IL-6 stimulates system A amino acid transporter activity in trophoblast cells through STAT3 and increased expression of SNAT2

H. N. Jones, T. Jansson, and T. L. Powell

Department of Obstetrics and Gynecology, College of Medicine, University of Cincinnati, Cincinnati, Ohio

Submitted 4 May 2009; accepted in final form 22 June 2009

Jones HN, Jansson T, Powell TL. IL-6 stimulates system A amino acid transporter activity in trophoblast cells through STAT3 and increased expression of SNAT2. Am J Physiol Cell Physiol 297: C1228–C1235, 2009. First published September 9, 2009; doi:10.1152/ajpcell.00195.2009.—Changes in placental nutrient transport are closely associated with abnormal fetal growth. However, the molecular mechanisms underlying the regulation of placental amino acid transporters are unknown. We demonstrate that physiological concentrations of the proinflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor (TNF-α) stimulate the activity of amino acid transporter system A, but not system L, in cultured human primary trophoblast cells. Both cytokines increased the gene and protein expression of the Na⁺–coupled neutral amino acid transporter (SNAT)2 isoform and upregulated SNAT1 protein expression. IL-6 increased Tyr705 phosphorylation of signal transducer and activator of transcription 3 (STAT3). In cells transfected with small interfering RNA (siRNA) targeting STAT3, the RNA and protein expression of SNAT2, but not SNAT1, was reduced and the stimulating effect of IL-6 on system A activity was abolished. Despite eliciting similar responses in amino acid transport activity and transporter expression, TNF-α effects on system A activity were not mediated through the JAK/STAT pathway. In conclusion, we have identified a novel regulatory pathway involving increased gene expression of the SNAT2 isoform mediated by a STAT-dependent pathway, which links IL-6 to increased activity of system A, a ubiquitously expressed transporter of neutral amino acids. From these new findings, we propose that upregulation of amino acid transporters by cytokines may contribute to increased placental nutrient transport and fetal overgrowth, which are commonly found in pregnancies complicated by maternal diabetes and obesity.

IN HUMAN PREGNANCY complicated by diabetes or obesity, maternal circulating levels of IL-6 and TNF-α are elevated (2, 3), the activity of placental amino acid transporters is increased (19), and fetal overgrowth is common (1, 5). Maternal IL-6 levels are correlated with fetal adiposity at birth (33), and this raises the possibility that cytokines may regulate placental nutrient transport and delivery to the fetus. Recent evidence suggests that alterations in placental system A and system L amino acid transporters play a key role in the development of pathological fetal growth (18, 19), which may lead to increased risk of the metabolic syndrome in later life (11).

Amino acid transport system A is ubiquitously expressed and mediates the cellular uptake of small, neutral amino acids by cotransporting sodium (27). There are three isoforms, Na⁺–coupled neutral amino acid transporter (SNAT)1, SNAT2, and SNAT4, encoded by the genes Slc38a1, Slc38a2, and Slc38a4 (15, 38), respectively. SNAT1 and SNAT2 operate via similar mechanisms (15, 38), while SNAT4 has a lower affinity for neutral amino acids and interacts with cationic amino acids in a sodium-independent manner resembling system y⁺L (14).

System A transports large neutral amino acids in a sodium-independent manner and consists of differing light chains [ι-type amino acid transporter (LAT)1 encoded by the gene Slc7a5 and LAT2 encoded by Slc7a9] associated with the heavy chain 4F2hc or CD98 (Slc3a2) (24).

System A amino acid transporter activity is highly regulated by many factors including insulin, cortisol, oxygen availability (20), and amino acid availability (20); however, the intracellular signaling mechanisms are poorly understood. Information on how cellular amino acid uptake is regulated by cytokines in mammalian cells is limited (10). IL-6 stimulates hepatic amino acid uptake in the rat (39), and TNF-α upregulates arginine uptake in human umbilical vein endothelial cells (35). Furthermore, IL-1β downregulated system A in a trophoblast cell line (37). However, these studies have provided no information on the cellular signaling mechanisms involved.

Isolation of human cytotrophoblasts from healthy term placentas (23) and culture for 90 h allows for differentiation of these cells to syncytiotrophoblasts as demonstrated by a rapid rise in human chorionic gonadotropin (hCG) secretion. We have used this model to study the effects of IL-6 and TNF-α on trophoblast amino acid uptake. Our data suggest that physiological concentrations of IL-6 and TNF-α increase the activity of the amino acid transporter system A but not system L. This is the first report that signal transducer and activator of transcription 3 (STAT3) may be involved in the regulation of system A amino acid transporter isoforms. Pregnancies complicated by pathological fetal growth, for example, intrauterine growth restriction (IUGR), diabetes, or obesity, are characterized by changes in maternal metabolic and inflammatory markers. We propose that increased levels of maternal inflammatory cytokines in pregnancies complicated by obesity will increase placental nutrient transporter activity and contribute to fetal overgrowth.

MATERIALS AND METHODS

Cytotrophoblast isolation. Cytotrophoblast cells were isolated from normal term placentas as reported elsewhere (23, 34). Tissue collection was approved by the Institutional Review Board of the University of Cincinnati College of Medicine. Using vimentin as a marker, we previously demonstrated (34) that these cells are not significantly contaminated by cells of mesenchymal origin, such as macrophages. Briefly, cells were plated and cultured for a total of 90 h with medium changes at 20, 44, and 66 h to allow cell differentiation. Cultured primary trophoblast cells were incubated with cytokines for 24 h, from 66 to 90 h after plating.

RNA interference. Dharmafect 2 transfection reagent (Thermo Scientific, Rockford, IL) and three proprietary small interference RNAs (siRNAs) (Sigma-Aldrich, St. Louis, MO), targeted against
STAT3 (1: 5′GGAUACGCUAUACAGCA, 2: 5′GGUACAUCAUUGGCUUUAAU, 3: 5′UGCAGUACUAGGCGAGA) or a noncoding scrambled sequence (sense: 5′GAUCAUCGUGGAGCAAGA) were added to cultured primary trophoblast cells after 20 h in culture, incubated for 24 h, and removed, and fresh medium was added to wells (9). Initially all three siRNAs were tested, and the construct that most efficiently silenced STAT3 was used in all further assays. At 66 h (total culture time) cultured primary trophoblast cells were treated with control medium, IL-6, or TNF-α for 24 h.

**Amino acid uptake assay.** System A activity was measured as Na⁺-dependent uptake of 14C-labeled 2-(methylamino)isobutyric acid (MeAIB; 10 μM), and system L amino acid transporter activity was determined as 2-aminoisobicycloc(2.2.1)heptane-2-carboxylic acid (BCH)-inhibitable uptake of 1-[3H]leucine (0.05 μM) (34). Cultured primary trophoblast cells in triplicate were incubated in buffers with and without Na⁺ (isosmotic choline replacement) and in the presence and absence of 1 mM BCH. Uptake of [1H]MeAIB and [1-14C]leucine was stopped after 8 min by rapidly rinsing cells with isotope-free, ice-cold isosmotic buffer. After washing, cells were incubated for 1 h in distilled water to lyse the cells and release the isotope. After lysis, residual cells did not retain radioactivity and were used to measure protein content by Bradford assay. Radioactivity in cell lysates was counted in a liquid scintillation counter, and mediated uptake was calculated as picomoles per minute times milligram of protein, using standards with known amounts of isotope.

**Western blot.** Protein expression of the system A transporter isoforms SNAT1, -2, and -4, STAT3, phospho-STAT3, and β-actin was analyzed with Western blotting. A polyclonal SNAT2 antibody was kindly provided by Dr. P. D. Prasad (Medical College of Georgia, Augusta, GA). Affinity-purified polyclonal anti-SNAT1 (raised against the peptide sequence VPEDDNISDNSDFT) and anti-SNAT4 (YGVEVEDHHAYSKV) antibodies were generated in rabbits by Eurogentec. Anti-SNAT3 and anti-phospho-STAT3 (Tyr705) antibodies were purchased from Cell Signaling, and anti-β-actin antibody was purchased from Sigma (St. Louis, MO). Protein concentrations were determined by Bradford assay, and Western blotting was performed. Briefly, 15 μg of total protein was loaded onto a 10% SDS-PAGE gel, and electrophoresis was performed at a constant 100 V for 2 h. Proteins were transferred onto nitrocellulose membranes overnight at a constant 30 V. Membranes were incubated with primary antibodies overnight (SNAT2, phospho-SNAT3) or for 1 h at room temperature (SNAT1 and -4, STAT3, GAPDH, β-actin), washed, and incubated with the appropriate secondary peroxidase-labeled IgG (1:1,000–1:5,000) for 1 h. After washing, bands were visualized with ECL detection reagents (GE Healthcare). Blots were stripped and reprobed for β-actin as a loading control. Analysis of the blots was performed by densitometry with Alpha Imager (Alpha Innotech, San Leandro, CA).

**Real-time PCR.** After exposure of cultured primary trophoblast cells to 0.002 ng/ml of either IL-6 or TNF-α or control medium for 24 h, total RNA was isolated with TRIzol reagent (Invivogen, Carlsbad, CA) and reverse transcribed with a Quantitect Reverse Transcription kit (Qiagen, Valencia, CA). Proprietary Quantitect Primer Assays for SNAT1, -2, and -4 and succinate dehydrogenase A (SDHA) were obtained from Qiagen. Quantitative PCR was performed in triplicate in 96-well plates according the standard Quantitect SYBR PCR protocol in a 7300 PCR system (Applied Biosystems, Foster City, CA). For analysis STAT3 and SNAT1, -2, and -4 were standardized against SDHA expression as an internal control previously identified as a stable housekeeping gene for expression studies in the placenta (28). As negative controls, preparations lacking RNA were used in the place of cDNA, RNAs were assayed by seven independent biological replicates. RNA levels are expressed as a ratio, using the ΔΔCₘ method (where Cₘ is threshold cycle) for comparing relative expression results between treatments in real-time PCR.

**ELISAs.** Measurement of hCG in the medium was measured with an ELISA kit (Immuno Biological Labs, Minneapolis, MN) according to a standard protocol at 20, 44, 66, and 90 h of culture. Cultured primary trophoblast cell medium was collected after 24-h incubation with 0.002 ng/ml of either IL-6 or TNF-α. IL-6 or TNF-α concentrations in medium were determined by colorimetric ELISA (Thermo Scientific, Rockford, IL) according to instructions provided by the manufacturer.

**Data presentation and statistics.** Data are presented as means ± SE. Statistical significance of differences between control and cytokine-treated cells was assessed with repeated-measures (RM) ANOVA or Student’s t-test. A P value <0.05 was considered significant.

**RESULTS**

In control cultured primary trophoblast cells there was a fivefold increase in hCG levels between 44 and 66 h, and hCG levels remained at or above this level at 90 h after plating, demonstrating cell differentiation and syncytialization (Fig. 1). Incubation of cultured primary trophoblast cells in IL-6 or TNF-α between 66 and 90 h or exposure of cultured primary trophoblast cells to siRNA and transfection agents between 20 and 44 h did not alter hCG production (Fig. 1), suggesting that cell differentiation and hCG production were unaffected by our experimental perturbations.

Sodium-dependent MeAIB uptake, representative of system A activity, increased twofold after 24-h incubation of cultured primary trophoblast cells with physiological concentrations of IL-6 (0.002 ng/ml; P = 0.016, n = 6 for each treatment). In contrast, IL-6 at higher concentrations did not stimulate system A activity (Fig. 2). In response to TNF-α treatment a dose-dependent increase in sodium-dependent MeAIB uptake was observed (Fig. 2; P = 0.004, n = 6 for each treatment). At physiological concentrations (0.002 ng/ml) TNF-α increased system A activity by 100%. BCH-inhibitable L-leucine uptake, representative of transport by system L, was unaltered by exposure of the cultured primary trophoblast cells to any concentration of TNF-α or IL-6 (Fig. 3).

Because of the marked stimulation of system A by both cytokines at physiological levels (0.002 ng/ml), we used this concentration for all further studies. After 24-h exposure of cultured primary trophoblast cells to 0.002 ng/ml of either IL-6 or TNF-α, total RNA and protein were isolated at 90 h after plating and analyzed by real-time PCR or Western blotting for SNAT1, -2, and -4 expression levels. Both RNA (Fig. 4A; RM ANOVA P < 0.01, n = 7) and protein (Fig. 4B; RM ANOVA P < 0.01, n = 7) levels for SNAT2 were increased after exposure to 0.002 ng/ml IL-6 or TNF-α. SNAT1 RNA expression remained at control levels (Fig. 4A); however, protein levels were significantly increased with exposure to both cytokines (Fig. 4B; RM ANOVA P < 0.01, n = 7). IL-6 and TNF-α had no significant effect on SNAT4 RNA or protein expression (Fig. 4).

At 66 h after plating, exposure of cultured primary trophoblast cells to IL-6 (0.002 ng/ml) for a further 24 h markedly increased the expression of phospho-STAT3 compared with control, whereas TNF-α had no effect. Total STAT3 expression remained at control levels after exposure to either IL-6 or TNF-α (Fig. 5; n = 7).

After 24-h incubation of cultured primary trophoblast cells at 20 h after plating with the siRNA that most efficiently silenced STAT3 (siRNA 1; Fig. 6B), STAT3 RNA (Fig. 6A; n = 4) and protein (Fig. 6C; n = 6) expression levels were
reduced by ~70\% by 90 h after plating. Similarly, in those cultured primary trophoblast cells treated with the STAT3 siRNA, SNAT2 RNA and protein expression were diminished (Fig. 6) but SNAT1 expression levels remained equal to control (transfection reagent alone) (Fig. 6). Suppressor of cytokine signaling 3 (SOCS3), a known IL-6-STAT3 target, was also reduced after STAT3 knockdown (Fig. 6). To ensure that reducing STAT3 levels with siRNA led to decreased phospho-STAT3 levels after IL-6 treatment we isolated protein from control, IL-6 treated, and siRNA IL-6-treated cultured primary trophoblast cells. Figure 7 demonstrates that silencing STAT3 abolished the IL-6-stimulated increase in phospho-STAT3 protein levels ($n = 5$). Amino acid uptake assays were performed on cultured primary trophoblast cells previously exposed to siRNA targeted to STAT3 or a scrambled RNA sequence. Incubation with scrambled siRNA did not affect basal sodium-dependent MeAIB uptake or the increase of system A activity in response to IL-6 at a total culture time of 90 h (Fig. 8). Silencing STAT3 did not alter basal sodium-dependent MeAIB uptake (Fig. 8). However, after STAT3 knockdown, the stimulatory effect of IL-6 on sodium-dependent MeAIB uptake was abolished (Fig. 8) (RM ANOVA $P < 0.01; n > 4$ for each treatment). However, the TNF-α stimulation of sodium-dependent MeAIB uptake persisted despite the knockdown of STAT3 (Fig. 8).

In a previous study of isolated human trophoblast cells, treatment with TNF-α increased secretion of IL-6 after only 30 min (26); therefore, we investigated the effects of 24-h stimulation on cytokine secretion. Exposure of cultured primary trophoblast cells for 24 h to 0.002 ng/ml IL-6 did not change (Student’s $t$-test; $n = 7$ for all treatments) TNF-α levels (8.9 ± 1.65 pg/mg; $n > 7$) compared with control cells (8.8 ± 2.7 pg/mg). Likewise, treatment with 0.002 ng/ml TNF-α for 24 h did not significantly alter IL-6 levels (343 ± 95 pg/mg) compared with control cells (266 ± 42 pg/mg).

**DISCUSSION**

In this study we demonstrate for the first time that physiological concentrations of IL-6 and TNF-α markedly stimulate the activity of amino acid transport system A in cultured...
An important novel aspect of the present study is the demonstration that the effect of IL-6 on system A activity is mediated by activation of the STAT3 signaling pathway, increasing the gene and protein expression of the system A transporter isoform SNAT2. This is in contrast to the mechanism of TNF-α stimulation of system A activity, which was mediated by increased protein expression of SNAT1 and SNAT2 without effect on STAT3 signaling and SNAT1 gene expression. Pregnancies complicated by diabetes or obesity are characterized by elevated proinflammatory cytokines, insulin resistance, increased placental amino acid uptake, and fetal overgrowth (1, 2, 19). This study identifies a molecular link between proinflammatory cytokines and placental amino acid transport and may provide novel insights into mechanisms causing fetal overgrowth in pregnant women with obesity or diabetes. STAT3 has not previously been implicated as a cellular mediator in the regulation of nutrient transport.

Our data demonstrate differential regulation of the SNAT isoforms by proinflammatory cytokines, since protein expression of SNAT1 and SNAT2 was upregulated by IL-6 and TNF-α whereas these cytokines did not affect SNAT4 expression. The finding that SNAT4 is regulated differently from the two other SNAT isoforms is consistent with observations reported in the literature. For example, a trophoblast-specific knockdown of the igf2 gene in mice alters the gene expression of SNAT4 but not of SNAT1 or SNAT2 (6, 36), and we recently reported (21) that a high-fat diet in pregnant mice caused a marked upregulation of placental SNAT2 but not SNAT4 expression. In the human placenta, SNAT4 gene expression is gestationally regulated but SNAT1 and SNAT2

Fig. 4. A: summary data of real-time PCR of system A Na⁺/H⁺-coupled neutral amino acid transporter (SNAT) isoforms SNAT1, SNAT2, and SNAT4 after incubation of cultured primary trophoblast cells with control medium (C), TNF-α (0.002 ng/ml), or IL-6 (0.002 ng/ml) for 24 h. Data are means ± SE for 7 placentas. IL-6 and TNF-α (RM ANOVA, P < 0.01) significantly increased SNAT2 gene expression (Tukey-Kramer multiple comparison posttests, *P < 0.05, **P < 0.01). B: representative Western blot of SNAT1, -2, and -4 and β-actin in control (C)-, 0.002 ng/ml IL-6-, or 0.002 ng/ml TNF-α-treated cultured primary trophoblast cells. C: summary data are means ± SE for cultured primary trophoblast cells isolated from 7 different placentas. IL-6 and TNF-α (RM ANOVA, P < 0.001) significantly increased SNAT1 and SNAT2 protein expression (Tukey-Kramer posttests, *P < 0.05, **P < 0.01).

Fig. 5. Representative Western blot and summary data of phospho-signal transducer and activator of transcription 3 (STAT3) (Tyr705), total STAT3, and β-actin in control (C)-, 0.002 ng/ml IL-6-, or 0.002 ng/ml TNF-α-treated cultured primary trophoblast cells. Summary data are means ± SE for cells isolated from 6 different placentas. IL-6 significantly increased phospho-STAT3 expression (RM ANOVA, P > 0.01, Tukey-Kramer posttest **P < 0.05).
mRNA levels do not change from first trimester to term (8). In addition, preliminary data from our laboratory indicate that SNAT2 protein expression is increased and SNAT4 protein expression remains unchanged in the placenta of obese women giving birth to large babies (N. Jansson, unpublished observation). In the present study both IL-6 and TNF-α increased SNAT1 and SNAT2 protein expression; however, only the changes in SNAT2 appeared to be mediated by STAT3 transcriptional activation. These findings suggest that the levels at which the SNAT isoforms are regulated differ and that there are likely to be distinct intracellular pathways involved in mediating cytokine regulation of amino acid transport system A.

In other cell types JAK-STAT signaling is a key intracellular pathway mediating the effects of IL-6 on gene transcription (31). TNF-α signal transduction usually involves activation of ERK (12) or NF-κB (25) signaling pathways, leading to increased gene transcription (12). However, TNF-α can also activate the JAK/STAT pathway in human B cells (29). Therefore, since our data suggested that the stimulation of system A activity in response to IL-6 and TNF-α involved increased SNAT2 gene expression, we investigated the role of the tran-

---

**Fig. 6.** A: summary of STAT3, SNAT1, and SNAT2 RNA expression after exposure of cultured primary trophoblast cells to control medium or siRNA targeted against STAT3 20 h after plating for 24 h, wash, and culture to 90 h. Data are means ± SE for cells isolated from 4 different placentas. Exposure to siRNA significantly (RM ANOVA, \( P < 0.01 \)) reduced STAT3 and SNAT2 RNA expression in control and IL-6-stimulated cells (Tukey-Kramer posttests, * \( P < 0.05 \)). B: representative Western blots of STAT3, SNAT1, SNAT2, and β-actin at 90 h of total culture time. Cultured primary trophoblast cells were incubated with control medium or Dharmafect transfection agent and 3 different siRNAs (1, 2, or 3) targeted against STAT3 20 h after plating for 24 h, washed, and cultured to 90 h. C: representative Western blots of suppressor of cytokine signaling 3 (SOCS3) and β-actin at 90 h of total culture time. Cultured primary trophoblast cells were incubated with control medium or Dharmafect transfection agent and siRNA 1 targeted against STAT3 20 h after plating for 24 h, washed, and cultured to 90 h. D: summary of STAT3, SOCS3, SNAT1, and SNAT2 protein expression after exposure of cultured primary trophoblast cells to control medium or siRNA 1 targeted against STAT3. Data are means ± SE for cells isolated from 6 different placentas. Exposure to siRNA 1 significantly (paired \( t \)-test ** \( P < 0.01 \)) reduced STAT3, SOCS3, and SNAT2 protein expression.

---

**Fig. 7.** Representative Western blot and summary data of phospho-STAT3, STAT3, and β-actin at 90 h of total culture time. Cultured primary trophoblast cells were incubated with control medium or Dharmafect transfection agent and siRNA targeted against STAT3 20 h after plating for 24 h, washed, and cultured to 66 h, at which time cells were exposed to control medium or control medium + 0.002 ng/ml IL-6. In IL-6-treated cultured primary trophoblast cells that were exposed to siRNA targeted against STAT3, phospho-STAT3 expression levels were significantly reduced compared with IL-6-treated control cells (RM ANOVA \( P > 0.01 \); Tukey-Kramer posttest ** \( P < 0.05 \)).

---

by 10.220.33.2 on June 22, 2017
scription factor STAT3 in these effects. A role for STAT3 in mediating the effects of IL-6 on SNAT2 was indicated by the increased Tyr705 phosphorylation of STAT3 in response to IL-6. In contrast, there was no change in phospho-STAT3 protein expression levels after 24-h exposure to TNF-α, suggesting that the effects of TNF-α were mediated through a distinct signaling pathway.

In the present study, TNF-α but not IL-6 elicited a dose response in sodium-dependent MeAIB uptake by cultured primary trophoblasts. A maximum effect was seen with 0.002 ng/ml IL-6, a physiologically relevant dose in overweight/obesity (40), and increasing IL-6 concentrations further did not have any additional effect on system A activity. It might be speculated that this is related to SOCS3 action since IL-6 treatment in many cell types increases SOCS3 expression (22), which inhibits further IL-6 action (22). Indeed, we observed an increase in SOCS3 expression following IL-6 (0.002 ng/ml), and it is possible that the higher doses of IL-6 caused a greater SOCS3 response and therefore dampened the response to IL-6.

To more directly explore the involvement of STAT3 signaling in IL-6- and TNF-α-stimulated system A activity we used RNA silencing techniques targeting STAT3 before treatment of cultured primary trophoblast cells with cytokines. Primary human trophoblast cells have been regarded as difficult to transfect; however, successful transfection of these cells with a lipid-based protocol was recently reported in the literature (9), and we used this approach in our study. hCG measurements confirmed that siRNA and transfection agents did not affect the normal differentiation of trophoblast cells in culture. Using siRNA targeted against STAT3, we achieved >70% knockdown at the mRNA and protein levels 46 h after exposure of cells to the transfection reagents and siRNA. We further confirmed the efficient knockdown of STAT3 by demonstrating a marked decrease in the protein expression of SOCS3, a canonical IL-6-STAT3 target gene, following exposure of the cultured primary trophoblast cells to siRNA targeted against STAT3. In STAT3-transfected cultured primary trophoblast cells, mRNA and protein levels of SNAT2, but not SNAT1, were reduced, suggesting that STAT3-mediated transcription maintains basal levels of SNAT2 in cultured primary trophoblast cells. Despite the reduction in SNAT2 protein expression after STAT3 silencing, basal system A activity was maintained. These data indicate that SNAT1 may be important in mediating basal system A activity. Alternatively, there may be a redistribution of existing SNAT2 transporters from intracellular compartments to the plasma membrane in response to the decreased SNAT2 gene expression after STAT3 silencing. This possibility is consistent with reports that SNAT2 is subjected to regulation by effects on membrane trafficking (16). Knocking down STAT3 before incubation of cultured primary trophoblast cells with IL-6 completely abrogated the stimulation of sodium-dependent MeAIB uptake, clearly demonstrating involvement of the STAT3 transcription factor in the IL-6 effect. Since STAT3 silencing caused a decrease in basal SNAT2 but not SNAT1 expression and since IL-6 specifically increased transcription of SNAT2 RNA, it is likely that the effect of STAT3 signaling on system A is increased transcription of SNAT2. Furthermore, these data indicate that the increase in SNAT1 protein expression subsequent to treatment with IL-6 is due to posttranscriptional regulation. However, the mechanisms involved in the effect of IL-6 on SNAT1 remain to be established.

In our study the treatment of cultured primary trophoblast cells with TNF-α for 24 h did not activate the STAT3 signaling pathway, as measured by Tyr705 phosphorylation of STAT. Furthermore, the stimulation of system A by TNF-α was not attenuated by STAT3 silencing. Previous studies indicate that the intracellular signaling pathway activated by TNF-α treatment may depend on the type of TNF-α receptor present in the cell (32). Further investigation is needed to identify the TNF-α receptors in human trophoblast cells. Our data indicate that chronic TNF-α treatment activates intracellular pathways other
than JAK/STAT to elicit stimulation of system A, increased transcription of SNAT2, and translation of SNAT1 and SNAT2. One such pathway may be via NF-κB, shown in placental JEG-3 cells to be activated by TNF-α (25) and involved in the regulation of transcription of amino acid transport systems in the lung and kidneys (4, 13). Interestingly, TNF-α treatment in embryonic kidney 293, HeLa, and ME180 cells also activates the phosphatidylinositol 3-kinase-AKT pathway in conjunction with NF-κB activation (30). The AKT pathway is associated with increased glucose transport (17) and also mammalian target of rapamycin (mTOR) pathway activation (41). We recently reported (34) that mTOR is a positive regulator of system A amino acid transport activity in cultured primary human trophoblast cells, raising the possibility that TNF-α regulates system A through mTOR signaling.

In a previous study with isolated human trophoblasts, treatment with TNF-α increased secretion of IL-6 after only 30 min (26). In our study, however, we did not observe stimulation of IL-6 secretion by 24-h exposure to TNF-α, and this discrepancy may be due to differences in the timing and concentration of the TNF-α treatment. In the previous study, cells were treated 48 h after plating, at which time the cells in our study were not fully differentiated to syncytiotrophoblast cells. We treated cells after 66 h in culture to ensure that cell differentiation had occurred; therefore our results represent TNF-α treatment in cultured, differentiated trophoblasts, not isolated cytotrophoblasts as described in the previous study (26). Furthermore, we used a concentration of TNF-α shown to stimulate the activity of system A, 0.002 ng/ml; this is much lower than the 200 ng/ml used by Li (26). The lower but physiological levels of TNF-α used in our study may not be sufficient enough to elicit the responses shown in the previous study (26) but are more relevant to our studies on transporter system A and the JAK/STAT pathway.

Identification of the signaling pathways mediating the regulation of amino acid transporters may provide important insight into the pathophysiology of a number of human diseases. We have shown that IL-6 and TNF-α are potent stimulators of system A activity, and we have identified a novel regulatory pathway involving increased ST3A3 phosphorylation and increased gene expression of the SNAT2 isoform, which links IL-6 to increased activity of the system A transporter. Since the system A transporter is ubiquitously expressed in placental explants, isolated primary cells and cell lines. Proc Natl Acad Sci USA 102: 19219 –19224, 2005.

References


