.002 ± 0.001 (30)
24	3.504 ± 0.126	0.457 ± 0.038	0.011 ± 0.001	0.009 ± 0.002	0.014 ± 0.002	0.66 ± 0.07	0.004 ± 0.002 (25)
48	7.776 ± 0.390	0.420 ± 0.040	0.015 ± 0.002	0.014 ± 0.003	0.021 ± 0.003	0.68 ± 0.11	0.003 ± 0.001 (16)

[a] Concentration after centrifugation at 100,000*g*.
[b] Measured after addition of 1% teric.
[c] Values obtained after adding 1% teric to the pellet obtained by centrifugation at 100,000*g*. Values in parentheses are expressed as percentage of the total.
[d] Mean ± 2SE values are indicated.

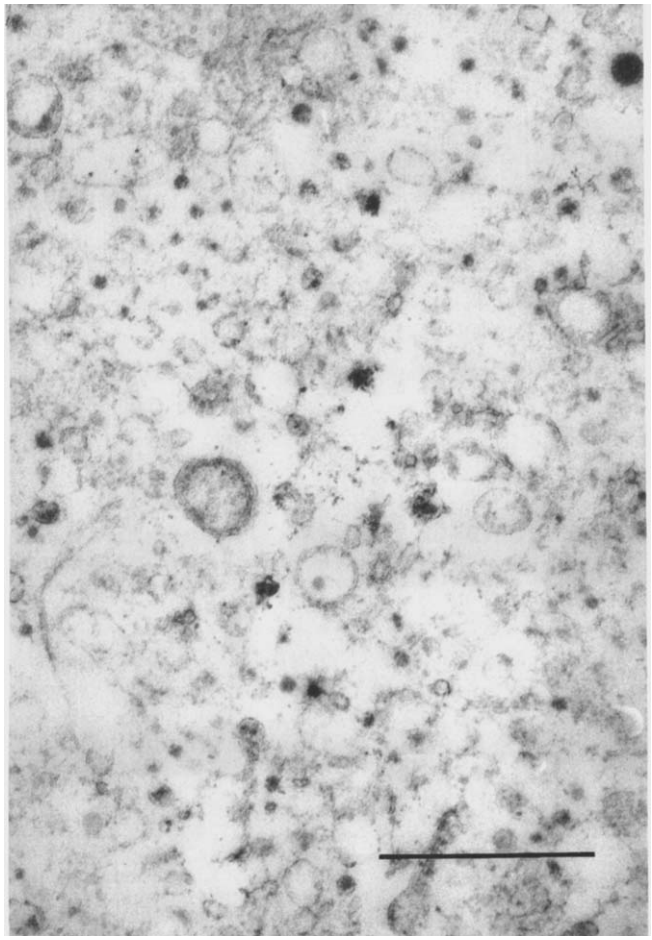

Figure 1. Electron micrograph (×38,000) of the pellet obtained by sectioning of the material pelleted after centrifugation of culture supernatant at 100,000*g* for 90 min. The dimension bar indicates 1 μm.

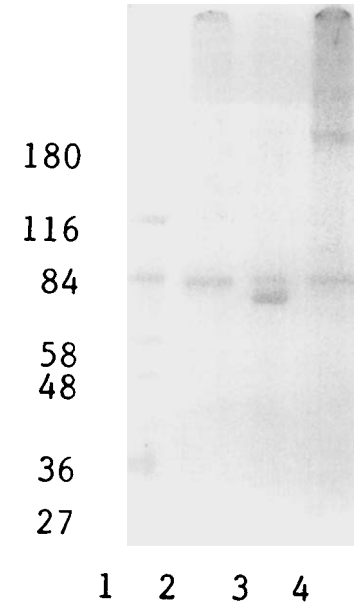

Figure 2. Comparison of immunoaffinity purified material from K562 culture supernatants (Lane 2) with similarly purified serum proteins (Lane 3) and material derived from solubilized K562 cells (Lane 4). All preparations were electrophoresed on SDS-PAGE under nonreducing conditions and the gel was stained with Coomassie brilliant blue. Molecular weight standards were run in Lane 1. The single peptide identified in Lane 2 (mol wt of 85 kDa) is similar to that in serum that has been identified previously as the soluble form of transferrin receptor (9). The smaller 75-kDa peptide in serum has been identified previously as transferrin, and the larger 190-kDa protein in cellular extracts has been identified as intact receptor dimer.

Isolation of Membrane Bound Transferrin Receptor. To isolate the cellular form of the receptor, the pellet remaining after the initial cell suspension centrifugation was solubilized by adding HBSS-Tris containing 1% teric (pH 7.4) and rotating continuously for 14 hr at 4°C. The material was then processed as described for the supernatant.

Biochemical Procedures. Samples were analyzed on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) by loading onto 4–20% gradient minigels. Electrophoresis was performed by the method of Laemmli (15) at 150 V for 1.5 hr. The gels were fixed in 40% methanol-10% acetic acid for 1 hr and

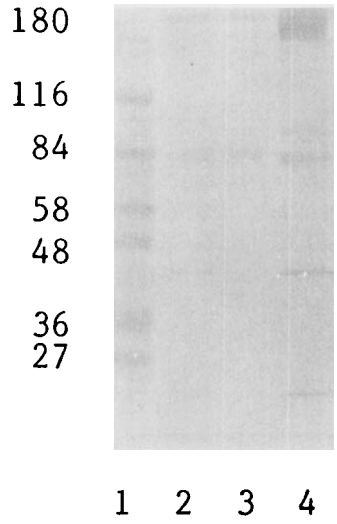

Figure 3. Confirmation of the identity of the peptides outlined in Figure 2 by immunological staining of material transblotted from SDS-PAGE. The lane order is as in Figure 2, with the protein present in supernatant (Lane 2) at 85 kDa being similar to the serum receptor (Lane 3). Intact dimer (190 kDa), monomer (95 kDa), and truncated monomer (85 kDa) are noted in the solubilized cellular preparation (Lane 4).

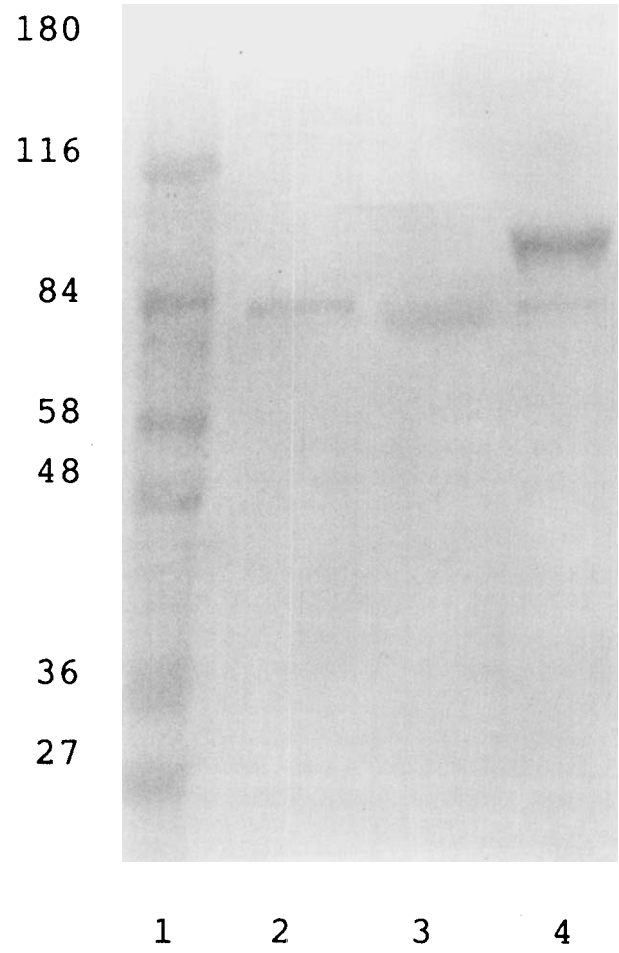

Figure 4. Immunological characterization of immunoaffinity-purified peptides from K562 supernatants (Lane 2), serum (Lane 3), and cellular extracts of K562 cells (Lane 4) after transblotting from SDS-PAGE after materials were prepared under reducing conditions. The 85-kDa peptide present in all three represents truncated monomer. The dominant 95-kDa peptide in the cellular material is the intact monomer.

stained with 0.2% Coomassie brilliant blue R-250 in 40% methanol-10% acetic acid for 1 hr and destained overnight.

To obtain Western blots, transblotting was performed immediately after electrophoresis at 250 mA for 1.5 hr by the method of Burnette (16). The nitrocellulose membrane was blocked by incubating in PBS containing 1% nonfat dry milk for 1 hr, and then incubating for 2 hr with monoclonal antitransferrin receptor antibody diluted in PBS containing 0.1% nonfat dry milk. The blot was thoroughly rinsed with PBS and incubated for an additional 2 hr with polyclonal goat anti-mouse IgG antibody conjugated with horseradish peroxidase diluted in PBS containing 0.1% nonfat dry milk. After more extensive washing, the antibody antigen complexes were detected employing 4-chloro-naphthol as the substrate for the peroxidase. Detection of glycoprotein was as described previously (9).

Amino acid sequence analysis was performed on protein obtained by electrophoresing in a 7.5–20% polyacrylamide gradient and transblotting onto polyvinylidine difluoride membrane, according to the procedure of Matsudaira (17). The desired band was sliced from the membrane and sequenced at the University of California sequencing facility, Department of Biological Chemistry, School of Medicine, University of California at Los Angeles.

Immunoassay. The concentrations of supernatant and solubilized cell receptor were measured with an enzyme-linked immunosorbent assay (ELISA). This assay was designed for the measurement of transferrin receptor in human serum and employs monoclonal antibodies developed against transferrin-saturated receptor isolated from human placenta (6). The assay protocol was the same as that used for serum measurements, except that 200 μl of supernatant was assayed directly and the solubilized cell pellet was diluted 1/20 directly in the well. The assay was standardized against purified transferrin-receptor complex that contained 54% receptor protein as determined by protein analysis and assay of unbound placenta receptor. Recovery studies in which transferrin receptor standard was added to the incubation medium gave a mean recovery of 107%.

Electron Microscopy. This was performed on sections of pellets obtained by ultracentrifugation. These were embedded in plastic after initial fixation with glutaraldehyde. Electron microscopy was performed with a Jeol 100S transmission electron microscope (Japanese Electron Optic Ltd., Tokyo, Japan).

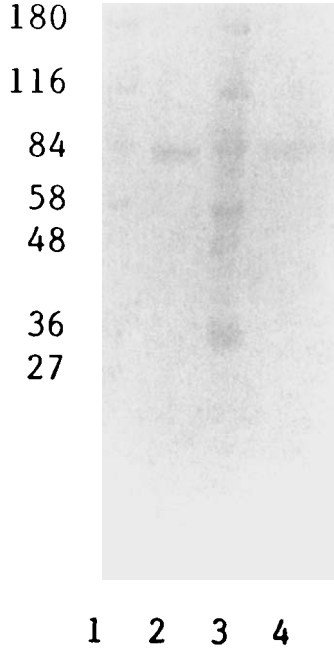

Figure 5. Glycoprotein detection using concanavalin A and horseradish peroxidase. Immunoaffinity-purified peptides from K562 supernatants (Lane 2) and serum (Lane 4) were transblotted onto a nitrocellulose membrane after SDS-PAGE. The peptides were prepared under reducing conditions. The membrane was sequentially incubated with concanavalin A, peroxidase, and 4-chloro-1 naphthol (9). Lanes 1 and 3 were molecular weight standards.

Results

ELISA measurements of the solubilized K562 cells and culture media obtained after 24-hr incubation in log phase growth demonstrated that relatively large amounts of transferrin receptor were released to the supernatant. The average content of cellular receptor in aliquots drawn from six separate incubations was 1,623 ng/aliquot, or 0.37 ± 0.01 (± 2SE) pg/cell (Table I). Direct assay of the cell-free supernatant (S_1) gave average receptor values of 66.3 ng/aliquot, or 0.016 ± 0.002 pg/cell. Thus, 4.2% of the cellular receptor was directly assayable in the culture supernatant. Following ultracentrifugation at 100,000*g* for 90 min, the supernatant (S_2) values fell to 62.8 ± 4.3 ng/aliquot, or 0.015 ± 0.002 pg/cell, the latter reflecting the concentration of soluble receptor. The total supernatant receptor, measured after the addition of 1% teric to the supernatant (S_3), averaged 90.1 ± 4.5 ng/aliquot, or 0.021 ± 0.002 pg/cell. Thus, an average of 70% of the released receptor ($S_2 \times 100/S_3$) was in a soluble form. These results also indicate that only a small amount of the particulate receptor, 3.5 (S_1-S_2) of 27.3 (S_3-S_2) ng/aliquot or 12.8%, was detected by direct assay of the untreated supernatant. Slightly lower estimates of particulate receptor were obtained by solubilizing the putative pellet obtained by ultracentrifugation. With this approach, an average of 16.6 ng/aliquot, or 18%, of the total supernatant receptor was recovered.

Studies were next performed to determine whether the proportion of soluble and particulate receptor released to the supernatant varied with time. These cultures were initiated with pelleted cells rather than log phase cell suspensions. A representative experiment is presented in Table II. Based on supernatant assays, the proportion of soluble receptor remained highly constant at 8, 24, and 48 hr, varying between 66% and 68%.

When the pellet obtained by ultracentrifugation of the supernatant was examined by electron microscopy, vesicular structures were seen (Fig. 1). However, the vesicles were heterogeneous in size and only a relatively small proportion were in the 50-nm diameter range reported for the exosomes released by maturing sheep reticulocytes (12).

The protein isolated from the K562 supernatant by affinity chromatography closely resembled the receptor isolated from human serum (Fig. 2). Receptor extracted from solubilized K562 cells displayed a major protein component at 190 kDa consistent with an intact receptor dimer. Serum receptor showed two proteins at 85 kDa and 75 kDa. The latter was identified as transferrin by immunoblotting with specific antisera (9). The K562 supernatant, containing minimal amounts of transferrin, consisted mainly of the 85-kDa protein. The mobility of this protein was the same as that of the protein isolated from human serum.

The components seen on electrophoresis were further characterized by immunostaining of Western blots using monoclonal antibodies against human transferrin receptor (Fig. 3). Immunoreactive proteins were observed with cellular receptor at 190 kDa, 95 kDa, and 85 kDa. The latter two components represent the monomeric forms of intact and truncated receptor, respectively. The major immunoreactive proteins observed in

Table III. N-Terminal Amino Acid Sequence of the K562 Supernatant Receptor and Serum Receptor Compared with Intact Transferrin Receptor Sequence of Residues 95–125[a]

		#	#
Supernatant		L-A-G-K-E-S-P-V-V-E-E-P-X-E-D-F-P-A-A-X-R-L-T-★★★
Serum		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-R-R-L-T-W-D-★★★	

[a] X indicates nonidentified residues. # indicates residues discrepant with the sequence deduced for intact receptor.

the supernatant of K562 cells and human serum both occurred at 85 kDa. When Western blots were performed on material electrophoresed on SDS-PAGE under reducing conditions (Fig. 4), the major immunoreactive protein from K562 cells corresponded to a molecular mass of 95 kDa and only a minor protein band was observed at 85 kDa. Again, the locations of the 85-kDa immunoreactive components in the supernatant of K562 cells and human serum were identical. The supernatant soluble receptor was documented to be a glycoprotein similar to the serum form of receptor (Fig. 5).

Amino acid sequence data provided further evidence that receptors isolated from human serum and from the supernatant of K562 cells were virtually identical. The first 23 amino acids of the K562 supernatant receptor and the first 19 amino acids from the serum receptor (9) are compared in Table III with the sequence of the intact transferrin receptor from residues 95–125. The latter were deduced from cDNA data of the human transferrin receptor gene (18). These results indicate that both serum and the supernatant from K562 cells contained a smaller fragment of the intact receptor, with the truncation occurring between arginine (position 100) and leucine (position 101).

Discussion

The first clear demonstration of the phenomenon of transferrin receptor release was obtained by studying the maturation of sheep reticulocytes *in vitro* (10, 11). When labeled antireceptor antibody was added to the immature reticulocytes, the radioactivity bound initially to the cells, but it was then progressively released to the supernatant over the following 24 hr. The released receptor was contained in small extracellular vesicles termed exosomes (12). Using electron microscopic studies, these 50-nm diameter particles have been shown to arise by budding at the internal surface of the endosome and are subsequently released with the transferrin binding site facing outward. These vesicular structures are, therefore, produced by a double membrane inversion, the first during initial endocytosis and the second during intravesicular budding phase. Exosomes have been detected in the circulation of anemic sheep and other animal species (14), but were seen under conditions of basal erythropoiesis only in sheep and then only in markedly reduced numbers. When exosomes were isolated with magnetic core beads coated with antibodies, they were found to contain several other plasma membrane activities (13, 14). It was inferred that the formation of exosomes represents a common mechanism to release specific plasma membrane proteins.

These early observations prompted a group of Japanese workers to search for the presence of transferrin receptor in human serum (3). Using an ELISA developed with monoclonal antibodies reacting with cell-surface human transferrin receptor, immunoreactivity could invariably be detected in human serum, even in patients with no red cell production. These initial observations have been confirmed by several groups, and the receptor has also been detected in rat serum (4–8). The serum transferrin receptor has recently been isolated by monoclonal antibody immunoaffinity and its chemical identity has been defined (9). It is, in fact, a truncated form of the intact membrane-bound receptor, with the truncation occurring between position 100 (arginine) and 101 (leucine). On SDS-PAGE, it has an apparent molecular mass of 85 kDa, as compared with the molecular mass of 95 kDa for the intact receptor monomer. The truncated receptor appears to be normally glycosylated.

The soluble protein detected by immunoassay in human serum is apparently distinct from the phenomenon of exosome production for several reasons. In unpublished studies of fresh human serum and plasma, we found minimal increase in assay values following solubilization with detergent, minimal reduction in receptor values following ultracentrifugation, and minimal measurable receptor in solubilized pellets obtained by centrifuging serum at 100,000*g* for 90 min. We have also been unable to detect vesicles by electron micrograph examination of pellets obtained by ultracentrifugation of fresh human serum or plasma. Solubilized receptor from exosomes produced by sheep erythrocytes has a molecular mass of 93 kDa, consistent with a monomer of intact receptor (11), whereas material isolated from human serum is a truncated monomeric form with a lower molecular mass of 85 kDa (9). Finally, exosomes have only been detected in significant numbers in the sera of anemic animals with pronounced reticulocytosis and not in other cell types (13). Therefore, the production of these structures would seem to be associated only with erythroid maturation. Because serum receptor is reduced by only 50% in patients with complete marrow aplasia (6), nonerythroid tissues contribute significantly to the circulating protein. However, it is possible that some of the truncated soluble receptor in human serum is derived from exosomes before or after their release from cells. The finding of some 85-kDa form in the solubilized cell preparations suggests that if proteolytic mechanisms are responsible, then these may be intracellular or cell-membrane associated.

Interestingly, we obtained evidence in the present study for simultaneous production by K562 cells of transferrin receptor in both soluble and particulate form. When the soluble protein was isolated by affinity chromatography, it proved to be identical biochemically with serum receptor. Both the serum receptor and soluble K562 supernatant protein had a mobility on SDS-PAGE corresponding to a molecular mass of 85

kDa. In contrast, the major immunoreactive protein observed with receptor isolated from the cellular K562 fraction was located at 190 kDa before reduction and at 95 kDa after reduction. Glycoprotein analysis indicated that the mass of soluble form of the receptor, like that of serum receptor, included the carbohydrate residues. Amino acid analysis provided more direct evidence of the identity of serum and K562 supernatant receptor; both proteins lacked the first 100 amino acid residues of intact receptor. Thus, the soluble form released in the supernatant by K562 cells, as with serum receptor, is an abbreviated form of intact receptor, with truncation occurring in the extracellular domain between arginine 100 and leucine 101. However, it must be pointed out that although culture supernatant and serum receptor appear biochemically identical, it remains to be documented that *in vitro* and *in vivo* mechanisms of production are completely analogous.

We also obtained evidence, albeit indirect, that a portion of the transferrin receptor released by K562 cells is in a particulate or membrane-bound form. Vesicles were seen in pellets obtained by ultracentrifugation of the supernatants, although these were sparse and more heterogeneous in size than those produced by sheep reticulocytes (12). Based on various manipulations of the supernatant, our ELISA was used to measure the distribution of released receptor in soluble and particulate form quantitatively. Assuming that the detergent-treated supernatant represents all of the released protein,and that values in the ultracentrifuged supernatant represent only the soluble form, then approximately 70% of released receptor is soluble. Membrane-bound receptor can be estimated by the increase in supernatant values following solubilization or from values obtained by solubilizing the postultracentrifugation pellet. These approaches demonstrated that 18–30% of released receptor was in particulate form after 24 hr of incubation. Only a small fraction of the particulate protein was measured directly by immunoassay (3.5 of 27.3, or 12.8%, in the log phase cultures at 24 hr).

The production of a soluble form of transferrin receptor by HL60 cells has been reported previously (19). The relationship between the transferrin receptor released by these cells and the serum form was not defined in that report, whereas in the present study it was demonstrated that serum receptor and receptor released by K562 cells are identical biochemically. More importantly, we have employed precise ELISA technology to demonstrate the existence of two separate forms of transferrin receptor in tissue culture supernatant and to assess their quantitative importance. Because of methodological differences, it is not possible to determine the similarities or differences in the nature or amount of receptor released by HL60 cells. However, additional studies with both of these *in vitro* models will assist in better defining factors governing the release of cellular transferrin receptor.

The K562 tissue culture system provides a useful model to examine the mechanisms controlling the production of soluble and membrane-bound transferrin receptor. While only a portion of the particulate form is measured by the assay, reliable measurements of the soluble form can be obtained by ultracentrifuging the K562 supernatant prior to analysis. The proportion released in vesicle form can be measured either as the increase in supernatant values following the addition of a detergent or by measurement of solubilized pellets obtained by ultracentrifugation. Additional studies will be required to define the precise relationship between these two forms of released receptor.

Although the production both *in vitro* and *in vivo* of soluble receptor is now well-established, little is known of its cell-biological or physiological significance. On the one hand, its production could result from random proteolysis, in which case the serum concentration would simply reflect total receptor synthesis or total body mass of receptor. Alternatively, its degradation may be regulated, allowing for an independent influence on cellular receptor content and serum receptor concentration. Both *in vitro* (20) and *in vivo* (7) studies have shown a close relationship between soluble receptor concentration and cellular receptor mass, indicating that unregulated proteolysis is more likely. There is no clear evidence to date that the serum receptor plays a physiological role in iron metabolism, but there are several possibilities that have not yet been fully examined. The mechanism by which tissue iron needs are conveyed to the mucosal cell of the gastrointestinal tract is still unknown and it is possible that the serum receptor might in some way be involved in the regulation of iron absorption. It has been shown recently that both the serum receptor concentration and iron absorption are increased in iron deficient patients (21). There are many other possible roles for the serum receptor in iron metabolism and examining these will be important in future studies.

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