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Molecular Cross-Talk at the Feto–Maternal Interface

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Molecular cross-talk at the feto–maternal interface occurs between many different cell types, including uterine leukocytes, extravillous trophoblast cells, and uterine spiral arteries, is essential for the establishment and maintenance of pregnancy. This review concentrates on human pregnancy and examines three main areas in which cross-talk occurs; immune tolerance, regulation of extravillous trophoblast invasion, and remodeling of the uterine spiral arteries.

For the purposes of this review, the feto–maternal interface will be defined as the interaction between the uterus, specifically the decidualized endometrium (decidua) and inner third of the myometrium, and the invasive extravillous trophoblast cells (EVT). Successful pregnancy requires a highly receptive endometrium during the implantation window, which involves decidualization and a symbiotic signaling process between the blastocyst and the mother (Aplin 2000). The decidua is comprised of luminal and glandular epithelium, stromal cells, spiral arteries, lymphatics, leukocytes, and fetal derived EVT.

There are three major processes in which feto–maternal cross-talk is essential for the maintenance of a successful pregnancy; establishment of immune tolerance, regulation of trophoblast invasion, and remodeling of the uterine spiral arteries.

IMMUNE TOLERANCE

In the first trimester of pregnancy, ~30%–40% of decidual stromal cells are leukocytes, primarily uterine natural killer (uNK) cells, macrophages and T lymphocytes (Bulmer et al. 1991) although other less abundant but functionally important endometrial leukocyte populations are also present including dendritic cells (Gardner and Moffett 2003), natural killer T (NKT) (Tsuda et al. 2001) cells and regulatory T cells (Heikkinen et al. 2004). Leukocytes are prominent at the implantation site where they are in close contact with invading EVT, spiral arteries, and each other.

Uterine Natural Killer Cells

uNK cells ($CD56^{bright}CD16^{-}$) account for 70% of first trimester decidual stromal leukocyte

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population (King et al. 1989; Williams et al. 2009). However, their levels diminish drastically toward the end of gestation (Williams et al. 2009; Bulmer et al. 2010). uNK are a rich source of a range of cytokines and angiogenic growth factors (Jokhi et al. 1994, 1997; Li et al. 2001; Rieger et al. 2001; Hanna et al. 2006; Lash et al. 2006a, 2010a).

uNK cells have the appropriate machinery for efficient cytotoxicity but their level of cytotoxicity is lower than the peripheral blood NK cells. Demonstration of their cytotoxicity includes activity against the cell target K562 (Kopcow et al. 2005), activation of NKp46, perforin polarization granule exocytosis, as well as efficient lysis of target cells (El Costa et al. 2008; Yagel 2009). Moreover, uNK cells are granular lymphocytes containing perforin, granzyme, and TIA-1, indicative of their cytolytic functionality (King et al. 1991). However, uNK cells are not cytolytic toward the fetal EVT cells; this can be explained in part by the fact that EVT selectively express HLA-C, HLA-E, and HLA-G. In addition, it has been shown that HLA-E stabilizes HLA-G for NK cell receptor interaction and that HLA-G binds to inhibitory NK receptors and suppresses immune responses, especially leukocyte Ig-like receptor LILRB1, on the NK cell surface (Apps et al. 2007; Yagel 2009).

Uterine Macrophages

Macrophages, one of the two antigen presenting leukocyte populations in the uterus, stably constitute up to 20%–25% of the leukocyte common antigen, $CD45^+$ leukocytes in the pregnant decidua (Bulmer and Johnson 1984; Lessin et al. 1988; Vince et al. 1990). In addition, they are the most abundant leukocyte within the myometrium.

Decidual macrophages most commonly reside in the stroma, in close proximity to EVT and in the vicinity of spiral arteries and are proposed to play a major role in endometrial decidualization in preparation for implantation (Lea and Clark 1991). Their close proximity to EVT has been suggested to promote monocyte differentiation into unique immunosuppressed macrophages, either by direct interaction or secretion of cytokines (Fest et al. 2007; Svensson et al. 2011). Macrophages are highly versatile and perform a multitude of functions including metabolic regulation of lipids, extracellular matrix (ECM) and vascular remodeling, tissue regeneration, inflammation, and fetal antigen recognition (Houser et al. 2011). They express a unique set of cell surface markers: CD14, CD68, and MHC class II antigen HLA-DR and their active state can be distinguished through expression of CD11c and CD86 (Hunt et al. 2006). Classically, macrophages can be differentiated into two subtypes; proinflammatory M1 or anti-inflammatory M2 (Martinez et al. 2009); however, it has been shown that decidual macrophages do not belong to either of these two subsets (Gustafsson et al. 2008; Svensson et al. 2011). In contrast, Houser et al. (2011) reported the presence of two distinct categories of decidual macrophages, according to their disparate pattern of CD11c complement receptor expression: $CD11c^{HI}$ and $CD11c^{LO}$. Additionally $CD11c^{HI}$ macrophages were shown to have more efficient APC function consistent with increased expression of lipid–antigen presenting CD1 molecule isoforms such as CD1a, CD1c, and CD1d compared with their $CD11c^{LO}$ counterparts (Houser et al. 2011).

Decidual macrophages are also key immunoregulators at the maternal–fetal interface under local environmental cues from different lymphocyte populations. Decidual macrophages not only regulate adaptive T cell responses, but are also proficient in monitoring innate NK cell responses. The production of important anti-inflammatory substances such as IL-10 (Heikkinen et al. 2003; Lidstrom et al. 2003), prostaglandin E_2 (PGE_2) (Parhar et al. 1989), and IDO (Heikkinen et al. 2003) points toward a key immunosuppressive role played by the decidual macrophages, resulting in fetal antigen tolerance throughout gestation.

Decidual macrophages have been shown to communicate with other decidual leukocyte populations such as uNK cells. It has been suggested that decidual macrophages may regulate uNK cell numbers alongside controlling their state of differentiation and activation via secre-

tion of IL-15, which in turn promotes NK cell proliferation and activation (Carson et al. 1994; Kitaya et al. 2000; Manaster et al. 2008). Macrophage activation/deactivation has been categorized into five groups (Gordon 2003) differentially regulated, respectively, via TLRs, IFNγ, Fc, and complement receptors, IL4/IL13, IL-10, TGFβ/IFNα/β or M-CSF. Amongst other factors, which create an immune-tolerant fetal environment are inhibitory members of the B7 family (B7-H1), ILT3, CD209 (DC-SIGN), MS-1, and factor 13 (reviewed by Hunt and Petroff 2008). Thus, the decidual microenvironment nurtures a unique decidual macrophage population, which may function to ensure fetal tolerance by inhibition of harmful immune responses.

T Lymphocytes

$CD3^+$ T lymphocytes comprise ~10% of the first trimester human decidual leukocyte population (Heikkinen et al. 2004). Among this $CD3^+$ subpopulation, 40%–75% are $CD8^+$ cytotoxic T lymphocytes (CTLs), ~30%–45% are $CD4^+$ helper T cells (Th) and a small number are mucosal T cells expressing T cell receptor (TCR)-γ/δ, TCRαβ+ and NKT cells (Bulmer et al. 1991; Vassiliadou and Bulmer 1996; Mjosberg et al. 2010; Tilburgs et al. 2010). Within the T cell population in the decidua basalis, ~50% of the $CD4^+$ T cells express an activated/memory $CD25^{dim}$ phenotype and ~40% of the $CD8^+$ T cells show an effector/memory $CD28^-$ cell surface phenotype (Tilburgs et al. 2006, 2008, 2010). Approximately 5% of the $CD4^+$ T cells, which show a $CD25^{bright}$ phenotype and express intracellular forkhead box transcription factor (FOXP)-3, are regulatory T cells (Tregs) (Tilburgs et al. 2008, 2010; Mjosberg et al. 2010). It has also been shown that amongst the helper T cells the majority (~5%–30%) are Th1 cells involved in cellular immunity. A small number of Th2 cells (~5%), involved in humoral immunity, whose elevated numbers are linked to normal pregnancy, and Th17 cells (~2%) are also detectable (Mjosberg et al. 2010). However, Th17 cell responses may be abrogated by uNK cells within the decidua (Fu et al. 2013). T cell population proportions alter during pregnancy: diminishing in early pregnancy but peaking again at term, their relative percentages increase in the third trimester (Tilburgs et al. 2006, 2010; Erlebacher 2013).

Dendritic Cells

The bone marrow derived $CD14^-$ dendritic cells are the second subset of antigen presenting cells in the decidua constituting 1.7% of $CD45^+$ cells during the first trimester of pregnancy (Gardner and Moffett 2003). They play a role in remodeling the cycling endometrium following through to either menstruation or implantation and are key to initiation and modulation of maternal–fetal immune responses (Bengtsson et al. 2004). $CD1a^-$ immature dendritic cells differentiate from their precursors and become proficient at antigen detection via toll like receptors (Reis e Sousa 2001), antigen processing and presenting, thus bridging the gap between the innate and acquired immune systems (Sallusto and Lanzavecchia 1999; Schulke et al. 2009). Engulfment and digestion of antigens and inflammatory cytokines induces migration of dendritic cells to lymphoid organs where they efficiently activate naïve T cells and regulate NK and B cells (Crow and Kunkel 1982; Banchereau et al. 2000). Mature dendritic cells express CD83 as well as an array of costimulatory molecules such as CD40, CD58, CD80, CD86, MHC molecules, and high levels of CC-chemokine receptor (CCR)-7 (Sallusto et al. 1995, 2000; Cella et al. 1999; Reis e Sousa 2001; Schulke et al. 2009). CCR-7 stimulates dendritic cell migration to secondary lymphoid organs, where they attract and present antigens to $CD4^+$ and $CD8^+$ T cells (Sallusto et al. 2000; Reis e Sousa 2001; Juretic et al. 2004).

In first trimester pregnant decidua, the majority of the dendritic cells express CD209, a marker of immature or inactive dendritic cells (Gardner and Moffett 2003; Kammerer et al. 2003; Rieger et al. 2004). Uterine dendritic cells interact with the other uterine leukocyte populations. It has been suggested that the majority of the decidual immature dendritic cells remain in close contact with uNK cells, although the

small proportion of mature dendritic cells aggregate around the $CD3^+$ T cells (Juretic et al. 2004). Evidence has suggested that interactions between immature dendritic cells and uNK cells leads to dendritic cell maturation or cell death, and that this outcome is dependent on the dendritic cell:uNK cell ratio. Indeed, it is in conditions of a low dendritic cell:uNK cell ratio that uNK cell activation takes place (Piccioli et al. 2002; Zitvogel 2002; Moretta 2005). It has been suggested that, in the uterus, IL-10 produced by dendritic cells may be crucial for generation of regulatory T cells (Tregs) (Akbari et al. 2001; Groux et al. 2004) and that it is the dendritic cell-Treg interaction that leads to tolerogenic dendritic cells functionality. Furthermore, it has been shown that activating the CD200R2 pregnancy-protective receptor on immature dendritic cells generates a Treg subset with the ability to suppress mixed lymphocyte responses and allograft rejection (Gorczynski 2006; Blois et al. 2007). Alongside their immunomodulatory role, dendritic cells also show proangiogenic functions through their ability to produce angiogenic growth factors such as VEGF-A, fibroblast growth factor (FGF)-2, endothelin (ET)-1, and chemokines such as CXCL8 (IL8) and CXCL12, thus highlighting their highly diverse and significant role at the fetal–maternal interface (Riboldi et al. 2005; Piqueras et al. 2006).

TROPHOBLAST

The human placenta is an intricate organ, which is made up from a variety of different specialist cell types and vascular networks, which allows it to achieve its main functional role of promoting fetal growth and viability. The major cell type of the placenta is the trophoblast cells which has three main subtypes; villous cytotrophoblast (CTB), syncytiotrophoblast and EVT (Gude et al. 2004; Fitzgerald et al. 2008). EVT and CTB can be distinguished by differential expression of various phenotypical markers such as cell adhesion molecules, integrins, growth factors, and HLA molecules (Norwitz et al. 2001). The villous cytotrophoblast cells fuse to form the multinucleated syncytiotrophoblast cell layer which covers floating chorionic villi in the intervillous space. In contrast, the cytotrophoblast cells of the anchoring villi differentiate from a proliferative phenotype into an invasive phenotype (EVT), anchoring the placenta to the underlying decidua (Irving et al. 1995). The EVT invade through the decidua as far as the inner third of the myometrium via two distinct pathways, interstitial and endovascular, forming four populations of EVT: interstitial mononuclear, interstitial multinuclear, intramural and endovascular. In interstitial invasion, EVT cells invade through the decidua and inner third of the myometrium, although in endovascular invasion EVT cells move up the lumen of the spiral arteries in a retrograde fashion, again ceasing in the inner third of the myometrium. Interstitial mononuclear and multinuclear EVT are found throughout the decidua and inner third of the myometrium, it is assumed that multinuclear interstitial EVT are formed from fusion of mononuclear interstitial EVT although the mechanism underlying this is not known. Endovascular EVT are found in the lumen of spiral arteries whereas intramural EVT are located embedded in fibrinoid material within the wall of spiral arteries during and after spiral artery remodeling.

Trophoblast Invasion

Cellular invasion is a complex process that is tightly regulated in EVT, unlike in metastatic cancers (Lala et al. 2002). In simple terms, there are three features of cellular invasion; attachment to the extracellular matrix (ECM), proteolytic breakdown of the ECM and then movement into that cleared space before reattachment. EVT are a naturally highly invasive cell type, although their ability to invade in in vitro models decreases with increasing gestational age, with EVT from 8 to 10 wk gestational age being twice as invasive as those from 12 to 14 wk, 16 to 20 wk, or term (Genbacev et al. 1996; Lash et al. 2006b). EVT invasiveness is associated with their phenotype, for example EVT express a unique repertoire of cell surface integrins, distinct from those expressed by CTB. In particular, EVT are characterized by the expression of $\alpha1\beta1$ and $\alpha5\beta1$ integrins whereas

CTB express α6β4 integrin (Damsky et al. 1992). This switch in integrin expression appears to be essential for the invasive phenotype of EVT (Damsky et al. 1994). In addition, EVT express a wide range of proteases, both secreted and cell surface associated, dipeptidyl peptidase IV (Sato et al. 2002), carboxypeptidase-M (Nishioka et al. 2003), matrix metalloproteinases (MMPs) (Bischof et al. 2000; Anacker et al. 2011), and the urokinase plasminogen activator (uPA) system (Chakraborty et al. 2002). The gelatinases, MMP-2 and MMP-9, appear to be the most important proteases secreted by EVT for their invasive behavior with the ratio of MMP-2 and MMP-9 altering throughout gestation (Shimonovitz et al. 1994; Bischof et al. 2000) and inhibition of MMP-9 completely inhibiting EVT invasion in vitro (Librach et al. 1991). Disruptions in the tightly controlled process of EVT invasion can lead to placental deficiencies which affect the maternal vascular homeostasis resulting in pregnancy complications such as early miscarriage (Khong et al. 1987; Hustin et al. 1990), late miscarriage (Ball et al. 2006), preeclampsia (Pijnenborg et al. 1991), fetal growth restriction (Khong et al. 1986), preterm birth (Kim et al. 2003), and placenta accreta (Khong and Robertson 1987; Hannon et al. 2012). Despite the importance of trophoblast invasion in pregnancy, very little is understood about the factors that control this process in vivo, although decidual factors are likely to play an important role (Fitzgerald et al. 2008; Knöfler and Pollheimer 2012).

The mechanisms underlying regulation of EVT invasion in humans likely differs from other species. Owing to ethical issues of studying early human pregnancy, several different in vitro assays have been developed to study this important process, these include modified Boyden chamber assays as well as decidua–placenta coculture assays. In addition, primary isolates of first trimester EVT are difficult to obtain for many research laboratories, and when they can be isolated yield small cell numbers limiting the level of experimentation. One other feature that limits their usefulness is their inability to proliferative in vitro, again limiting the time frame of experiments or the ability to successfully molecularly manipulate their expression profiles. Therefore, several trophoblast-like cell lines have been developed including choriocarcinoma cell lines, JEG-3, JAR and BeWo, as well as stably transfected primary isolates, e.g., HTR-8/SVneo and SGHPL-4. The validity of these cell lines for use as models of EVT and CTB has been widely discussed by the research community (Genbacev and Miller 2000; Frank et al. 2000, 2001; King et al. 2000; Shiverick et al. 2001; Morrish et al. 2002). Caution must also be taken in interpreting results using these different cell lines as they often respond in a different manner to external stimuli from primary EVT isolates, e.g., their invasive response to low oxygen varies considerably from primary EVT isolates and their response may also vary depending on the time frame of the experiment (Lash et al. 2006b, 2007). In addition, many of the commonly used cell lines display vastly different expression profiles as determined by mRNA array studies (Bilban et al. 2010).

Regulation of EVT Invasion

Many different factors have been proposed to play a role in regulating EVT invasion of the decidua and inner third of the myometrium including different decidual cell types, environmental factors, growth factors, and cytokines.

Decidual Cell Regulation of EVT Invasion

EVT cells are naturally invasive, particularly up to ~12 wk gestation (Genbacev et al. 1996; Lash et al. 2006b). They achieve this not only through the secretion of proteases but also by responding to a variety of autocrine and paracrine signals, some of which are stimulatory whereas others are inhibitory. It is likely that the balance between inhibitory and stimulatory factors alters with gestational age thereby regulating EVT invasiveness. Angiogenic growth factor and cytokine secretion profiles of total decidual cell isolates, uNK cell isolates, EVT, and CTB have been examined at 8–10 wk and 12–14 wk gestation (Lash et al. 2006a, 2010a,b; Naruse et al. 2010). Total decidual secretion of keratinocyte

growth factor (KGF), angiopoietin (Ang)-2, and ICAM-1 is decreased with increasing gestational age (Lash et al. 2006a). uNK cell secretion of Ang-2 and VEGF-C decreased with increasing gestational age (Lash et al. 2006a) whereas secretion of interleukin (IL)-1β, IL-6, IL-8, granulocyte macrophage colony stimulating factor (GMCSF), and interferon (IFN)-γ is increased with increasing gestational age (Lash et al. 2010a). EVT secretion of IL-8, IL-13, and RANTES also increased with gestational age (Naruse et al. 2010). In addition, CTB secretion of Ang-2, sVEGF-R1, IL-1β and IL-8 increased with increasing gestational age (Lash et al. 2010b; Naruse et al. 2010).

The decidua, and to a lesser extent the myometrium, is a rich source of paracrine factors that regulate the extent of EVT invasion including cytokines, growth factors, and proteases. In situ zymography for gelatinases (MMP-2 and MMP-9) and uPA suggests that protease levels are lower in the myometrium than the underlying decidua (Naruse et al. 2009a,b). This may contribute to the lack of EVT invasion past the inner third of the myometrium. In vitro, cell culture supernatants from total decidual cell isolates (6–9, 8–10, or 12–14 wk gestational age) stimulates trophoblast (placental villous explants, JEG3, and B6TERT cell line) invasion in fibronectin or Matrigel transwell assays, via mechanisms associated with altered protease activity (Wright et al. 2006; Zhu et al. 2009; Lash et al. 2010c). Several studies have investigated the role of isolated decidual cell types, including uNK cells, macrophages, CD8 T lymphocytes, and decidual stromal cells, on EVT invasion.

One study has investigated the effect of in vitro decidualized endometrial stromal cells on the invasiveness of JEG3 and ACH-3P cell lines; showing a stimulation of invasion that was associated with increased MMP-2 and MMP-9 activity in the ACH-3P cell line and decreased TIMP-1, TIMP-2, and TIMP-3 mRNA in the JEG-3 cell line (Godbole et al. 2011).

The uNK cells have long been proposed to play a role in regulating EVT invasion. Hanna et al. (2006) showed that IL-15 stimulated uNK cells stimulated the invasion of isolated first trimester CTB cells in the Matrigel transwell invasion assay, an effect mediated in part by IL-8 and IP-10. In contrast, Hu et al. (2006) showed that IL-15 stimulated uNK cells inhibited EVT outgrowth and migration from first trimester placental villous explants, an effect mediated by secreted IFN-γ. Lash et al. (2010c) showed that when uNK cell supernatants (unstimulated) and the placental villous explants used in Matrigel transwell invasion assays were both taken from 12–14 wk gestation then EVT invasion was stimulated, an effect mediated in part by IL-8 (De Oliveira et al. 2010). When explants and cell culture supernatants from 8–10 wk gestation were used there was no effect on invasion. The increase in invasion at 12–14 wk gestation was associated with increased secretion of proteases and decreased apoptosis. These data suggest that the role of uNK cells on EVT invasion is very dependent on gestational age and may depend on the local production of factors. Indeed, coculture of uNK cells and EVT isolated from the same patient decreases secretion of Ang-1, VEGF-C, IL-6, IL-8, and TGF-β1, irrespective of gestational age (Lash et al. 2011).

Decidual macrophages have also been proposed to play a role in regulating EVT invasion, although studies with isolated decidual macrophages have been limited and results have been extrapolated from the use of peripheral blood macrophages, which may differ phenotypically (Gustafsson et al. 2008). Renaud et al. (2005) showed that peripheral blood macrophages activated with lipopolysacharride inhibit the invasion of the trophoblast cell line HTR-8/SVneo, via TNF-α and increased PAI-1. However, nonactivated macrophages had no effect on trophoblast invasion. In addition, Huang et al. (2006) showed that macrophages derived from the THP-1 cell line inhibited HTR-8/SVneo invasion, although a mechanism was not investigated. One pilot study has examined the effect first trimester decidual macrophages from women at high and low risk of developing PE or FGR based on uterine artery Doppler analysis (Cartwright in Lash et al. 2009). They reported that macrophage cell culture supernatants from low risk pregnancies promoted a greater level of trophoblast cell line (SGHPL-4)

invasion than those from higher risk pregnancies.

$CD8^+$ T lymphocytes are a minor leukocyte population in early pregnancy decidua (Williams et al. 2009). $CD8^+$ T lymphocyte supernatants also stimulate EVT invasion in an explant Matrigel transwell invasion assay but only when stimulated with PHA-P (Scaife et al. 2006; De Oliveira et al. 2010).

Taken together, the decidua, and its component cell types, appears to be a stimulator of EVT invasion. Although other factors likely play a role, IL-8, IP-10 and TNFα have been identified as molecular mediators of this response.

Hormone Regulation of EVT Invasion

Both pregnancy-specific and general hormones appear to play roles in regulating EVT invasion. The best well studied of the pregnancy-associated hormones is human chorionic gonadotropin (hCG). Yagel et al. (1993) showed reduced invasion of first trimester CTB into amnion after treatment with hCG that was associated with reduced collagenase and uPA activity. In contrast, Saleh et al. (2007) showed that hCG (with or without contaminating epidermal growth factor, EGF) stimulated invasion of SGHPL-5 cells in a Matrigel transwell assay. Similarly, SGHPL-5 and first trimester EVT cell migration and invasion were stimulated by hCG via a mechanism involving signaling through ERK and AKT pathways and increased MMP-2 activity (Prast et al. 2008). Hyperglycosylated hCG has also been shown to stimulate isolated first trimester EVT invasion in a Matrigel invasion assay (Fournier et al. 2011).

Other hormones that may play roles in regulating EVT invasion include thyroid hormone (Oki et al. 2004), placental growth hormone (Lacroix et al. 2005), progesterone (Goldman and Shalev 2006), corticotropin releasing hormone (Bamberger et al. 2006), gonadotropin-releasing hormone (GnRH) I and II (Liu et al. 2009, 2010).

Local hormone concentrations likely play a role in regulation of interstitial EVT invasion, although these actions may differ depending on gestational age.

Cytokine and Growth Factor Regulation of Trophoblast Invasion

Cytokines are commonly accepted to be produced by immune cells, although they are also produced in the decidua and placenta (Bowen et al. 2002). For many years, the role of different, individual, cytokines in regulating EVT invasion has been investigated. These cytokines are either decidual and/or trophoblast products with trophoblast also expressing the appropriate receptors.

Cytokines and growth factors which have been shown to stimulate trophoblast invasion include, but are not limited to, IL-1β (Librach et al. 1994), leukemia inhibitory factor (LIF) (Fitzgerald et al. 2005), IL-8 (De Oliveira et al. 2010; Jovanović et al. 2010), IL-15 (Zygmunt et al. 1998), IL-17 (Pongcharoen et al. 2006), CXCL12 (Zhou et al. 2008), CXCL16 (Huang et al. 2006), epidermal growth factor (EGF) (Bass et al. 1994), heparin binding EGF (HB-EGF) (Leach et al. 2004).

In contrast, several cytokines and growth factors have been shown to inhibit trophoblast invasion and include, but are not limited to, IL-10 (Yamamoto-Tabata et al. 2004), IL-12 (Karmakar et al. 2004), IL-24 (Cheng and Zou 2008), CXCL14 (Kuang et al. 2009), TGF-β2 (Lash et al. 2005), TGF-β3 (Lash et al. 2005), IFNγ (Lash et al. 2006c), VEGF-A (Lash et al. 1999; Fitzpatrick et al. 2003), endocrine gland-derived VEGF (EG-VEGF or prokineticin 1) (Hoffmann et al. 2009).

Several cytokines and growth factors appear to show differing effects dependent of the cell type and assay used for study. IL-6 has been shown to stimulate both first trimester CTB and HTR-8/SVneo cell invasion (Jovanović and Vićovac 2009). In contrast, Fitzgerald et al. (2005) and Champion et al. (2012) found no effect of IL-6 on invasion of JEG3 or EVT from placental explants respectively. IL-11 is a member of the IL-6 superfamily and has been shown to stimulate first trimester CTB migration (Paiva et al. 2007) but inhibit HTR-8/SVneo cell line invasion (Paiva et al. 2009). It has also been shown to stimulate JEG3 invasion (Suman et al. 2009). Transforming growth fac-

tor (TGF)-β1 has been shown to inhibit migration and invasion of first trimester CTB or EVT isolates, or from explants (Graham et al. 1992, 1993, 1994; Irving and Lala 1995; Lash et al. 2005) as well as HTR-8/SVneo and NCP cells (Graham 1997; Zhao et al. 2006). However, it has no effect on JEG3, JAR and BeWo cells (Graham et al. 1994; Fitzgerald et al. 2005). It has also been shown to stimulate invasion of SGHPL-4 cells (Tse et al. 2002). TNFα has been shown to have no effect on first trimester CTB invasion (Bass et al. 1994). However, later studies have shown an inhibition of migration and invasion of first trimester EVT, HTR-8/SVneo and first trimester EVT from explants (Todt et al. 1996; Bauer et al. 2004; Renaud et al. 2005; Huber et al. 2006; Otun et al. 2011).

Signaling Molecules and Nuclear Receptors in Regulation of EVT Invasion

There are several recent reviews on the role of various signaling molecules and pathways in the regulation of trophoblast invasion (Fitzgerald et al. 2010; Knöfler 2010; Sonderegger et al. 2010; Knöfler and Pollheimer 2012) and therefore these will not be reviewed in detail here. The two best described signaling pathways for regulation of EVT invasion are the wnt signaling pathway (Sonderegger et al. 2010) and the STAT3 pathway (Fitzgerald et al. 2008).

SPIRAL ARTERY REMODELING

Remodeling of the uterine spiral arteries is one of the most important maternal adaptations to pregnancy (Pijnenborg et al. 2006). During spiral artery remodeling, the blood vessels supplying the uterus undergo significant alterations that result in the decidual and superficial myometrial portions of the vessels losing their musculoelastic wall, which is replaced by fibrinoid and intramural EVT (Pijnenborg et al. 2006). This remodeling process allows for maternal blood that is not under vasoactive control to be delivered to the fetal–placental unit. The underlying pathology of preeclampsia and fetal growth restriction is associated with reduced spiral artery remodeling, likely from reduced EVT invasion (Khong et al. 1987; Pijnenborg et al. 1991). Spiral artery remodeling is often described in terms of its sequential morphological features which include vascular smooth muscle cell (VSMC) separation, endothelial cell swelling, vessel dilatation, endovascular and/or interstitial EVT invasion, transient loss of endothelial cells, VSMC loss, fibrinoid deposition, presence of intramural EVT, and regeneration of the endothelium (Pijnenborg et al. 2006). Although EVT are absolutely required for completion of successful spiral artery remodeling the initial steps occur in the absence of EVT, and may be mediated by uNK cells and macrophages. However, the molecular triggers of spiral artery remodeling are not known and exactly how EVT contribute to this process is also not known.

uNK cells and macrophages have been observed to aggregate around the uterine spiral arteries and arterioles in endometrium in early human pregnancy (Bulmer and Lash 2005; Smith et al. 2009). uNK cells secrete an array of angiogenic growth factors including vascular endothelial growth factor (VEGF)-C, placental growth factor (PLGF), angiopoietin (Ang)1 and Ang2, with VEGF-C and Ang2 levels decreasing with increasing gestational age (8–10 wk vs. 12–14 wk) (Li et al. 2001; Hanna et al. 2006; Lash et al. 2006a). A recent in vitro study has shown that uNK cell supernatants (8–10 wk gestational age only) could induce disorganization of two different vessel models via a mechanism likely dependent on their secretion of Ang2 (Robson et al. 2012). These data suggest a role for uNK cells in the early stages of spiral artery remodeling. The role of macrophages is less well defined although they are a potential source of MMPs involved in vessel wall ECM breakdown. In addition, it has been shown that, although VSMCs within the wall of remodeling vessels are not lost by apoptosis, they do migrate into the surrounding stroma (Bulmer et al. 2012). VSMC migration into the stroma appears to be facilitated by the presence of EVT, although the molecular triggers of this process are not yet defined they may include TGF-β1 and PDGF-BB (Bulmer et al. 2012). In addition, the ultimate fate of these migrated VSMCs is unclear although it is hypothesized that they

undergo apoptosis and are phagocytosed by decidual macrophages.

For effective spiral artery remodeling to occur, the successful interplay of a number of different cell types is required, uNK cells with VSMCs and EVT, EVT with VSMCs and endothelial cells, and macrophages with VSMCs. Much more research is required to fully understand the extent of this cross-talk and the molecular mediators involved.

CONCLUSIONS

There is considerable interplay between different cells of the maternal–fetal interface, particularly involving the local immune cells to establish immune tolerance toward the placenta and developing fetus as well as in regulating EVT invasion and spiral artery remodeling. Many layers of communication are required for the successful establishment and continuation of pregnancy, which are likely often unique in humans. Although not all of the molecular communication signals are fully understood, we are starting to understand the cellular components of this communication and establish tools for their greater study.

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