

Regulation of transferrin receptor expression and distribution in in vitro cultured human cytotrophoblasts

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Abstract

During gestation the transplacental iron transport is very important to the fetus. Iron uptake by the placenta can be studied in cultured cytotrophoblasts. The influence of culture time and human diferric transferrin on the number and distribution of transferrin receptors (TfRs) was investigated in human cytotrophoblasts. Cytotrophoblasts cultured for 2.5 h had few TfRs (0.28 pmol/mg protein). With time, total TfR amounts increase (4.14 pmol/mg protein at 70 h). They increase to a higher level in cells cultured in iron-poor medium, indicating that iron has an effect on the TfR synthesis/breakdown ratio. TfRs were distributed between two 'active' (located at the cell surface and intracellularly) and one 'inactive' (located intracellularly) receptor pools. TfR distribution among these pools was modulated by culture time and iron. Trophoblasts regulated iron uptake by variation of number of surface TfRs via changes in total TfRs and redistribution of TfRs among the receptor pools.

Key words: Iron; Transferrin receptor; Placenta; Cytotrophoblast

1. Introduction

The principal function of transferrin (hTf) is the transport of iron in the circulation [1,2,3]. hTf is taken up by the cells via receptor-mediated endocytosis [4,5]. hTf

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binds to transferrin receptors (TfRs) which are clustered in coated pits [6]; the coated pits are internalized by forming endosomes. These vesicles become acidic [4,7], iron is released from transferrin and the apo hTf-TfR complex is recycled back to the cell surface. In response to the environmental pH of 7.4 apo hTf dissociates from its receptor leaving both ready to be re-used. This mechanism of iron uptake is among others [8,9] found in trophoblasts [10].

A variable percentage of TfRs are found within cells [11,12]. TfRs participating in the endocytic cycle are found both on the cell surface and within cells. Apart from these two metabolically active subgroups a further TfR pool has been proposed by Hirose-Kumagai and Akamatsu [13]. This pool, also located intracellularly, is functionally inactive.

In several cell types TfR and ferritin synthesis are simultaneously, though inversely, controlled by the intracellular iron concentrations [14]. Regulation takes place at the translational level by means of iron responsive elements on the mRNA for TfR and ferritin [15]. This regulation mechanism, however, has not yet been identified in trophoblasts.

Because high intracellular iron concentrations are potentially dangerous and the fact that transplacental transport of iron increases enormously during pregnancy, the uptake of iron has to be balanced very precisely. Furthermore, the fetus does not suffer from iron-induced anaemia, even if the maternal iron stores are depleted [16–18], so a regulation mechanism for iron transport is likely to exist [19]. Bierings et al. [20] suggested that the iron transport may be regulated by the alteration of surface TfR numbers similar to the variation of surface TfRs by HeLa cells [21].

Surface TfR numbers could be regulated firstly by a change in the synthesis/breakdown ratio and secondly by redistribution of TfRs among (functionally) different pools. Both mechanisms might occur simultaneously. Redistribution of TfRs has been confirmed in several cell types [22–26] and might be influenced by the number of TfRs located in intracellular pools [27].

The cell layer primarily involved in the transplacental transport of iron is the syncytium formed by syncytiotrophoblasts. In situ syncytiotrophoblasts differentiate out of cytotrophoblasts which are proliferative throughout gestation. During this differentiation process, cells start with the production of specific hormones (hCG, hPL and SP-1) [28,29].

As a model of the maternal fetal interface cultured cytotrophoblasts were used. Cytotrophoblasts in culture do not proliferate though they differentiate into syncytiotrophoblast-like cells which can be concluded from the production of syncytiotrophoblast-specific hormones [28,30–32] and the expression of TfRs [10,33,34].

Biochemical differentiation and morphological differentiation do not necessarily occur simultaneously [30,33]. Even the parameters of the biochemical differentiation do not necessarily parallel each other [20].

In cultured cytotrophoblasts, TfR expression not only depends on culture time but also on the supplementation of iron [20,35]. In this study we investigated the influence of culture time (differentiation state) and hTf-2Fe availability on total TfR expression and on the distribution of TfRs between endocytic active and inactive TfR pools. The relevance for the regulation of iron transfer and the maintenance of low intracellular (free) iron concentrations is discussed.

2. Materials and methods

2.1. Chemicals

Calcium and magnesium free solution of Earle's Balanced Salts (CMFS), fetal calf serum (FCS), Medium 199 (M199), penicillin, streptomycin and amphotericin were obtained from Flow Labs., (ICN Biomedicals, Zoetermeer, Netherlands). Gentamycin was from Schering Corp., USA, trypsin (1:250 t.c.) from Sigma Chemical Company, St. Louis, MO and DNase grade II from Boehringer Mannheim, Germany. Apo transferrin was from Behring Werke, Marburg, Germany. Na¹²⁵I was obtained from Radiochemical Centre Amersham, UK. Iodo-Gen was from Pierce Chemical Co., USA. All chemicals were of the highest purity available.

2.2. Iodination

Fully saturated transferrin was obtained using iron-nitrilotriacetate (Fe-NTA) (ratio 1 mol Fe to 2 mol NTA) and a 10-fold molar excess of bicarbonate as the synergistic anion (buffered in 0.1 M Tris-HCl, pH 8.2). The excess of Fe-NTA was removed on a PD-10 Sephadex column (Pharmacia, Uppsala, Sweden) and by extensive dialysis against Tris-HCl buffer (pH 8.2). The iron saturation of the transferrin was checked by measuring the E470/E280 ratio which was always 0.045, indicating full saturation. One milligram of this diferric transferrin was incubated with ¹²⁵I (0.5 mCi) for 20 min at room temperature in a glass vial coated with 100 µg Iodo-Gen. Free ¹²⁵I was separated from radiolabelled transferrin on a PD-10 Sephadex column, followed by extensive dialysis against phosphate buffered saline (PBS) (136.8 mM NaCl, 2.68 mM KCl, 8.10 mM Na₂HPO₄, 1.14 mM KH₂PO₄; pH 8.2). The final specific activity of the diferric ¹²⁵I-labelled transferrin varied between 230 and 535 × 10⁶ counts/min per mg protein.

2.3. Trophoblast-cell isolation

Normal human placentae were obtained (from the Department of Obstetrics, Academic Hospital Rotterdam/Dijkzigt, Rotterdam) within 0.5 h after spontaneous delivery. These placentae were essentially processed as described by Hall et al. [36] in combination with a Percoll gradient described by Kliman et al. [28], except for the addition of CaCl₂·2H₂O (1 mM) and MgSO₄·7H₂O (0.8 mM) to the enzyme solution [20]. By this procedure a cell population was isolated consisting of at least 95% cytotrophoblasts [28] which has been reconfirmed in our laboratory by immunocytochemical staining using a panel of monoclonal antibodies [33]. Furthermore the percentage of hCG, hPL and SP-1 producing cells was revealed by immunocytochemical staining. [33,36]. In the present experiments one culture dish was routinely checked for the number of cells positive for hCG production. In all experiments at least 95% of the cells became positive for hCG production after 40 h. Cells obtained were counted (using a Burkert counting-chamber), diluted to 6 × 10⁵ cells/ml in culture medium and plated out in 35 mm Falcon culture dishes (Greiner and Söhne, Germany) (1.5 × 10⁶ cells/dish). Culture medium consisted of 80 volume % M199 (Flow Labs), 20 volume % FCS, 4 mM L-glutamine, 0.3 mg/ml gentamycin, 50.0 IU/ml penicillin, 50.0 µg/ml streptomycin and 2.50 µg/ml amphotericin. Osmolality was 280–300 mmol/kg, pH 7.4. The medium was sterilized by filtration through a 0.22-µm Millipore-GS filter (Millipore SA Molsheim, France). Cultures were incubated at 37°C in humidified 5% CO₂/95% air.

2.4. Cell culture conditions

Cells were allowed to recover from the isolation procedure for 18–24 h and subsequently washed twice with M199 to remove non-adherent cells. The cells used for TfR determination after 2.5 h were handled in a similar way. Cell culture was continued in fresh, identical culture medium or the same medium supplemented with human diferric transferrin (hTf-2Fe) in humidified 5% CO₂/95% air at 37°C. The culture medium was changed every 24 h. The 0-passage of cells was used for the experiments, which were generally started 18–20 h after cell isolation.

2.5. Binding essays

To minimize the interference of residual receptor bound transferrin, cells were pre-incubated for 15 min (at 37°C in 5% CO₂/95% air) in M199 without additives. Finally they were re-chilled to 4°C and washed twice with PBS.

Surface transferrin receptor population. To measure the number of cell surface TfRs, cells were incubated for 1.5 h at 4°C (long enough to reach equilibrium) with a concentration of 125 nM ¹²⁵I-labelled diferric transferrin (¹²⁵I-hTf-2Fe). A transferrin concentration of ten times K_D would give a 90% saturation [37]. The concentration used by us was 60 times K_D . Under these conditions full saturation was reached [36]. Non-specific binding was measured by addition of a 100-times excess of unlabelled diferric transferrin and never exceeded 25% of the total binding of ¹²⁵I-hTf-2Fe. Non-specific binding amounting up to 25% is rather high for ligand concentrations used in Scatchard analysis. However, at the ligand concentrations used, it is not extreme. The cells were then washed three times with ice-cold PBS to remove unbound ¹²⁵I-hTf-2Fe. Finally cells were lysed by addition of distilled water and collected with a rubber 'Policeman'. Surface bound radioactivity was assessed using a Packard 500C autogamma spectrometer. TfRs were calculated from the amount of specifically bound ¹²⁵I-hTf-2Fe.

Total transferrin receptor population. Cultured cells were washed three times with ice-cold PBS and lysed in 0.5 ml 0.1% Triton X-100. Lysis was checked using an inversion microscope (CK2, Olympus, Japan). The detergent 'Triton X-100' has widely been used in receptor binding studies and no effects have been reported on plasma membrane receptors [13]. Cell suspensions were collected in pre-weighed tubes and homogenized by sonication for 10 s in melting ice. Tubes were re-weighed to ascertain the sample volume. Of the cell lysates portions of 0.1 ml were taken for protein determination. Cell lysates were incubated with ¹²⁵I-hTf-2Fe (final and saturating concentration 125 nM) for 1 h at room temperature. Ammonium sulfate was added in a 1:1 volume ratio resulting in a 30% (NH₄)₂SO₄ solution to precipitate the ¹²⁵I-hTf-2Fe-TfR complex [38]. Samples were filtered through 1.2-μm glass microfiber filters (GF-C, Whatman) [38]. Filters were rinsed four times with 30% (NH₄)₂SO₄. Radioactivity on the filters was measured as described above. TfR number was calculated from the amount of specifically bound ¹²⁵I-hTf-2Fe. This method was carefully checked for precipitation of unbound ¹²⁵I-hTf-2Fe (<0.5%) (results not shown).

Transferrin receptors participating in the endocytic cycle. Cells were incubated for 1 h at 37°C in medium M199 supplemented with 250 nM ¹²⁵I-hTf-2Fe and suc-

cessively washed three times with PBS (pH 7.4) at 4°C. To remove surface bound hTf-2Fe, acid/neutral washes were carried out at 4°C; cells were firstly incubated for 10 min with sodium acetate buffer (pH 4.5) and secondly for 10 min with PBS (pH 7.4). This procedure was repeated twice. Finally the cells were lysed in distilled water and collected with a rubber 'Policeman'. Radioactivity in the cell lysates and the combined acid/neutral washes was determined as described above. Non-specific binding was determined by binding of ^{125}I -hTf-2Fe in the presence of a 100-times excess of unlabelled hTf-2Fe. The number of surface-bound TfRs was derived from the acid/neutral wash labile ^{125}I -hTf-2Fe. The number of intracellular TfRs was calculated from the amount of radioactivity resistant to the acid/neutral washes.

2.6. Protein determination

A homogenous sample was obtained by sonication for 10 s in melting ice. Protein concentration in the samples containing distilled water was determined according to Bradford [39] with human transferrin as a standard. The samples containing Triton X-100 were analyzed according to Wang and Smith [40].

2.7. DNA-determination

DNA contents of the samples were determined with the Nucleo-100 kit obtained from Sanbio BV, The Netherlands [41].

2.8. hCG-determination

Culture medium hCG concentrations were measured using an ES-600 auto-analyzer (Boehringer Mannheim, Germany). The medium was removed at indicated times and replaced by identical culture medium. Prior to analysis the medium was centrifuged for 5 min at $1,200 \times g$. Supernatants were used for hCG determination.

2.9. Statistics

To estimate the significance of the results the Student's *t*-test was used in experiments with only two groups, the Student-Newman-Keuls test for experiments with more than two groups and for the experiments on receptor distribution the χ^2 test was used.

3. Results

3.1. Validity of DNA and protein measurements as reference parameters

As depicted in Fig. 1, hCG production strongly increased after approximately 40 h and the total hCG production was comparable with previously presented data [28,29]. Together with the absence of TfRs and the fact that over 95% of the cells were hCG-positive after 40 h in culture, this was taken as evidence that the initially attached cell population was highly enriched with cytotrophoblasts.

Culture-dish protein content varied (from 50 up to 150 μg per dish) between samples isolated from different placentae. The variance in protein concentration between samples prepared from cells isolated from one placenta was small and

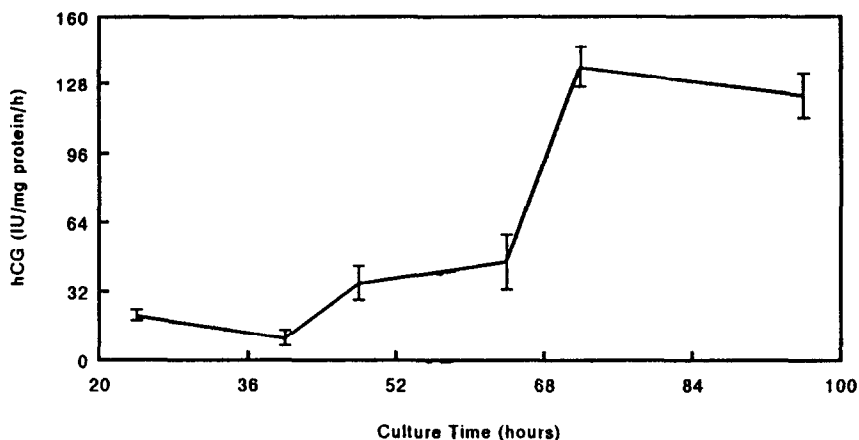


Fig. 1. hCG production by cytotrophoblasts in culture. Cells were isolated as described in Materials and methods and cultured in standard culture medium (control, —). The medium was removed at indicated time points, centrifuged and hCG concentrations were measured in the supernatants using an ES-600 Boehringer Mannheim autoanalyzer. The medium was replaced by identical culture medium. Calculated were the hCG productions per hour, related to the dish protein content, during the period previous to the removal of the culture medium.

therefore acceptable (S.D. maximal 10% of mean dish protein content; constant with time).

Up to 72–90 h after isolation, protein concentration remained stable and then fell significantly ($\alpha_T < 0.01$). Protein concentration was independent of culture medium supplementation with hTf-2Fe (Fig. 2).

DNA concentration also declined (from 2.0 to 1.5 $\mu\text{g}/\text{dish}$) after 72 h of culture ($\alpha_T < 0.05$) indicating cell loss. Nevertheless, DNA concentration was more stable than dish protein content which is depicted in Table 1 showing protein/DNA ratios. It can be concluded that cultured trophoblasts are viable for at least a period of up to 72–90 h. Nevertheless, cultures for up to 160 h were possible without appreciable loss of protein and DNA if the placentae were obtained within 15 min of parturition.

3.2. Transferrin receptor amounts

The number of surface TfRs increased with time and were influenced by addition of hTf-2Fe to the medium (Fig. 3A), a process which was reversible (Fig. 3B). Cells cultured in iron-poor medium increased the number of surface TfRs to a significantly higher level (Fig. 3A) ($\alpha_T < 0.05$). Replacement of iron-fortified medium by iron-poor medium led to a number of surface TfRs highly comparable with that of cells permanently cultured in iron-poor medium (Fig. 3B). In these experiments the number of surface TfRs was significantly higher ($\alpha_T < 0.01$) in cells cultured in iron-poor medium. In the cells cultured for 2.5 h, surface TfRs were not detectable with the procedure used. Non-specific binding in these cells was always comparable with the total binding of ^{125}I -hTf-2Fe.

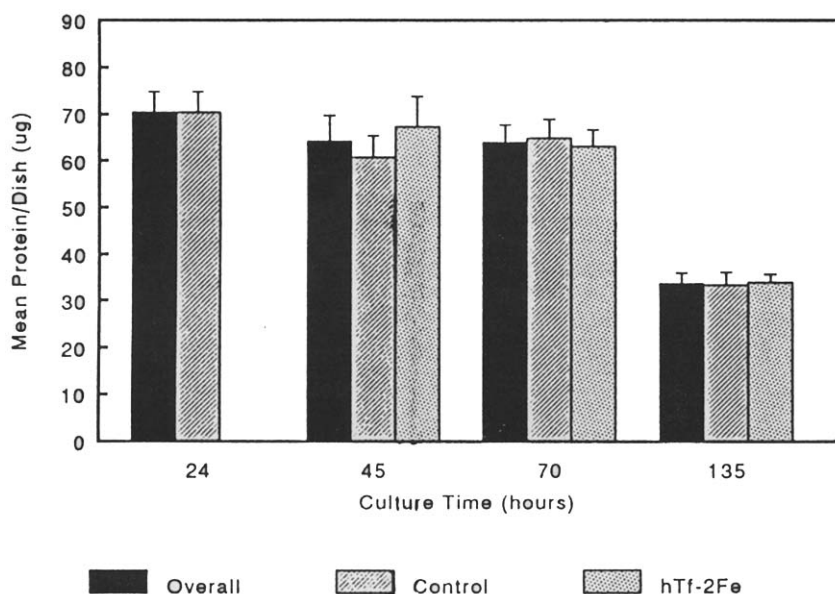


Fig. 2. Protein content of culture dishes. Influence of culture time and hTf-2Fe. Shown are the results of one experiment of a total of three. Although different in protein quantity (see Results) all three experiments were highly similar for the trend of protein loss. Trophoblasts were isolated and cultured as described in Materials and methods. At indicated culture times cells were harvested and lysed in distilled water. Depicted are the mean protein contents (\pm S.D.) calculated from five dishes per series except for the overall means which were calculated from ten dishes.

Table 1
Mean protein/DNA ratios. Influence of culture time and hTf-2Fe

| Culture time (day) | Experiment 1 | | | Experiment 2 | | | Experiment 3 | | |
|-----------------------|--------------|------|----|--------------|------|----|--------------|------|---|
| | Mean | S.D. | n | Mean | S.D. | n | Mean | S.D. | n |
| 1 | 23.2 | 2.6 | 10 | 30.7 | 0.5 | 5 | 29.6 | 1.1 | 6 |
| 2 | 22.3 | 5.0 | 9 | 31.9 | 2.7 | 9 | 30.2 | 4.0 | 6 |
| 3 | 23.7 | 2.6 | 10 | — | — | — | 28.3 | 2.5 | 6 |
| 4 | — | — | — | 22.3 | 1.9 | 13 | — | — | — |
| 5 | — | — | — | 14.0 | 1.7 | 17 | 15.4 | 2.1 | 6 |
| 6 | 15.0 | 1.9 | 10 | — | — | — | — | — | — |
| 7 | — | — | — | 14.1 | 1.9 | 20 | — | — | — |

Reproduced are the mean protein/DNA ratios (\pm S.D., n) of three highly similar experiments. Trophoblasts were isolated and cultured as described in Materials and methods. At indicated culture times (days) cells were harvested and lysed in distilled water. Dish protein- and DNA-contents were determined as described in Materials and methods.

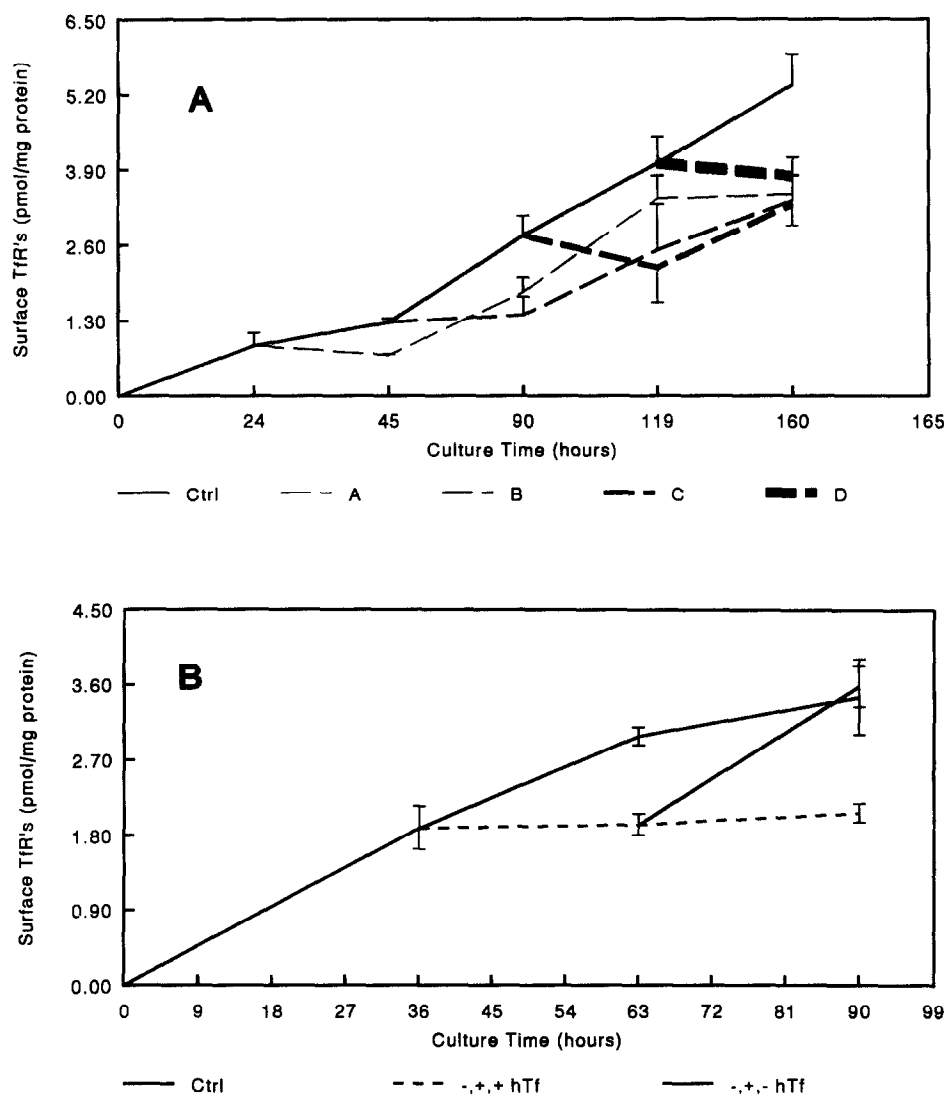


Fig. 3. Surface transferrin receptors. Influence of culture time and hTf-2Fe. (A) Trophoblasts were isolated as described in Materials and methods and cultured in iron-poor medium (—, control series). After indicated culture periods culture was continued in medium containing hTf-2Fe (---, 0.1 mg/ml; Series A-D). Bars represent 1 S.D. Transferrin receptors were determined as described in Materials and methods. Results were corrected for non-specific binding by using parallel cultures incubated with a 100-times excess of unlabelled hTf-2Fe. (B) Culture conditions were identical to those described under Fig. 3A (control series: - - -; hTf-2Fe series: -, +, +). In order to invest the reversibility of the regulation mechanism the hTf-2Fe fortified medium of five culture dishes was replaced by iron-poor medium at 65 h (-, +, - series). Bars represent two standard deviations. Transferrin receptors were determined as described in Materials and methods. Results were corrected for non-specific binding by using parallel cultures incubated with a 100-times excess of unlabelled hTf-2Fe.

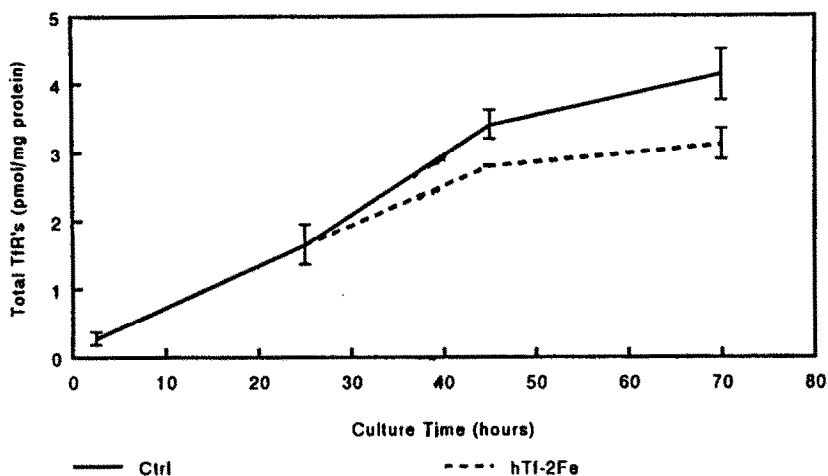


Fig. 4. Total transferrin receptors. Influence of culture time and hTf-2Fe. The results given are of one experiment highly similar to a total of three. Trophoblasts were isolated as described in Materials and methods and cultured in iron-poor medium (—). From 24 h on culture was continued in either iron-poor medium (—) or diferric human transferrin (hTf-2Fe) fortified medium (---) (0.1 mg/ml). At indicated culture times cells were harvested, lysed in 0.1% Triton X-100 and total TfRs were determined as described in Materials and methods. Results were corrected for aspecific binding by using parallel cultures incubated with a 100-times excess of unlabelled hTf-2Fe.

The total number of TfRs increased with time (Fig. 4). This increase was more clear cut if cells were cultured in iron-poor medium ($\alpha_T < 0.02$). In the cells cultured for 2.5 h very small numbers of TfRs were determined (0.28 pmol, ± 0.03) TfRs/mg protein).

3.3. Transferrin receptor distribution

With time there was a redistribution of TfRs among the functionally different compartments in the trophoblast cell. Figure 5 shows the results obtained in one of three studies. The data show that about 45% of all TfRs actively participated in the endocytic cycle. The other part is stored and functionally inactive. This distribution was stable with time unless cells were cultured in iron-poor medium. In this case the percentage of active TfRs increased to about 56, which was significantly higher than the 46% of the hTf-2Fe cultured series ($P < 0.02$). After 24 h about 30% of the TfRs were located on the cell surface. With time a significantly increasing part (up to 46%) of the active TfRs were found on the cell membrane ($P < 0.05$), an increase which depended on hTf-2Fe supplementation of the culture medium. Finally, the percentage of surface-bound TfRs (of the total of TfRs) was significantly higher ($P < 0.05$) if cells were cultured in iron-free medium. The results indicate that this was mainly caused by an increase in the number of TfRs actively participating in the endocytic cycle.

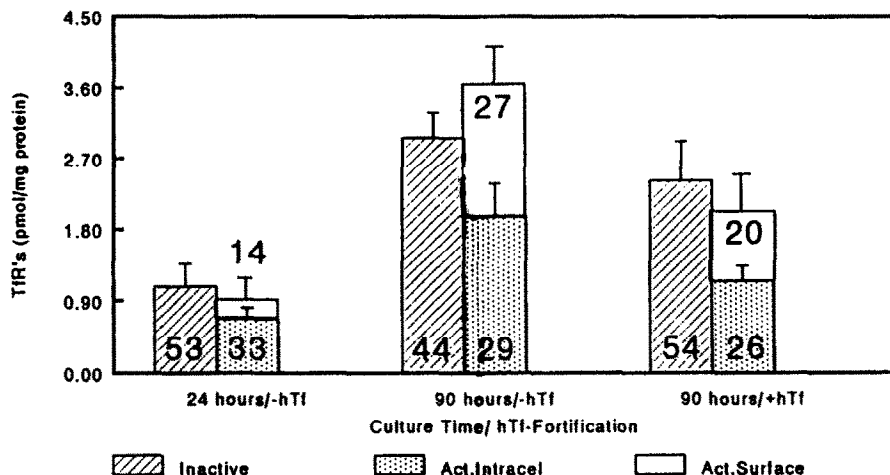


Fig. 5. Transferrin receptor distribution. Influence of culture time and hTf-2Fe. Shown are the results obtained in one experiment which is highly similar to a total of three. Trophoblasts were isolated and cultured as described in Materials and methods and cultured in iron-poor medium. After 24 h culture was continued in either iron-poor medium or human diferric transferrin (hTf-2Fe) supplemented medium (0.1 mg/ml). TfRs were determined as described in Materials and methods. Results were corrected for aspecific binding by using parallel cultures incubated with a 100-times excess of unlabelled hTf-2Fe. TfR sub-pool percentages are given by the numbers in the figure.

4. Discussion

Placental transport of iron has to be balanced very accurately. The first step in this process is the uptake of iron by syncytiotrophoblasts, which can be studied in vitro cultured cytotrophoblasts [10,20,33,34,36].

In culture, cytotrophoblasts express TfRs [10,33,34] and start with the production of β -hCG [28,30–32]. The extent of TfR expression as well as of β -hCG production depends on cell quality which, on its turn, depends on the time loss between delivery of the placenta and the start of the isolation procedure [42]. In general, trophoblasts could be cultured for 72–90 h without significant loss of protein and DNA (Fig. 2, Table 1). Mean dish protein contents varied with cell isolation but were comparable with those published elsewhere [36,43]. In some experiments the culture period could be extended to approximately 8 days, though only if the placentae were obtained within 15 min after delivery. Because the protein-DNA ratio was stable during 72 h (Table 1) both can be used as standard for cell number. The combined loss of both protein and DNA, after approximately 90 h, indicated cell loss.

In our studies TfR expression as well as hCG production increased until day 4–5 (Fig. 1). After this period hCG production fell [31] suggesting a metabolic/biochemical change whereas TfR expression continued to increase or at least stabilized.

The rate of increase in surface TfRs was not essentially influenced by the availability of iron (Fig. 3A). Replacement of the standard culture medium by medium containing human diferric transferrin (hTf-2Fe) transiently reduced surface TfR numbers. Soon, however, the normal rate of increase was resumed (Fig. 3A), a process which was reversible (Fig. 3B). The rate of increase, as well as the numbers of TfRs, were highly comparable with those found by Kennedy et al. [34]. Their results showed that syncytium formation [30] is related to a reduction in surface TfR density. This seems to be supported by the earlier findings of van der Ende et al. [24] who demonstrated that cAMP analogs or cAMP-phosphorylase inhibitors induce BeWo cells to fuse and simultaneously to reduce surface TfR numbers. However cAMP-analogues, as such, neither stimulated the formation of syncytia nor modulated surface TfR numbers in cytotrophoblasts cultured in M199, though hCG production was increased 5- to 10-fold [20]. Furthermore, cytotrophoblasts *in vivo* lack TfRs [44,45]. We therefore think that the culture conditions used by Kennedy et al. [34] might induce differences in biochemical differentiation independent from the morphological differentiation.

Similar to the surface TfRs, total TfR numbers increased with time as depicted in Fig. 4, particularly if cells were cultured in iron-poor medium. Preliminary results suggested a stronger increase of total TfRs if cells were cultured in hTf-2Fe supplemented medium [35]. However with the carefully tested procedure described above, these results could not be reconfirmed. Based on the results on total TfR numbers we conclude that iron affects the TfR synthesis/breakdown ratio. However these results do not exclude the possibility of TfR redistribution, a process which, as a result of cell differentiation, has been demonstrated before in choriocarcinoma [24] and K562 erythroleukemic cells [25,46]. No changes in TfR amounts nor in TfR distribution, however, were found in HL-60 human leukemic cells during differentiation [47] and in different grades of macrophage activation [12], respectively.

Time and hTf-2Fe had different effects on the TfR synthesis/breakdown ratio and receptor redistribution (Fig. 5). Surface TfR numbers expressed as a percentage of the active TfR subpopulation increased significantly with time ($P < 0.05$) and were not influenced by the availability of iron. This might be an effect of cell differentiation. The final distribution is highly comparable with that in BeWo cells [24,48]. The size of the active TfR population, expressed as a percentage of total TfRs, was constant if, similar to the normal physiological situation, iron was available. Iron shortage caused TfRs to redistribute between the inactive and active receptor pool.

Within the pool of active TfRs there was a shift of TfRs to the surface, independent of iron availability, and therefore most likely caused by a process of differentiation similar to that in BeWo choriocarcinoma cells [24,47]. Surface TfRs might be increased to a pre-fixed percentage of the active receptor pool which has been suggested before [20].

The variation in the number of surface TfRs due to iron availability was independent of the differentiation process and can be explained by the shift of TfRs between the inactive and active pool. If, within the active TfR subpopulation, the distribution of TfRs over cell surface and interior remains stable this shift will automatically lead to an increase in surface TfRs.

Trophoblasts could regulate iron uptake (and by this way transplacental iron transport) by variation of surface TfR numbers via changes in total TfR amounts and via redistribution of TfRs among the receptor pools. This is in accordance with the *in vivo* situation of iron-deficient mothers carrying fetuses with normal iron stores. Nevertheless, the results discussed in this article do not prove that there is indeed a causal relationship between the number of surface TfRs and iron uptake.

The effects of a differentiating iron transport system and of a regulation mechanism for iron uptake may interfere and should therefore be considered if transplacental iron transport processes are studied.

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