Demonstration of Transferrin Receptors on Human Placental Trophoblast

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It has been postulated that the transplacental passage of maternal iron to the developing fetus requires binding of maternal transferrin to the trophoblast. We have therefore examined the ability of the human placenta to bind transferrin in vitro. Transferrin was demonstrated on trophoblast of human chorionic villi by immunohistologic methods. Moreover, after removal of transferrin bound in vivo by treatment of tissue with chaotropic solution or phosphate-buffered saline, freshly added transferrin was shown to bind in vitro in the same characteristic distribution. These findings suggest that placent al iron transport is initiated by uptake of maternal transferrin iron to specific trophoblast binding sites.

DURING human pregnancy, the normal fetus acquires approximately 300 mg iron by a process of placent al transfer, which is thought to involve active transport mechanisms. Previous studies have indicated that the major source of such iron is that bound to maternal transferrin. In order to reconcile the large fetal requirement for iron with the avidity of its binding to transferrin in the maternal circulation, it has been suggested that human trophoblast expresses specific receptor sites that bind maternal transferrin. We have shown in immunohistologic studies of the human placenta that abundant quantities of transferrin are indeed present on the apical aspect of trophoblast; this has been a constant finding in all villi of placenta examined, both mature and immature. Furthermore, transferrin has been localized to the microvilli of the trophoblast by immunoelectron microscopy. Nevertheless, the origin of such trophoblast transferrin, whether maternal or fetal, has not yet been established. The direct contact established in hemochorial placentation between syncytiotrophoblast and the maternal circulation is consistent with binding of maternal transferrin to this fetal structure, but no direct evidence for this concept has yet emerged. In this study we have investigated binding of transferrin to human placental tissue in vitro; the results demonstrate the presence of specific binding sites for maternal transferrin on the syncytiotrophoblastic cell membrane.

MATERIALS AND METHODS

Tissues and Antisera

Human full-term normal placentae were obtained within 30 min of delivery, and small cubes of tissue were removed and immediately snap-frozen in liquid nitrogen-cooled isopentane. The immunoglobulin (Ig) fraction of a rabbit antiserum to human transferrin (Dakopatts A/S, Denmark) gave single precipitin lines on immunodiffusion (ID) and immunoelectrophoresis (IEP) against normal human serum (NHS) and human transferrin solution. The specificity for transferrin was further confirmed by absorption and displacement studies, as previously described. Rabbit antiserum to human transfoblast antigens were absorbed with normal human serum to remove unwanted antibody activities and checked for trophoblast specificity by immunohistologic studies using a panel of normal human tissues, both before and after absorption with trophoblast membranes. Rabbit antiserum to human albumin, fibrinogen, IgG, and alpha-2-macroglobulin were obtained from Dakopatts, and a rabbit antiserum to NHS was purchased from Cappell Laboratories, Cochranville, Pa. Fluorescein isothiocyanate (FITC) conjugated sheep antiserum to rabbit Ig (Wellcome Laboratories, Research Triangle Park, N. C.) with a fluorescein-to-protein ratio of 2000:1 was employed as the secondary antibody. The specificity of all commercial antisera was confirmed by ID and IEP against the appropriate antigen and NHS. The working dilutions of these antisera have been determined previously.

Transferrin

Purified human apotransferrin containing less than 0.05% ferric iron (Sigma Chemicals, St. Louis, Mo.) in solution at 1–10 mg/ml in 0.15 M phosphate-buffered saline (PBS) pH 7.4, formed single precipitin lines on ID and IEP against the rabbit antiserum to NHS and human transferrin (Dakopatts). Furthermore, a single peak in the included volume was obtained upon chromatography in PBS on Sephadex G-150 (Pharmacia Fine Chemicals, Piscataway, N.J.) and a single band was observed upon sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis in the presence of 2-mercaptoethanol. Normal human serum (NHS) was also used as a source of transferrin (2–4 mg transferrin/ml serum, 25%–40% iron saturated).

Immunohistology and Microscopy

Cryostat placental sections were examined by an indirect immunofluorescence method in which tissues were reacted first with antibody for 20 min, washed 3 times for 10 min in 0.15 M PBS, pH 7.4, reacted with FITC-labeled second antibody and similarly
washed, and mounted in 50% glycerol in PBS, pH 7.4. In control experiments, the first antibody was replaced by normal rabbit Ig (serum control) or PBS (conjugate control). Unwanted fluorescence was minimized by solid-phase immunoabsorption of the FITC-conjugate with NHS. Sections were examined using a Zeiss Universal fluorescence microscope fitted for transillumination with an XBO 75 xenon light source, Triyda condensor, and appropriate FITC filter system. Photographs were taken with an Olympus automatic camera using Kodak Tri-X film.

Removal and Replacement of Trophoblast Transferrin

Placental sections were subjected to washing in PBS and ammonium thiocyanate solutions under different conditions of molarity, temperature, and time, and subsequently reacted with rabbit antitransferrin and FITC sheep antirabbit Ig. Parallel sections were similarly washed in PBS or ammonium thiocyanate, incubated for 10 min with NHS (neat and at increasing dilutions to 1:10,000 in PBS) or transferrin solution (0.0001-2.5 mg/ml), and reacted with transferrin antiserum and conjugate.

RESULTS

The characteristic and highly reproducible fluorescence pattern of transferrin obtained on human placenta is illustrated in Fig. 1A. In contrast, serum and conjugate controls were completely negative. In order to demonstrate uptake of transferrin by human trophoblast in vitro, transferrin bound in vivo was removed so that binding sites were made available. This was accomplished in two ways using methods described above. Detectable transferrin was removed with the use of 0.5 M ammonium thiocyanate for 10 min at 20°C or by washing in 0.15 M PBS, pH 7.4, for 72 hr at 4°C, the PBS being replaced every 8 hr. When

Fig. 1. (A) Immunofluorescence of transferrin on mature human trophoblast. Placental sections reacted with rabbit antiserum to human transferrin and FITC-sheep antiserum to rabbit Ig (×250). (B) Removal of transferrin using ammonium thiocyanate. Placental sections were washed in 0.5 M ammonium thiocyanate for 10 min, then reacted with rabbit antitransferrin and FITC sheep anti-rabbit Ig. Note absence of fluorescence (×250). (C) Restoration of transferrin fluorescence pattern. Placental section was depleted of transferrin (as for B), incubated with human transferrin solution (21 mg/ml), then reacted with rabbit antitransferrin and FITC-sheep anti-rabbit Ig. An identical pattern was obtained when similar sections were incubated with undiluted normal human serum (×250). Abbreviations: ivs, intervillous space; v, villus.
sections thus treated were reacted with antiserum to transferrin, the fluorescent pattern of transferrin was seen to be abolished (Fig. 1B). These washing procedures did not cause any apparent morphological change in the tissue nor any reduction in the expected intensity or distribution of fluorescence with antisera to other proteins, including albumin, IgG, fibrinogen, and human trophoblast antigens, for which placental distribution has been defined previously.4,7

Subsequent incubation of similar transferrin-depleted tissues with freshly added transferrin or NHS at all dilutions used, followed by reaction with antisera to transferrin and FITC-conjugate, completely restored the fluorescence pattern of transferrin (Fig. 1C). Moreover, the intensity of fluorescence did not vary appreciably with the concentration of the transferrin source, nor were there discernible differences in patterns obtained with apotransferrin and the partially iron-saturated protein present in NHS. The specificity of this binding reaction was demonstrated by control experiments employing antisera to other human proteins, including IgG, alpha-2-macroglobulin, and albumin. These antisera yielded patterns distinct from that observed with antiserum to transferrin,* and in addition, no differences in the distribution of fluorescence could be discerned following incubation of transferrin-depleted tissue with NHS.

**DISCUSSION**

The direct demonstration in this study of the ability of transferrin-depleted villous trophoblast to bind transferrin, but not other serum proteins in vitro provides evidence for the presence of specific syncytiotrophoblast membrane binding sites for transferrin. Although quantitative data concerning binding were not obtained with this immunofluorescence method, the detection of binding concentrations of protein 10,000 times lower than serum levels is compatible with high affinity of this reaction, and the presence of specific transferrin receptors. Since maternal serum iron required by the fetus for growth and development must be obtained in competition with maternal erythropoietic centers, placental transferrin receptors would thus provide a mechanism whereby iron could be diverted to the trophoblast.

Transferrin itself has not been shown to traverse the placenta in significant amounts,12 and the immediate fate of iron concentrated at the trophoblast membrane is unclear. Iron uptake by immature erythrocytes has been shown to involve receptor binding and internalization of transferrin–iron complexes, followed by intracellular dissociation of ferric ions, and release of the carrier protein.13 The results of the present study provide preliminary support for the suggestion that similar events occur at the level of the trophoblast.

The ability of transferrin to chelate iron efficiently not only is vital to its role as an iron carrier but also gives it bacteriostatic activity against many microorganisms to which iron is essential for growth. It is therefore possible that transferrin bound at the trophoblast membrane may play an important part in the defense of the trophoblast against maternal bloodborne infection, in addition to its contribution to the effective delivery of iron to the fetus.

**REFERENCES**

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