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DRUG METABOLISM & DISPOSITION

Special Section on New Era of Transporter Science: Unraveling the Functional Role of Orphan Transporters—Minireview

Impact of Inflammation and Infection on the Expression of Amino Acid Transporters in the Placenta: A Minireview

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ABSTRACT

Amino acid transporters expressed in the placenta help to regulate the transfer of amino acids from maternal to fetal circulation. Nutritional or hormonal factors are known to potentially impact the expression of amino acid transporters in the placenta. A relatively new field of inquiry has demonstrated that inflammation, whether associated with infection or not, also alters the expression of amino acid transporters in the placenta. Indeed, studies over the past 15 years have demonstrated that malaria, viral and bacterial models of infection, pre-eclampsia, and direct administration of proinflammatory cytokines can alter placental amino acid transporter expression. Although such studies have largely focused on System A and System L transporters, other transporters are also affected. p38 mitogen-activated protein kinase (p38 MAPK), signal transducer and activator of transcription 3 (STAT3), mammalian target of rapamycin C1 (mTORC1), and AMP-activated protein kinase (AMPK) signaling have all been implicated in these changes, but the underlying mechanisms remain to be fully elucidated. Furthermore, the implications of such changes warrant further investigation. This review will summarize studies that have investigated the impact of inflammation on placental amino acid transporter expression, identify questions that remain unanswered, and propose future areas of research to advance the field. As amino acid transporters are now being considered for drug targeting and drug delivery, furthering our understanding of the regulation of these transporters during disease states will be of increasing clinical value.

SIGNIFICANCE STATEMENT

Although this is a relatively new field of research, multiple studies have demonstrated that inflammation alters placental amino acid transporter expression. This review will serve to summarize, for the first time, studies in this field and identify gaps in current knowledge as research in this area moves beyond identifying changes in transporter expression to investigating the implications of such changes and the mechanisms underlying them.

Introduction

During pregnancy, the placenta plays an integral role in supporting fetal growth and health. Proper growth of the fetus requires protein synthesis using maternally derived amino acids (Vaughan et al., 2017). This is especially important during early development when the fetus lacks the ability to synthesize its own amino acids. Moreover, amino acids are used for fetal production of ATP and other important biomolecules, including neurotransmitters and nucleotides. As such, placental transport of amino acids is strongly linked with fetal growth and development.

The human placenta expresses over 20 different amino acid transporters (Gaccioli and Lager, 2016). These transporters are localized to the maternal-facing microvillous and fetal-facing basolateral membranes of the syncytiotrophoblast, which is the transporting epithelium of the placenta. Belonging to the solute carrier (SLC) superfamily, these transporters are classified as either accumulative, exchanger, or facilitated transporters (Vaughan et al., 2017) (Fig. 1). Accumulative transporters mediate net uptake of amino acids into the placenta. This creates a transmembrane amino acid gradient that drives the placental uptake of other amino acids via exchangers, which use an antiport mechanism to exchange intracellular nonessential amino acids for essential amino acids (Cleal et al., 2018). Lastly, facilitated transporters mediate diffusion of amino acids down their concentration gradients to efflux accumulated amino acids into fetal circulation. The interplay of these three transport mechanisms results in a net uptake of amino acids by the fetus.

Figure 1 provides examples of the different transporters belonging to each transport mechanism and their proposed localization; however, the

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ABBREVIATIONS: AMPK, AMP-activated protein kinase; ASCT, alanine/serine/cysteine transporter; CAT, cationic amino acid transporter; EAAT, excitatory amino acid transporter; 4F2HC, 4F2 cell-surface antigen heavy chain; IFN, interferon; IL, interleukin; IV, intervillositis; LAT, large neutral amino acid transporter; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; PBPK, physiologic-based pharmacokinetic; PE, pre-eclampsia; PHT, primary human trophoblast; Poly(I:C), polyinosinic:polycytidylic acid; SLC, solute carrier; SNAT, sodium-coupled neutral amino acid transporter; STAT3, signal transducer and activator of transcription 3; TAUT, taurine transporter; TNF, tumor necrosis factor.

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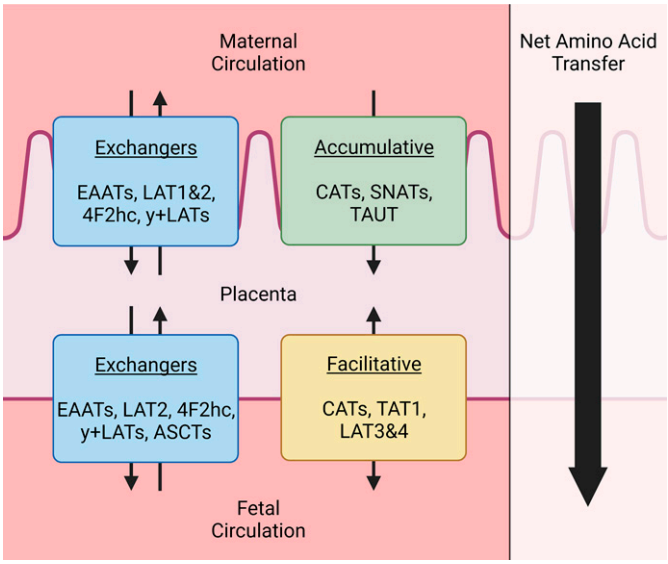

Fig. 1. Overview of amino acid transport systems in the placenta. Exchangers, accumulative, and facilitative transporters work in concert to drive a net transfer of amino acids from maternal to fetal circulation. Examples of transporters in each transport category are provided along with their speculated localization in humans on either the maternal-facing microvillous membrane or fetal-facing basolateral membrane. BAT: bidirectional amino acid transporter; TAT1: T-type amino acid transporter 1. 4F2hc covalently links to members of the SLC7A6 and SLC7A7 families, including LAT1 and LAT2, to form heteromeric amino acid transporters. Y + L amino acid transporters (y + LATs) belong to the y + L system of amino acid transporters that transports both neutral and basic amino acids.

exact localization of many amino acid transporters in the placenta remains unclear. The two most extensively studied amino acid transport systems include System A and System L. System A consists of sodium-coupled neutral amino acid transporters (SNATs) encoded by *SLC38A1* (SNAT1), *SLC38A2* (SNAT2), and *SLC38A4* (SNAT4) (Vaughan et al., 2017; Cleal et al., 2018). SNATs are expressed on the microvillous membrane and transport small amino acids, such as serine, glycine, and alanine, into the placenta. System L includes the large neutral amino acid transporters (LATs) encoded by *SLC7A5* (LAT1) and *SLC7A8* (LAT2) (Cleal et al., 2018). Although both LAT1 and LAT2 show predominant expression and activity on the microvillous membrane, LAT2 is also expressed on the basolateral membrane at lower levels. As exchangers, LATs bring essential amino acids, such as leucine, into the placenta by exchanging them for nonessential amino acids (Vaughan et al., 2017). Although less well-studied, other amino acid transport systems also exist in the placenta (reviewed by Jansson, 2001; Cleal and Lewis, 2008; Cleal et al., 2018).

Most amino acid transporters show affinity for multiple amino acids, often with overlapping substrate specificity (Table 1). Moreover, given the interplay between accumulative, exchanger, and facilitated transporters, placental amino acid transfer is more accurately conceptualized as a system as opposed to focusing on individual transporters (for a detailed explanation, see Cleal et al., 2018). As such, changes in placental amino acid transporter expression have the capability to influence placental amino acid transport as a whole. A number of drugs are also substrates of amino acid transporters, including L-DOPA, melphalan, baclofen, and gabapentin, all substrates of LAT1 (Scalise et al., 2018). Furthermore, the upregulation of amino acid transporters in some diseases makes them appealing novel drug targets and useful for drug delivery (Zhang et al., 2020; Zeden et al., 2021). For example, turning an existing chemotherapeutic into an amino acid-mimicking prodrug or modifying nanocarriers to display transporter ligands can be used to leverage selective delivery into target cells (Zhang et al., 2020). As this new field of drug discovery grows, it will be important to understand how these transporters are regulated in disease states and what implications this could have for maternal-to-fetal transfer of new xenobiotics.

Multiple factors influence placental amino acid transporter expression. Maternal nutrition, metabolic disorders, age, and substance use are associated with altered placental amino acid transporter expression (Gaccioli and Lager, 2016; Castillo-Castrejon and Powell, 2017; Vaughan et al., 2017; Johns et al., 2020). Furthermore, in vitro treatment of placental cells with glucose, amino acids, or hormones affects the regulation of amino acid transporters. However, research investigating the impact of inflammation or infection on these transporters in the placenta has been limited to a handful of studies that have yet to be summarized. Thus, the purpose of this review is to provide an overview of studies that have investigated the impact of inflammation (either in the presence or absence of infection) on the expression of amino acid transporters in the placenta.

Brief Historical Perspective

Inflammation Associated with Infection. Two types of active infection, malaria and ascending placentitis, have been studied for their impact on placental amino acid transporters. The impact of malaria was first studied in 2013 by Boeuf et al. in a cohort of Malawian women, the rationale being that malaria during pregnancy is associated with fetal growth restriction and low birth weight, which could be a result of altered placental transport of nutrients imperative for fetal growth (Chandrasiri et al., 2014). In addition to comparing malaria-infected to noninfected placentas, Boeuf et al. further stratified placentas by the presence or absence of intervillositis (IV), which involves recruitment of maternal immune cells to the intervillous blood spaces, resulting in localized inflammation (Boeuf et al., 2013). This study reported that transcript levels of *SNAT1* were significantly decreased in infected placentas, but only in the presence of IV; infected placentas with no sign of IV had similar *SNAT1* levels to noninfected placentas. A similar trend was observed for *SNAT2* but did not reach significance. Furthermore, upon isolating microvillous membrane vesicles from the collected placentas, this study reported significant decreases in System A activity from placentas with malaria either with or without IV, and treatment of the choriocarcinoma cell line BeWo with conditioned media from infected erythrocytes also inhibited System A activity. Together, these results imply that malaria during pregnancy impacts System A activity and expression, which could have implications for fetal amino acid access. Indeed, this study also showed that malaria infection was associated with alterations in the levels of several amino acids in fetal cord blood. We have also examined the impact of malaria on placental amino acid transporter expression. Using a mouse model of malaria during pregnancy, we observed a significant reduction in transcript levels of *Lat1*, *4F2 cell-surface antigen heavy chain* (*4F2hc*) (the second half of the LAT1 heterodimer), and *cationic amino acid transporter* (*Cat*)*1* in the placenta on gestational day 19 (Najjar et al., 2019). Thus, it appears that the impact of malaria on amino acid transporters in the placenta likely extends beyond System A.

The second type of infection investigated in this context is ascending placentitis, an ascending bacterial infection that results in placental inflammation. El-Sheikh Ali et al. demonstrated that compared with gestational age-matched controls, horses with placentitis showed significant downregulation of transcript levels of 42 SLC transporters, including the amino acid transporters alanine/serine/cysteine transporter (*Asct*)*1*, *4F2hc*, *bidirectional amino acid transporter 1* (Bat1), and *Snat7* (El-Sheikh Ali et al., 2021). Although the implications of these changes were not further examined, the

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authors speculated that this could result in decreased transport of nutrients to the fetus.

Although studies investigating the impact of active infections on placental amino acid transporter expression are limited, several studies have used animal models that mimic bacterial or viral infection. Bacterial infection is often simulated through administration of the toll-like receptor (TLR) 4 agonist bacterial lipopolysaccharide (LPS), whereas viral infection is often mimicked using the synthetic double-stranded RNA molecule polyinosinic:polycytidylic acid (poly(I:C)), which is a TLR3 agonist. In vitro treatments of primary human trophoblasts (PHTs) with either LPS or poly(I:C) has been shown to significantly induce mRNA levels of *SNAT1* and *SNAT2*, increase protein expression of SNAT1 (SNAT2 protein expression was not measured), and increase System A activity (Liong and Lappas, 2017). In contrast, neither treatment was found to impact System L activity (expression was not assessed), thereby demonstrating the specificity of different amino acid transport systems in their response to infectious stimuli.

We recently examined the impact of poly(I:C) on placental amino acid transporter expression in vivo. As poly(I:C) administration during pregnancy leads to phenotypic changes in offspring that are consistent with neurodevelopmental disorders, such as autism and schizophrenia (Haddad et al., 2020), we hypothesized that this could, in part, be due to altered placental transport of amino acids, which are required for proper fetal brain development. Indeed, poly(I:C) administration to pregnant rats resulted in a significant induction of transcript levels of *Cat2b*, *Asct1*, *Snat1*, *Snat2*, *4F2hc*, and *Taut* (McColl and Piquette-Miller, 2019). In line with results in poly(I:C)-treated primary human trophoblasts (Liong and Lappas, 2017), *Lat1* and *Lat2* were unaltered. We also examined the protein expression of several of these transporters and saw a significant decrease in membrane expression of ASCT1 and excitatory amino acid transporter (EAAT)2, and an increase in taurine transporter (TAUT). Furthermore, the levels of multiple amino acids in the fetal brains of poly(I:C)-treated dams were significantly altered, demonstrating a functional change in amino acid transport. Therefore, models of bacterial and viral inflammation are associated with changes in placental amino acid transporter expression; however, how well these changes translate to true infection in humans remains unclear.

Noninfectious Inflammatory Conditions. Inflammation in the absence of infection has also been shown to alter amino acid transporters. A number of studies have investigated the impact of pre-eclampsia (PE). Although technically a hypertensive disorder of pregnancy, PE is associated with chronic immune activation that results in a proinflammatory state (Cornelius, 2018). The interest in this area of study is not surprising given that PE is highly associated with intrauterine growth restriction, which could stem from abnormalities in amino acid transport (Phipps et al., 2019). As PE is difficult to fully recapitulate in animal models, most studies in this area have looked at human placentas. Indeed, Aiko et al. examined protein expression and localization of ASCT2, LAT1, and 4F2HC in placentas from pregnancies complicated by PE (Aiko et al., 2014). Although the localization of 4F2HC, LAT1, and ASCT2 was similar in PE and control placentas, the staining intensity of 4F2HC and LAT1 was found to be significantly higher in PE placentas. This is, in part, consistent with an earlier study that reported no change in *ASCT2* transcript levels in placentas from individuals with PE (Chen et al., 2006). Additionally, as compared with controls, significantly increased protein expression of y+LAT1 has been reported in PE placentas with or without intrauterine growth restriction complications, whereas levels of SNAT5 were unchanged (Huang et al., 2018).

Stojanovska et al. investigated placental amino acid transporter expression in a mouse model of PE. They used a "double-hit" PE model in which mice are administered soluble Fms-like tyorosine kinase-1 (sFlt-1) on gestational day 8.5 and LPS on gestational day 10.5, resulting in phenotypes consistent with human PE, such as increased urinary protein excretion and blood pressure (Stojanovska et al., 2019). At gestational day 18.5, placental transcript levels of *Snat1* were significantly decreased in female but not male PE placentas while *Snat2*, *Snat4*, *Asct1*, and *4F2hc* were unaltered in either sex. On the other hand, levels of proline and threonine were significantly decreased in the plasma of male fetuses from PE dams, implying that transporter systems other than those investigated could be affected. Of note, this study demonstrates that inflammation-mediated changes in the expression of amino acid transporters in the placenta is not only transporter-specific, but also sex-specific.

In addition to inflammatory conditions that occur during fetal development, it appears that assisted reproductive technologies can also alter placental function, including the expression of amino acid transporters. Yao et al. demonstrated that preimplantation genetic diagnosis, in which a single blastomere is removed from an early-stage embryo for detection of genetic abnormalities, significantly increases mRNA expression of proinflammatory cytokines in the placenta on gestational day 18.5 in mice (Yao et al., 2016). This inflammation was accompanied by significant downregulation of transcript levels of *Snat1*, *Lat2*, and *Cat3*, and significant downregulation of CAT3 protein expression (SNAT1 and LAT2 protein expression was not examined). Furthermore, the fetal/placental ratio in the biopsied group was significantly lower on gestational day 18.5, implying that fetal growth was likely affected. Thus, even environmental fluctuations that occur very early in development can influence placental amino acid transporter expression.

Proinflammatory Cytokines. The aforementioned studies clearly demonstrate that inflammation, whether a result of infection or not, can alter the expression of amino acid transporters in the placenta. However, as inflammation or infections during pregnancy are often associated with the activation of multiple inflammatory processes and signaling pathways, it is difficult to dissect the underlying mechanism(s) or mediator(s) responsible for subsequent changes in amino acid transporter expression. In contrast, treatment of placental cell lines with individual proinflammatory cytokines in vitro offers the opportunity to examine the impact of individual inflammatory mediators. As such, in vitro studies of this nature have been used to expand our understanding of how inflammation alters amino acid transporter expression.

The vast majority of such studies have focused on the impact of tumor necrosis factor (TNF)-α, which is induced in a large majority of infectious and inflammatory conditions. Jones et al. originally demonstrated that treatment of PHT isolated from healthy term placentas with physiologic concentrations of TNF-α (0.002 ng/mL) resulted in a dose-dependent increase in System A activity (Jones et al., 2009). In contrast, System L activity was unaffected. Further examination revealed that TNF-α significantly induced *SNAT2* mRNA levels as well as SNAT1 and SNAT2 protein expression, with no changes in SNAT4 mRNA or protein. A later study by Aye et al. demonstrated that pretreatment of PHT with a mitogen-activated protein kinase (MAPK) inhibitor selective for p38 signaling prevented the TNF-α-mediated increase in System A activity (Aye et al., 2015). Furthermore, inhibiting p38 MAPK completely abolished the TNF-α-mediated induction of SNAT2 protein and alleviated the induction of SNAT1. Thus, it appears that TNF-α induces System A activity through p38 MAPK-dependent induction of SNAT1 and SNAT2. In contrast to findings in PHT, treatment of female primary murine trophoblasts with TNF-α has been shown to significantly decrease *Snat2* mRNA expression, whereas expression in male trophoblasts was unaffected (Claycombe-Larson et al., 2020). Although species or sex-specific differences may explain this discrepancy, it is important to note that the concentration of TNF-α used in the murine trophoblast study was significantly higher than that used to treat the PHT [100 ng/mL (Claycombe-Larson et al., 2020) versus 0.002 ng/mL (Jones et al., 2009; Aye et al., 2015)].

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The impact of TNF-α on amino acid transporter expression has also been examined in primary human umbilical vein endothelial cells (HUVECs), which serve as a model of the fetal endothelium of the placental barrier. Knyazev et al. demonstrated using microarray that treatment of HUVECs with supraphysiological levels of TNF-α (2 ng/mL) significantly and substantially induced *CAT2* mRNA levels by a factor of almost 5 (Knyazev et al., 2017). The authors speculated that this could be due to an induction of noncanonical nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) signaling, which was also upregulated, as the gene encoding CAT2 (*SLC7A2*) contains a binding site for RelB in its enhancer/promoter regions; however, this was not tested experimentally.

Lastly, Broekhuizen et al. examined the effect of supraphysiological levels of TNF-α (80 ng/mL) in combination with interferon (IFN)-γ on LAT1, LAT2, and ASCT2 expression in chorionic plate arteries isolated from placentas either with or without PE (Broekhuizen et al., 2020). In contrast to treatment of healthy arteries, cytokine stimulation significantly induced *LAT1* mRNA expression in arteries from placentas with PE (*LAT2* and *ASCT2* were unaffected in both). This is consistent with previous reports that LAT1 expression is increased in placentas with PE (Aiko et al., 2014) and implies that PE may increase the susceptibility of LAT1 to changes by inflammatory mediators, such as TNF-α and IFN-γ.

Although the impact of TNF-α on transporter expression has been studied the most extensively, a handful of studies have examined the impact of other proinflammatory cytokines, including interleukin (IL)-6 and IL-1β. Consistent with TNF-α treatment, treatment of PHT with physiologic levels of IL-6 (0.002 ng/mL) significantly induced System A activity as well as *SNAT2* mRNA and SNAT1 and SNAT2 protein expression, whereas System L activity and SNAT4 expression were unaffected (Jones et al., 2009). However, in contrast to treatment with TNF-α, IL-6 significantly induced phosphorylation of signal transducer and activator of transcription 3 (STAT3), and silencing of STAT3 abolished IL-6-mediated increases in System A activity, whereas TNF-α-mediated increases were unaffected. Furthermore, STAT3 silencing significantly reduced SNAT2 mRNA and protein expression, while leaving SNAT1 unchanged. Thus, although TNF-α-mediated induction of System A appears to occur via p38 MAPK signaling, IL-6-mediated induction likely occurs through STAT3-mediated increases in SNAT2, demonstrating that the regulation of amino acid transporter expression and activity during inflammation can be cytokine-dependent.

Lastly, despite being induced by both TNF-α and IL-6, treatment of the choriocarcinoma cell line BeWo-b30 with a supraphysiological concentration of IL-1β (5 ng/mL) significantly decreased System A activity as well as *SNAT1* and *SNAT2* mRNA levels (Thongsong et al., 2005). In contrast, treatment of primary murine trophoblasts with IL-1β had no significant effect on *Snat2* mRNA (Claycombe-Larson et al., 2020). These discrepancies may be the result of using a human immortalized cell line as opposed to primary murine trophoblasts. However, as before, the concentration of IL-1β used to treat the murine trophoblasts was 20-fold higher (100 ng/mL) (Claycombe-Larson et al., 2020) than that used to treat the BeWo-b30 cells (Thongsong et al., 2005). To conclude, TNF-α, IL-6, and IL-1β have all been shown to impact System A activity or expression in different manners and through varying underlying mechanisms. However, whether and how these cytokines affect other amino acid transporters is much less well-studied.

Key Recent Advances

Although research investigating the impact of inflammation on amino acid transporters in the placenta began about 15 years ago, the majority of the work conducted in this field has taken place quite recently. While many of the described studies have laid the groundwork in this field of inquiry, efforts have begun to explore the underlying mechanisms behind these changes. Until recently, this area has focused on transcriptional regulation of transporters during inflammation (Jones et al., 2009; Aye et al., 2015), despite the fact that amino acid transporters are often regulated post-translationally via membrane trafficking. As reviewed by McCracken et al., LAT1, SNAT2, CAT1, 4F2HC, and ASCT2 have all been shown to be regulated through post-translational trafficking via PI3K signaling, sphingolipids, or post-translational modifications, such as ubiquitination and phosphorylation (McCracken and Edinger, 2013). However, research concerning the post-translational regulation of amino acid transporters in the placenta has been limited. To this point, the mammalian target of rapamycin (mTOR)C1 pathway has been found to regulate the plasma membrane trafficking of SNAT2 and LAT1 in PHT through Nedd4-2-mediated ubiquitination (Rosario et al., 2016). A more recent study in PHT also implicated mTORC2 in SNAT2 and LAT1 membrane trafficking (Jansson et al., 2020). It appears that similar mechanisms also impact the expression or localization of other amino acid transporters as we recently demonstrated that mTORC1 inhibition reduces membrane localization of EAAT2 and ASCT1 in the choriocarcinoma cell line JAR (McColl and Piquette-Miller, 2021). We further extended this mechanism to show that activation of AMP-activated protein kinase (AMPK), which inhibits mTORC1, similarly affects membrane trafficking. We had previously reported that maternal immune activation with poly(I:C) significantly reduced EAAT2 and ASCT1 protein expression in the placentas of rats (McColl and Piquette-Miller, 2019). Given that we observed activation of AMPK and inhibition of mTORC1 in these same placentas (McColl and Piquette-Miller, 2021), it is possible that this could contribute to the downregulation of EAAT2 and ASCT1, thus providing the first mechanistic link between inflammation and mTORC1/AMPK-mediated changes in placental amino acid transporter expression. Further research exploring post-translational regulation of other placental amino acid transporters in the context of inflammation will be required to fully elucidate this mechanism.

Current Challenges and Knowledge Gaps

Given that this field of research is relatively new, a number of areas remain to be fully explored. For example, only one of the studies described above has examined the impact of infection on placental amino acid transporter expression in humans in vivo (Boeuf et al., 2013). Although this study investigated malaria infection, a myriad of other infections can occur during pregnancy, including chorioamnionitis, influenza, HIV, cytomegalovirus, and Zika. Moreover, only a handful of studies have been conducted in animal models of infection. Thus, with the exception of malaria and insights gained from rats given poly(I:C), the impact of infection during pregnancy on placental amino acid transporter expression is relatively uncharacterized. This is of particular relevance given the current COVID-19 pandemic.

A second area that requires further investigation is the implication of inflammation-mediated changes in amino acid transporter expression for offspring development and pregnancy outcomes. However, a better understanding of how changes in certain transporters may influence outcomes will require a more thorough understanding of the physiologic role of the transporters under normal conditions. Whereas the roles of LATs and SNATs in the placenta are fairly well established, knowledge of the expression and localization of other amino acid transporters is less well defined (Cleal et al., 2018). Furthermore, the physiologic role that they serve in the placenta is much less clear. Although amino acid transport across the placenta should be thought of as a system, rather than in terms of contributions from individual transporters, knowledge of how individual transporters contribute to the system, as a whole, is required to

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TABLE 1

Summary of studies examining the impact of inflammation on amino acid transporter expression in the placenta

Amino acid substrates are taken from Cleal et al., 2018 unless otherwise specified. Drug substrates are italicized and taken from Zhang et al., 2020. ↑ signifies increased expression, ↓ signifies decreased expression, ↔ signifies no change in expression, and X signifies that expression was not assessed. When possible, the approximate magnitude of change is provided as percent increase or decrease. (F) denotes a change in female placentas, (M) denotes a change in male placentas.

Transporter	Substrates	Model	Condition/ Treatment	mRNA	Protein	Reference
EAAT2 (*SLC1A2*)	Aspartate, glutamate	Rats	Poly(I:C)	↔	↓45%	(McColl and Piquette-Miller, 2019)
ASCT1 (*SLC1A4*)	Alanine, serine, cysteine, threonine	Mice	Pre-eclampsia	↔	X	(Stojanovska et al., 2019)
		Rats	Poly(I:C)	↑90%	↓75%	(McColl and Piquette-Miller, 2019)
		Horses	Ascending placentitis	↓	X	(El-Sheikh Ali et al., 2021)
ASCT2 (*SLC1A5*)	Alanine, serine, cysteine, glutamine, threonine, asparagine	Human chorionic plate arteries	Pre-eclampsia (plus TNF-α and IFN-γ)	↔	X	(Broekhuizen et al., 2020)
		Humans	Pre-eclampsia	X	↔	(Aiko et al., 2014)
				↔	X	(Chen et al., 2006)
4F2hc (*SLC3A2*)	Chaperone subunit for LAT1 and LAT2	Humans	Pre-eclampsia	X	↑25%	(Aiko et al., 2014)
		Mice	Pre-eclampsia	↔	X	(Stojanovska et al., 2019)
			Malaria	↓65%	X	(Najjar et al., 2019)
		Rats	Poly(I:C)	↑50%	X	(McColl and Piquette-Miller, 2019)
		Horses	Ascending placentitis	↓	X	(El-Sheikh Ali et al., 2021)
TAUT (*SLC6A6*)	Taurine	Rats	Poly(I:C)	↑75%	↑75%	(McColl and Piquette-Miller, 2019)
CAT1 (*SLC7A1*)	Arginine, histidine, lysine	Mice	Malaria	↓70%	X	(Najjar et al., 2019)
CAT2 (*SLC7A2*)	Arginine, histidine, lysine	HUVEC	TNF-α	↑380%	X	(Knyazev et al., 2017)
		Rats	Poly(I:C)	↑40%	X	(McColl and Piquette-Miller, 2019)
CAT3 (*SLC7A3*)	Arginine, histidine, lysine	Mice	Preimplantation genetic diagnosis	↓30%	↓60%	(Yao et al., 2016)
LAT1 (*SLC7A5*)	Leucine, isoleucine, valine, phenylalanine, tyrosine, tryptophan, methionine, histidine, *L-DOPA, melphalan, baclofen, gabapentin*	Humans	Pre-eclampsia	X	↑	(Aiko et al., 2014)
		Human chorionic plate arteries	Pre-eclampsia (plus TNF-α and IFN-γ)	↔	X	(Broekhuizen et al., 2020)
		Mice	Malaria	↓80%	X	(Najjar et al., 2019)
LAT2 (*SLC7A8*)	Leucine, isoleucine, valine, alanine, serine, threonine, cysteine, phenylalanine, tyrosine, tryptophan, asparagine, histidine, methionine, glutamine, glycine	Human chorionic plate arteries	Pre-eclampsia (plus TNF-α and IFN-γ)	↔	X	(Broekhuizen et al., 2020)
		Mice	Preimplantation genetic diagnosis	↓25%	X	(Yao et al., 2016)

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TABLE 1 *continued*

Transporter	Substrates	Model	Condition/ Treatment	mRNA	Protein	Reference
BAT1 (*SLC7A9*)	Arginine, histidine, lysine, phenylalanine, tyrosine, tryptophan, threonine, methionine, valine, isoleucine, leucine	Horses	Ascending placentitis	↓	X	(El-Sheikh Ali et al., 2021)
SNAT1 (*SLC38A1*)	Glutamine, alanine, asparagine, cysteine, histidine, serine	Primary human trophoblasts	TNF-α	↔	↑70-80%	(Jones et al., 2009; Aye et al., 2015)
			IL-6	↔	↑80%	(Jones et al., 2009)
			Poly(I:C)	↑50%	↑45%	(Liong and Lappas, 2017)
			LPS	↑160%	↑60%	
		BeWo-b30 cells (M)	IL-1β	↓40%	X	(Thongsong et al., 2005)
		Human placentas	Malaria	↓50%	X	(Boeuf et al., 2013)
		Mice	Preimplantation genetic diagnosis	↓20%	X	(Yao et al., 2016)
			Pre-eclampsia	↓50% (F) ↔ (M)	X	(Stojanovska et al., 2019)
		Rats	Poly(I:C)	↑35%	X	(McColl and Piquette-Miller, 2019)
SNAT2 (*SLC38A2*)	Alanine, asparagine, cysteine, glutamine, glycine, histidine, methionine, proline, serine	Primary human trophoblasts	TNF-α	↑50%	↑70%	(Jones et al., 2009)
				↔	↑70%	(Aye et al., 2015)
			IL-6	↑80%	↑55%	(Jones et al., 2009)
			Poly(I:C)	↑100%	X	(Liong and Lappas, 2017)
			LPS	↑100%	X	
		BeWo-b30 cells (M)	IL-1β	↓15%	X	(Thongsong et al., 2005)
		Human placentas	Malaria	↓50% (trend)	X	(Boeuf et al., 2013)
		Primary murine trophoblasts	TNF-α	↓40%	X	(Claycombe-Larson et al., 2020)
			IL-1β	↔	X	
		Mice	Pre-eclampsia	↔	X	(Stojanovska et al., 2019)
		Rats	Poly(I:C)	↑30%	↓20% (trend)	(McColl and Piquette-Miller, 2019)
SNAT4 (*SLC38A4*)	Alanine, asparagine, cysteine, glycine, serine, threonine	Primary human trophoblasts	TNF-α	↔	↔	(Jones et al., 2009; Aye et al., 2015)
			IL-6	↔	↔	(Jones et al., 2009)
		Mice	PE	↔	X	(Stojanovska et al., 2019)
SNAT5 (*SLC38A5*)	Glutamine, histidine, asparagine, serine	Humans	Pre-eclampsia (with or without IUGR)	X	↔	(Huang et al., 2018)
SNAT7 (*SLC38A7*)	Glutamine, histidine, alanine, serine, asparagine (Hagglund et al., 2011)	Horses	Ascending placentitis	↓	X	(El-Sheikh Ali et al., 2021)

HUVEC human umbilical vein endothelial cell; IUGR, intrauterine growth restriction.

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understand how changes in their expression may impact fetal access to amino acids. Thus, basic research characterizing the totality of amino acid transporter systems in the placenta will be required before the full implications of their dysregulation can be elucidated. On a related note, much of the research into how inflammation affects placental amino acid transporter expression has focused on SNATs and LATs, likely because their role in the placenta is more well defined. In contrast, studies investigating the impact of inflammation on other amino acid transporters are limited.

Lastly, technical challenges in measuring protein expression of amino acid transporters in the placenta may be hindering work in this area. Many of the described studies investigated changes in transporter expression at the mRNA level only. Given that these transporters are often regulated post-translationally, changes in mRNA expression may not reflect changes in membrane expression, which is what determines functionality. Indeed, in our study, many of the transporters showed inflammation-mediated increases in mRNA levels but decreased protein expression (McColl and Piquette-Miller, 2019). Studies have reported difficulty in quantifying transporters via traditional immunoblotting methods either due to low expression (Liong and Lappas, 2017) or a lack of commercially available antibodies (McColl and Piquette-Miller, 2019). Understandably, this has likely hindered characterization of transporter changes at the protein level.

Perspectives on Future Directions

As described above, future research in this field should focus on investigating the impact of infection on transporter expression in vivo and elucidating underlying mechanisms at the post-translational level. Given the technical challenge of detecting some transporters at the protein level using immunoblotting, especially for less well-characterized transporters, there is a need for more sensitive and robust ways of quantifying amino acid transporter protein expression in the placenta. This could include targeted quantitative proteomics using mass spectrometry, which provides absolute quantification of transporters. Studies utilizing proteomics for absolute quantification of membrane transporters in the placenta are rare. A recent study used targeted proteomics to quantify a number of ATP-binding cassette transporter and SLC transporters in membrane fractions from the human placenta throughout gestation (Anoshchenko et al., 2020). Although they did not examine amino acid transporters specifically, they did include a number of other SLC drug and neurotransmitter transporters. Recently, targeted quantitative proteomics has been used to quantify some amino acid transporters in other tissues, particularly the blood-brain barrier (Al-Majdoub et al., 2019; Bao et al., 2020; Omori et al., 2020). While these studies provide peptide probe sequences corresponding to a number of amino acid transporters, additional studies will be required to determine whether these methods are translatable to the placenta. This would allow for true quantification of amino acid transporters at the membrane during inflammation and could also contribute to further characterization of the expression and localization of such transporters under physiologic conditions.

In addition to characterizing changes in transporter expression during inflammation and infection, beginning to determine the implications of such changes is an important area of future research. Given the important roles of amino acids in fetal growth and neurodevelopment, understanding how changes in placental transporter expression impact fetal access to amino acids is critical. This could be elucidated by quantifying changes in amino acid levels in fetal tissues (for animal studies) and cord blood (in human studies) during inflammation or examining amino acid transport in placenta-specific cell lines and membrane vesicles in vitro. Computational modeling may also aid in predicting how changes in transporter expression alter placental amino acid transport. One such approach could be the use of physiologic-based pharmacokinetic (PBPK) modeling, as recent advances have been made to incorporate transporter-mediated differential influx and efflux rates across the syncytiotrophoblast in PBPK models of placental drug transfer (Liu et al., 2021). However, such modeling requires extensive knowledge of the contribution and kinetics of specific transporters for the substrate in question as well as the abundance of such transporters in the placenta, which would make its application to modeling amino acid transport difficult. Indeed, such modeling has only recently begun for substrates of other highly characterized drug transporters in the placenta, such as P-glycoprotein (Anoshchenko et al., 2021). As such, it seems unlikely that PBPK modeling of placental amino acid transport would be feasible in the near future.

An alternative (and likely more viable) approach is to combine placental perfusion studies with either compartmental or geometric modeling. Doing so allows for placental uptake and release of transporter substrates at both sides of the maternal-fetal interface to be measured or manipulated with the use of competitive and specific transporter inhibitors. Such models have already been used to study placental amino acid transport (reviewed by Lewis et al., 2020) and therefore offer a more feasible method for investigating the impact of altered amino acid transporter expression on placental amino acid transport.

Examining the downstream impacts of changes in amino acid transport on fetal neurodevelopment, fetal growth, and placenta sufficiency will also be an important area of study. Lastly, as novel therapeutics targeting amino acid transporters continue to be developed (Zhang et al., 2020; Zeden et al., 2021), understanding how their transport across the placenta may be altered during inflammation will be critical for ensuring safe use during pregnancy. Depending on the specificity of novel drugs for individual amino acid transporters and progress made toward characterizing amino acid transporters in the placenta, this could be an avenue of research for which PBPK modeling may be more appropriate.

Conclusions

To conclude, a number of studies have demonstrated that various forms of inflammation alter the expression of amino acid transporters in the placenta (Table 1). While multiple different transporters appear to be affected, the majority of research has focused on changes in System A (SNATs) and System L (LATs). Mechanistic studies have implicated p38 MAPK, STAT3, and more recently mTOR and AMPK signaling in inflammation-mediated regulation of transporter expression, but further studies will be required to fully elucidate underlying mechanism(s), particularly for other types of amino acid transporters. Given that amino acids transported across the placenta are required for fetal growth and development, inflammation-mediated changes in their transport may have implications for fetal outcomes; however, studies investigating the implications of such changes are lacking.

Authorship Contributions

Participated in research design: McColl, Piquette-Miller.

Performed data analysis: McColl, Hurtarte.

Wrote or contributed to the writing of the manuscript: McColl, Piquette-Miller.

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