Development and polarization of cationic amino acid transporters and regulators in the human placenta

PAUL T.-Y. AYUK, COLIN P. SIBLEY, PAUL DONNAI, STEPHEN D’SOUZA, AND JOCELYN D. GLAZIER

Academic Unit of Child Health, University of Manchester and Department of Obstetrics and Gynecology, St. Mary’s Hospital, Manchester M13 0JH, United Kingdom

Ayuk, Paul T.-Y., Colin P. Sibley, Paul Donnai, Stephen D’Souza, and Jocelyn D. Glazier. Development and polarization of cationic amino acid transporters and regulators in the human placenta. Am J Physiol Cell Physiol 278: C1162–C1171, 2000.—We have investigated L-arginine transport systems in the human placental syncytiotrophoblast across gestation using purified microvillous (MVM) and basal (BM) plasma membrane vesicles. In MVM from first-trimester and term placentas, L-arginine transport was by systems y⁺ and y⁻L. In BM (term placenta), however, there was evidence for system y⁻L only. The Michaelis constant of system y⁻L was significantly lower (P < 0.05) in first-trimester compared with term MVM and lower in term MVM compared with BM (P < 0.05). There was no functional evidence for system b₀L in term MVM or BM. Cationic amino acid transporter (CAT) 1, CAT 4, and 4F2hc were detected using RT-PCR in placentas throughout gestation. rBAT was not detected in term placentas. An ~85-kDa and an ~135-kDa protein was detected by Western blotting in MVM under reducing and nonreducing conditions, respectively, consistent with the 4F2hc monomer and the 4F2hc-light chain dimer, and their expression was significantly higher (P < 0.05) in term compared with first-trimester MVM. These proteins were not detected in BM despite functional evidence for system y⁻L. These data suggest different roles for 4F2hc in the development and polarization of cationic amino acid transporters in the syncytiotrophoblast.

CAT 1; CAT 4; 4F2hc; rBAT; L-arginine; gestational change

THE ELUCIDATION OF the molecular basis of cationic amino acid transport over the last decade has significantly informed the understanding of the physiology and pathophysiology of membrane transport. First the murine ecotropic retroviral receptor ERR 1 was shown to mediate cationic amino acid transport (26, 39), and recent studies have shown that this protein [now described as cationic amino acid transporter (CAT) 1], along with other isoforms, comprises the molecular basis of the ubiquitous cationic amino acid transport system y⁺ (7, 24). Later, the related glycoproteins 4F2hc (equivalent to the antigen CD98; see Ref. 1) and rBAT (homologous to D2 or NBAT; see Refs. 2 and 41) were shown to induce cationic amino acid uptake on expression in Xenopus oocytes. 4F2hc and rBAT are now thought to be regulators of amino acid transport that require the presence of light chain proteins to form the functional transporter (14, 37, 40). Recently, proteins have been identified as the putative light chains, which form heterodimers with 4F2hc and mediate the transport of cationic and some neutral amino acids (37). Derangement in the glycoproteins or their associated light chains may form the molecular basis of the transport disorders lysinuric protein intolerance (4F2hc/system b₀L; see Ref. 37) and cystinuria (rBAT/system b₀L; see Ref. 40). The study of cationic amino acid transport has been given added impetus in recent years by the discovery of the physiological and pathophysiological roles of nitric oxide (32) and the dependence of cellular nitric oxide synthesis on L-arginine transport (3, 8, 13).

This has raised the possibility of a coupling of cationic amino acid transporters to nitric oxide synthase (31). In the placenta, nitric oxide has been shown to be the most important regulator of fetoplacental perfusion (4). In addition, adequate placental amino acid transfer to the fetus is vital for normal intrauterine development and may be altered in complicated pregnancy (27, 29).

In the human placenta, the syncytiotrophoblast is the transporting epithelium between maternal blood in the intervillous space and fetal blood in the umbilical capillaries. It comprises a microvillous (apical; MVM) and a basal (BM) plasma membrane through which solutes are transferred to effect materno-fetal exchange. It is becoming increasingly clear that there are important changes in the expression and activity of syncytiotrophoblast transporters over the course of gestation (35). However, cationic amino acid (L-lysine) transport has only been investigated in MVM and BM vesicles prepared from term placentas (17–19). Furthermore, much of the characterization, which suggested systems y⁺ and y⁻L in MVM and systems y⁺, y⁻L, and b₀L in BM, was undertaken before the recent and more precise definition of the properties of cationic amino acid transport systems (summarized in Table 1). Finally, the temporal (over gestation) and vectorial (MVM vs. BM) relationship between cationic amino acid transporter activity and the expression of CAT isoforms, 4F2hc, and rBAT has not been investigated in the human placenta and has not been reported previously in any human epithelium.

Here, we report 1) the characterization of cationic amino acid (L-arginine) transport systems in term
placental MVM and BM and first-trimester MVM using the properties outlined in Table 1; 2) the expression of mRNA for CAT 1, CAT 4, 4F2hc, and rBAT in first-trimester, second-trimester, and term placentas using RT-PCR, and 3) the expression of 4F2hc in MVM and BM from term placentas and MVM from first-trimester placentas. The data show significant differences in the affinity of system y^+ L between first-trimester and term MVM and between term MVM and BM. These differences are accompanied by significant differences in the expression of 4F2hc in MVM across gestation and between term MVM and BM.

**MATERIALS AND METHODS**

**Vesicle Preparation**

Placentas from normal term singleton pregnancies were collected as soon as possible after delivery (within 30 min). Placentas were also collected from pregnancies terminated surgically for psychological reasons during the first trimester (7–11 wk from last menstrual period) under Clause 2 of the United Kingdom Abortion Act of 1967. The collection of first-trimester placentas was approved by the Hospital Research Ethics Committee, and written informed consent was obtained. First-trimester tissue was collected on ice over a period of up to 3 h, pooled to give a total of at least 20 g starting material, and used to prepare MVM.

First-trimester and term MVM were prepared as described previously (22, 29). Term placental BM were prepared by a modification of the method of Kelley et al. (25) as recently described (21). Purity of MVM and BM was determined by measuring the enrichment for alkaline phosphatase activity (a marker for MVM) and dihydroalpinenol (DHA) binding (a marker for BM) in the membrane fractions as described (21, 22). The protein concentration in the membrane fractions and placental homogenate was determined by the method of Lowry et al. (28).

**Transport Assays**

Vesicles were suspended in intravesicular buffer (50 mM KCl, 50 mM choline chloride, 100 mM mannitol, and 20 mM HEPES-Tris, pH 7.4) and stored at 4°C. Transport assays were performed within 24 h of vesicle isolation. Vesicles were incubated in 4 µM valinomycin (Sigma) for 1 h at room temperature before transport assays, which were performed at room temperature (22–24°C).

Timed uptakes of [3H]arginine (0.2 µM, specific activity 1.0 mCi/ml; New England Nuclear) were performed in the presence or absence of Na^+ using 100 µl extravesicular buffer (EVB-Na^+: 50 mM KCl, 50 mM NaCl, 100 mM mannitol, and 20 mM HEPES-Tris, pH 7.4); or EVB-K^+: 100 mM KCl, 100 mM mannitol, and 20 mM HEPES-Tris, pH 7.4) containing 0.24 µM [3H]arginine. Uptake was initiated by the addition of 20 µl vesicle suspension (75–160 µg protein) and intravesicular [3H]arginine was separated by rapid filtration (17).

Strategy for the identification of transport systems. The criteria outlined by Deves and Boyd (11; see Table 1) were used to determine the contribution of different cationic amino acid transport systems to the uptake of L-arginine.

**TIME COURSE AND Na^+ DEPENDENCE OF L-ARGININE UPTAKE.** [3H]arginine (0.2 µM) uptake was measured at various time points (15, 30, 45, and 60 s; 2, 5, 10, 60, and 120 min) in the presence and absence of Na^+. [3H]arginine uptake in the presence and absence of Na^+ was compared using the paired t-test.

**DETERMINATION OF THE NUMBER OF TRANSPORT SYSTEMS, MICHAELIS CONSTANT, AND MAXIMAL VELOCITY.** Uptake of [3H]arginine (0.2 µM) was measured at 30 s (found in the time course experiments to be on the linear part of the uptake curve) in the presence of various concentrations of unlabeled L-arginine (0.1 µM–20 mM). Data were analyzed using computer models of the Michaelis-Menten equation with one and two transport components (Graphpad Prism 2.01), and the mean sum of squares of the regression lines was compared using the F-test. A two-transport-system model was accepted only if the mean sum of squares of the regression line was significantly lower (P < 0.05) than that of the one-system model. Otherwise, a one-transport-system model was accepted. Kinetic characteristics [Michaelis constant (K_m)] and maximal velocity (V_max)] of the transport systems and non-carrier-mediated uptake (C_Arg) were determined. The affinity of transport systems (log K_m) was compared using the Student's t-test.

**IDENTIFICATION OF SYSTEM y^+ L.** System y^+ L is a lower-affinity higher-capacity cationic amino acid transport system that does not transport neutral amino acids such as glutamine (Table 1). It was identified from the K_m (determined above) and from the observation of neutral amino acid (L-glutamine/ L-leucine; Table 1) insensitive [3H]arginine uptake. Therefore, uptake of [3H]arginine (0.2 µM) was measured in the presence of varying concentrations of unlabeled L-glutamine or leucine. Data were analyzed using nonlinear regression with a one-transport-system model. The component of [3H]arginine uptake that was insensitive to glutamine inhibition (C_Glu) was determined and compared (Mann Whitney U-test) with non-carrier-mediated [3H]arginine uptake (C_Arg determined above).

**IDENTIFICATION OF SYSTEMS y^- L AND b^0+.** Systems y^- L and b^0+ are higher-affinity cationic amino acid transport systems

![Table 1. Distinguishing characteristics of cationic amino acid transport systems](image-url)
that also transport neutral amino acids such as L-glutamine and L-leucine. Therefore, [3H]arginine uptake was measured as described in IDENTIFICATION OF SYSTEM Y'. The presence of glutamine/leucine-inhibitable [3H]arginine uptake was indicative of the presence of transport systems y' L or b0'.

**DISTINCTION BETWEEN SYSTEMS Y' L AND B0**. Neutral amino acid transport by system y' L is relatively Na⁺-dependent, whereas transport by system b0' is Na⁺-independent (Table 1). System b0' transports L-cystine, whereas system y' L does not (Table 1). Therefore, to distinguish between these two transport systems, [3H]arginine (0.2 µM) uptake was measured at 30 s (initial rate) in the presence of varying concentrations of unlabeled L-glutamine in the presence (EVB-Na⁺) and absence (EVB-K⁺) of Na⁺. Data were analyzed using nonlinear regression with a one-transport-system model, and the inhibition constant (Kᵢ) of glutamine in the presence and absence of Na⁺ was determined. An increase in the Kᵢ of glutamine when Na⁺ was replaced by K⁺ was positive identification of cationic amino acid transport system y' L. Similar experiments were undertaken using L-leucine. The presence of system b0' was positively tested for by determining L-cystine inhibition of [3H]arginine uptake. [3H]arginine uptake was measured at 30 s in the presence of varying concentrations of L-cystine (cysteine at 0.1 µM - 10 mM in the presence of 10 mM diamide; see Ref. 20). The observation of L-cystine-inhibitable [3H]arginine uptake would be indicative of transport system b0'. Kᵢ values are presented as the mean (95% confidence intervals).

In summary, transport system y' L was identified from the Kᵢ and the observation of glutamine-insensitive [3H]arginine uptake, and system y' L was identified from the Kᵢ and the observation of glutamine/leucine-inhibitable [3H]arginine uptake with an increase in Kᵢ for glutamine/leucine when Na⁺ was replaced by K⁺. System b0' was identified by the Kᵢ, Na⁺ independence with respect to neutral amino acid transport, and cystine sensitivity.

**RT-PCR**

RT-PCR was used to determine whether the cationic amino acid transporters CAT 1 and CAT 4, and regulator proteins 4F2hc and rBAT, were expressed by the human placenta at independence with respect to neutral amino acid transport, was replaced by K⁺ for glutamine/leucine when Na⁺ was replaced by K⁺. System b0' was identified by the Kᵢ, Na⁺ independence with respect to neutral amino acid transport, and cystine sensitivity.

RT-PCR

RT-PCR was used to determine whether the cationic amino acid transporters CAT 1 and CAT 4, and regulator proteins 4F2hc and rBAT, were expressed by the human placenta at different gestations. One gram of villous placenta was homogenized in 10 ml of 4 M guanidinium thiocyanate buffer and was stored at −80°C until required. RNA was extracted according to the method of Chomczynski and Sacchi (6). RNA integrity was confirmed by the presence of discrete 28S and 18S ribosomal RNA bands on electrophoresis through a 1.2% agarose/6.3% formaldehyde gel before the RT reaction.

Primers. Primers (20-mers) were designed from Genbank sequences as follows: transport system y', CAT 1/ERR 1 (Genbank accession no. X59155), 5' (1033)-ATCTGCCTTCATC-GCTACTT-3', 5' (1548)-TCTCTGCCTCTGGTAAAAC-3' and CAT 4 (Genbank accession no. A 000730), 5' (226)-ATGGTGCGGCTGGTCTCTA-3', 5' (510)-TGCGGATGCT- GTGCTGAAC-3'. Primer dimer/hairpin formation. These primers would be expected to generate cDNA fragments of 535 bp for CAT 1, 304 bp for CAT 4, 905 bp for 4F2hc, and 597 bp rBAT. PCR was performed over 30 cycles at an annealing temperature of 55°C for all primer pairs except CAT 4, in which case an annealing temperature of 60°C was used. All cDNA samples were run in 10 ml of 4 M guanidinium thiocyanate buffer and was stored at −80°C until required. RNA was extracted according to the method of Chomczynski and Sacchi (6). RNA integrity was confirmed by the presence of discrete 28S and 18S ribosomal RNA bands on electrophoresis through a 1.2% agarose/6.3% formaldehyde gel before the RT reaction.

**RESULTS**

**Membrane Purity**

First-trimester and term MVM were enriched (mean ± SE) 19.6 ± 1.3 (n = 10) and 17.6 ± 0.7 (n = 37)-fold for alkaline phosphatase activity, respectively (P = 0.19), whereas BM vesicles were enriched 1.6 ± 0.2-fold for alkaline phosphatase activity and 37.7 ± 3.7-fold (n = 14) for DHA binding.

**Time Course and Na⁺ Independence**

In all membrane fractions studied, [3H]arginine uptake was saturable, linear to 60 s, and Na⁺ independent (data not shown).

**Number of Transport Systems and Kᵢ and Vₘₐₓ**

Figure 1 shows [3H]arginine (0.2 µM) uptake at 30 s in the presence of varying concentrations of unlabeled L-arginine in term placental MVM and BM (Fig. 1A) and first-trimester MVM (Fig. 1B). In both MVM fractions, the mean sum of squares of the regression line with the two-transport-system model was significantly lower than with the one-transport-system model (P < 0.0001). The kinetic characteristics of the transport systems are shown in Table 2. With respect to the higher-affinity system in MVM, log Kᵢ was in the first trimester was significantly lower than log Kᵢ at term (P < 0.05). Log Kᵢ, Vₘₐₓ, and Vₘₐₓ were not significantly different between the first trimester and term. In BM, however, the mean sums of squares of the regression line with the two-transport-system model were not significantly different from that with the one-system model (P > 0.05). Data were therefore fitted to a one-system model with kinetic characteristics as shown in Table 2. The Kᵢ of the BM transport system was significantly different from that of the term MVM.
transport systems (P < 0.05). There was minimal nonsaturable [3H]arginine uptake in all membrane fractions: C Arg = 0.028 ± 0.004 and 0.027 ± 0.004 pmol·mg protein⁻¹·30 s⁻¹ for first-trimester and term MVM and 0.029 ± 0.003 pmol·mg protein⁻¹·30 s⁻¹ for term BM, representing 3.3, 3.6, and 8.2% of total uptake, respectively.

Identification of System y⁺

Figure 2 shows [3H]arginine uptake at 30 s in the presence of varying concentrations of unlabeled L-glutamine in MVM and BM from term placentas. Forty percent of [3H]arginine uptake by MVM was insensitive to glutamine inhibition. The noninhibitable component (CGlN = 0.32 ± 0.01 pmol·mg protein⁻¹·30 s⁻¹, n = 4) was significantly higher than non-carrier-mediated [3H]arginine uptake (CArg = 0.027 ± 0.004 pmol·mg protein⁻¹·30 s⁻¹, n = 11, P < 0.0001). Using the Michaelis constant (Km) for the high-affinity system in MVM, the inhibition constant (Ki) for L-glutamine was 115 and 470 µM for MVM and BM, respectively. Data are means ± SE for n = 4 and 3 for MVM and BM, respectively.

Table 2. Kinetic characteristics of placental MVM and BM cationic amino acid transport systems

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Km1, µM</th>
<th>Vmax,L, pmol·mg protein⁻¹·30 s⁻¹</th>
<th>Km2, µM</th>
<th>Vmax2, pmol·mg protein⁻¹·30 s⁻¹</th>
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<tbody>
<tr>
<td>MVM</td>
<td></td>
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<tr>
<td>First trimester</td>
<td>10</td>
<td>4.5 (3.9–5.2)*</td>
<td>16.7 (13.9–19.4)</td>
<td>291 (130–653)</td>
<td>167.6 (58.4–276.8)</td>
</tr>
<tr>
<td>Term</td>
<td>11</td>
<td>7.6 (5.9–9.7)</td>
<td>20.3 (13.5–27.1)</td>
<td>224 (119–419)</td>
<td>228.5 (132.5–324.5)</td>
</tr>
<tr>
<td>Term BM</td>
<td>5</td>
<td>48.4 (42.7–55.0)*</td>
<td>79.2 (69.1–89.3)</td>
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Data are mean values with 95% confidence intervals in parentheses; n, no. of placentas. MVM, microvillous membrane; BM, basal plasma membrane; Vmax, maximal velocity; 1 and 2, transport systems 1 and 2, respectively. [3H]arginine (0.2 µM) uptake was measured in the presence of 0.1 µM–20 mM unlabeled L-arginine, and data were analyzed using nonlinear regression with a one- or two-transport-system model. There was evidence for two transport systems (1 and 2) in MVM (first trimester and term) but only one transport system in BM. *P < 0.05, unpaired Student’s t-test; first-trimester MVM and term BM vs. term MVM.
Distinction Between Systems y\textsuperscript{+}L and b\textsuperscript{0+}

The effect of Na\textsuperscript{+} replacement (by K\textsuperscript{+}) on glutamine inhibition of [\textsuperscript{3}H]arginine uptake by MVM (first trimester) and BM (term) is shown in Figs. 3 and 4, respectively. In both membrane fractions, there was an increase in \(K_i\) when Na\textsuperscript{+} was replaced by K\textsuperscript{+} from 100 µM (75–132 µM) to 1,845 µM (1,477–2,305 µM), the extent of inhibition was however, not Na\textsuperscript{+} dependent. Data are means ± SE, and \(K_i\) are mean values [95% confidence interval (CI)] for \(n=3\) placentas.

RT-PCR

RT-PCR using 4F2hc specific primers revealed a cDNA product of 905 bp, as expected, in first-trimester, second-trimester, and term placental tissue (Fig. 6A). CAT 1 and CAT 4 were also uniformly expressed in placental tissue from all gestational ages, with a cDNA product of 535 and 304 bp, respectively, as expected for the primers used (Fig. 6, B and D). rBAT expression was evident in all first-trimester and some second-trimester placental tissue but was uniformly absent in all term placental tissue studied (Fig. 6C). Total RNA from renal tissue was used as a positive control for rBAT expression, and a 597-bp cDNA product was observed as expected for these primers.

Western Blotting

Figure 7, A and B, shows the results of Western blotting using anti-CD98 (4F2hc) antibody with term placental MVM and BM under reducing (Fig. 7A) and nonreducing (Fig. 7B) conditions. A specific ~85-kDa band was observed under reducing conditions in MVM but not in BM. Under nonreducing conditions, an ~135-kDa specific band was observed in MVM but not in BM. The bands that were observed in BM persisted when the primary antibody was preincubated with blocking peptide and when protein electrophoresis was performed under reducing and nonreducing conditions (Fig. 7C), indicating that these bands represented nonspecific antibody binding. A specific ~85-kDa band was also observed in MVM samples from first-trimester placentas under reducing conditions (Fig. 8, A and B). The mean density of this band in term MVM was 1.9 times that in first-trimester MVM (\(P=0.037\), \(n=4\) each). Under nonreducing
conditions, multiple bands were observed in first-trimester and term MVM; a predominant band was observed at \( \sim 135 \) kDa, and at least two bands were observed at \( \geq 207 \) kDa (Fig. 9A). To determine the specificity of these bands, the same term MVM sample was run in all eight lanes, the membrane was bisected, and one-half was used as a negative control (no primary antibody, Fig. 9B). This confirms the specificity of the two bands at \( \sim 135 \) and \( > 207 \) kDa under nonreducing conditions. An \( \sim 85 \)-kDa band was not detected in any MVM samples under nonreducing conditions. The mean density of the \( \sim 135 \)-kDa band in term MVM was 1.7 times that of first-trimester MVM (P = 0.038, n = 4 each).

**DISCUSSION**

In this study, we have characterized human placental cationic amino acid transport systems using kinetic and substrate inhibition studies, RT-PCR, and Western blotting. In MVM from first-trimester and term placentas, we have evidence for two transport systems: a higher-affinity, lower-capacity system and a lower-affinity, higher-capacity system. The MVM data also showed a neutral amino acid-sensitive and a neutral amino acid-insensitive \([3H]arginine transport system, which is supportive of the two-transport-system model. The neutral amino acid-sensitive component showed a significant reduction in its affinity for neutral amino
acids when Na\(^+\) was replaced by K\(^+\). The observations that \(^{3}H\)arginine uptake by MVM was Na\(^+\) independent and was not inhibited by \(\gamma\)-cystine indicated that systems B\(^0\) and \(B^\gamma\) were not expressed in MVM. On these bases, we have identified the lower-affinity system in MVM as system \(y^\gamma\) and the higher-affinity system as system \(y^\text{L}\). This is the first characterization of cationic amino acid transport systems in first-trimester placental MVM. The conclusions on term MVM are broadly similar to those of Furesz et al. (18).

In BM, we have kinetic evidence for one transport system only. The observation of homogeneity using kinetic studies was confirmed by substrate inhibition studies. We observed that \(^{3}H\)arginine uptake by BM was almost totally inhibited by \(\gamma\)-glutamine and \(\gamma\)-leucine, with the noninhibitable component representing only 6.4% of total carrier-mediated uptake. This could represent a glutamine-insensitive transport system undetectable by kinetic studies because of its relatively small contribution to total uptake. Alternatively, this could be indicative of slight contamination of BM by MVM.

The \(^{3}H\)arginine transport system in BM has a \(K_m\) that is higher than that for system \(y^\text{L}\) in MVM found in this study and higher than that previously reported for system \(y^\text{L}\) in the placenta (18) or other membranes.
The observation in the report was completely inhibited by L-leucine, L-alanine, and L-phenylalanine. The conclusion of Furesz et al. (17) that system 1 in their study was system y^+L rather than system b^0. Further work is required to localize the site of rBAT expression in the placenta of early pregnancy. CAT 4 mRNA has previously been shown to be expressed in placenta (36), and this study provides the first demonstration that CAT 4 mRNA is expressed in this tissue throughout gestation. The presence of system y^+ activity in MVM vesicles would suggest that CAT 4 is localized to the MVM rather than the BM.

Earlier reports of cationic amino acid transport systems of the term human placenta identified the presence of transport systems y^+, y^-L, and b^0 in purified BM using radiolabeled l-lysine. In the first report, Furesz et al. (17) identified systems y^+ (system 1) and system b^0 (system 2) in BM. System y^-L was identified from kinetic studies (K_m) and by the observation that neutral amino acids (2 mM) inhibited [H]lysine (20 µM) uptake in a relatively Na^+-dependent manner. System b^0 (system 2) was isolated by using a lower concentration of tracer (0.2 µM [H]lysine) and by saturating system 1 with 10 mM l-homoserine in the presence of Na^+. System 2 was found to be completely sensitive to l-leucine and l-methionine inhibition. In essence, therefore, both systems 1 and 2 in their study could be completely inhibited by neutral amino acids in the presence of Na^+. This is precisely in line with our observation that virtually all [H]arginine uptake by BM is sensitive to neutral amino acid (l-glutamine/l-leucine) inhibition.

The conclusion of Furesz et al. (17) that system 1 in their study was system y^+ is not consistent with what is now known about this transport system. In seminal papers, Kim et al. (26) and Wang et al. (39) reported that the murine ecotropic retrovirus receptor mediated cationic amino acid transport; expression in Xenopus oocytes resulted in increased l-arginine, l-lysine, and l-ornithine uptake. The uptake of l-leucine, l-glutamine, l-alanine, and l-phenylalanine was not increased, even in the presence of Na^+. Other studies have shown that system y^+ interacts only very weakly, if at all, with l-leucine in the presence of Na^+ (K, for inhibition of 1 µM lysine uptake = 30.36 mM in erythrocytes; see Ref. 10). The observation in the report by Furesz et al. (17) that BM [H]lysine uptake was completely inhibited by l-leucine, l-alanine, and l-phenylalanine is therefore not consistent with system y^-L activity. Their identification of system 2 as system b^0 was understandable, as system y^-L had not been discovered. Therefore, the data of Furesz et al. (17, 18) on l-lysine uptake by term BM are not inconsistent with our own on l-arginine uptake. However, in the light of more recent knowledge of the characteristics of the different transport systems, as well as the PCR data, the interpretation is that only system y^-L is present in BM.

With the use of Western blotting and an anti-CD98 (4F2hc) antibody, an ~85-kDa band was identified in MVM under reducing conditions. An ~135-kDa band was identified under nonreducing conditions, consistent with earlier reports on 4F2hc and 4F2hc-light chain dimer, respectively (23), with two putative light chains (y^-LAT-1 and y^-LAT-2) identified that are associated with 4F2hc by disulfide bonds and which mediate system y^-L amino acid transport (37). Because other light chains complex with 4F2hc, it is also possible that the ~135-kDa band represents additional 4F2hc-light chain complexes.

In addition to the previously reported ~135-kDa heavy chain-light chain dimer, we also observed a specific band at ~207 kDa. To the best of our knowledge, this is the first report of 4F2hc multimers with this molecular weight. With the use of rat renal brush border membranes and membranes from NBAT homologous to rBAT-injected oocytes under nonreducing conditions and anti-NBAT antibodies (40), bands of 180 to ~200 kDa were observed, and it was suggested that these could represent NBAT dimers or higher NBAT aggregates. In a more recent report, Torrents et al. (37) observed an 85- and 169-kDa band from oocytes after 4F2hc and y^-LAT-1 cRNA injection ([35S]methionine labeling, and immunoprecipitation with anti-4F2hc monoclonal antibody and electrophoresis. They suggested that the 169-kDa band represented 4F2hc homodimers. These data suggest that 4F2hc and NBAT are capable of forming homodimers and heterodimers and may form larger structures. The ~207-kDa band reported here may therefore represent higher 4F2hc aggregates. Alternatively, it could represent associations between 4F2hc and other proteins mediating different, non-transport-related activities such as cell fusion (34) and integrin function (16). These proteins would have to be ~120 kDa in size, and to date no protein of this size associated with 4F2hc by disulfide bonds has been reported. Thus it is possible that 4F2hc in the MVM serves additional functions other than mediating amino acid transport.

We did not observe 4F2hc monomers in MVM under nonreducing conditions, in contrast to the situation with NBAT in the rat renal brush-border membrane, where an 85-kDa band and a 135-kDa band were observed under nonreducing conditions. The 85-kDa band made up 50–85% of the total NBAT-containing species (40). It has been suggested that 4F2hc is expressed on the cell surface as a monomer on its own and plays a role in the trafficking of the light chain to the plasma membrane (33). Our failure to detect 4F2hc monomers under nonreducing conditions suggests that its role, or at least the stoichiometry of interaction with
the light chain, may be different in the syncytiotrophoblast.

In BM, we found that neither the ~85-kDa nor the ~135-kDa band was detected under reducing or nonreducing conditions, granted that this may have been limited by the sensitivity of Western blots. However, there is no doubt that 4F2hc is expressed at a much lower level in BM compared with MVM. This contrasts with the exclusive basolateral localization in other epithelia such as the kidney and intestine (11). This failure to detect 4F2hc despite the presence of kinetically defined system y\(^{-}\)-L is a novel observation as far as we are aware. Kinetic studies show that the \(K_{m}\) for system y\(^{-}\)-L is significantly higher in term BM compared with MVM and in term MVM compared with first-trimester MVM. These differences may be as a result of 1) differences in light chain expression between first-trimester and term MVM and between term BM and term MVM and/or 2) differences in the heavy chain-light chain stoichiometry. We observed that the expression of the ~85-kDa 4F2hc monomer and the ~135-kDa 4F2hc heterodimer were significantly higher in term compared with first-trimester MVM. There is now strong evidence for the existence of multiple putative light chains (30, 37) and evidence that different light chains may be expressed in different tissues in the same species (33). Further work is needed to identify the native light chain(s) in the syncytiotrophoblast and to examine their structural and functional interaction with 4F2hc.

We conclude that human placental cationic amino acid transport is by systems y\(^{-}\) and y\(^{-}\)-L in first-trimester and term MVM and by system y\(^{+}\)-L only in term BM. The lower affinity and higher capacity of system y\(^{+}\) in addition to the favorable transmembrane potential difference means that it will be the predominant system in MVM in vivo. However, system y\(^{-}\)-L, being an amino acid exchanger, may generate higher intracellular cationic amino acid concentrations (5). In the light of evidence for the intracellular compartmentalization of \(\text{L-arginine metabolism (8, 9), the roles of system y}\(^{-}\)-L in supplying different intracellular compartments require further investigation. We propose that the net materno-fetal transfer of cationic amino acids across the human placenta is driven by the Na\(^{+}\)-dependent uptake of neutral amino acids in BM by system y\(^{-}\)-L in exchange for cationic amino acid transfer to the fetus. The observed differences in the expression of 4F2hc in MVM across gestation, and between MVM and BM at term, may in part account for the different kinetic properties of system y\(^{-}\)-L in these membrane fractions. The newly described >207 kDa 4F2hc aggregates described here suggest that the 4F2hc-light chain interaction may be more complex than previously thought. The molecular basis and functional significance of these multimers await sequencing of the native light chain(s) in the syncytiotrophoblast.

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Address for reprint requests and other correspondence: J. Glazier, Academic Unit of Child Health, Univ. of Manchester, St. Mary’s Hospital, Hathersage Road, Manchester M13 0JH, UK (E-mail: jgglazier@fs1.mci.man.ac.uk).

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