Expression and secretion of antiviral factors by trophoblast cells following stimulation by the TLR-3 agonist, Poly(I: C)

Vikki M. Abrahams1,4, Todd M. Schaefer2, John V. Fahey2, Irene Visintin1, Jacqueline A. Wright2, Paulomi B. Aldo1, Roberto Romero3, Charles R. Wira2 and Gil Mor1,5

1Department of Obstetrics, Gynecology & Reproductive Sciences, Yale University School of Medicine, New Haven, CT, 2Department of Physiology, Dartmouth Medical School, Lebanon, NH and 3The Perinatology Research Branch, National Institute of Child Health and Human Development, Bethesda, Maryland and Detroit, MI, USA
4To whom correspondence should be addressed at: Department of Obstetrics, Gynecology & Reproductive Sciences, Reproductive Immunology Unit, Yale University School of Medicine, New Haven, CT 06520, USA. Email: vikki.abrahams@yale.edu
5To whom correspondence should be addressed at: Department of Obstetrics, Gynecology & Reproductive Sciences, Reproductive Immunology Unit, Yale University School of Medicine, New Haven, CT 06520, USA. Email: gil.mor@yale.edu

BACKGROUND: During pregnancy, the placenta may become exposed to micro-organisms, such as viruses, which may pose a substantial threat to the embryo/fetus well-being. Recent insight into the immunological capabilities of the trophoblast suggests that the placenta may function as an active barrier by recognizing and responding to pathogens through Toll-like receptors (TLRs). METHODS: The objective of this study was to determine whether the engagement of TLR-3 with viral dsRNA by first-trimester trophoblast could induce the production of factors necessary to generate an antiviral response. Therefore, trophoblast cells were exposed to the TLR-3 agonist, Poly(I: C). RESULTS: We report that following stimulation with Poly(I: C), first-trimester trophoblast cells produce interferon β (IFNβ) and secretory leukocyte protease inhibitor (SLPI), as well as the intracellular factors 2',5'-oligoadenylate synthetase (OAS), Myxovirus-resistance A (MxA) and apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like 3G (APOBEC3G). This response is TLR-3 specific because the TLR-4 ligand, lipopolysaccharide (LPS), had no effect on the production of these antimalter factors. Furthermore, we describe a positive feedback mechanism in which IFNβ enhances the antiviral response by promoting the production of OAS, MxA and APOBEC3G. CONCLUSIONS: These findings suggest that trophoblast cells are able to recognize and specifically respond to viral products in a highly regulated fashion and that the placenta may be pivotal in the control of viral infections at the maternal–fetal interface.

Key words: antimicrobial/infection/placenta/pregnancy/Toll-like receptor

Introduction

During implantation and throughout pregnancy, the endometrium develops into an immunologically unique site that accepts the semi-allogenic fetus and placenta, while maintaining host defence against infectious micro-organisms. Although these functions are thought to be facilitated by innate immune cells present within the decidua (Mor et al., 2005), it is also thought that the trophoblast actively participates in preventing allore cognition (Munn et al., 1998; Loke and King, 2000; Abrahams et al., 2004b; Moffett and Loke, 2004) and in the control of micro-organisms that may injure the embryo/fetus (Guleria and Pollard, 2000; Abrahams and Mor, 2005). Viral infections pose a major threat to a pregnancy, gaining access to the placenta by either ascending into the uterus from the lower tract or via the maternal circulation (Pereira et al., 2005). It is known that the placenta and fetal membranes have natural antiviral properties, as evidenced by their ability to produce type I interferons and antiviral peptides (Bulmer et al., 1990; Toth et al., 1990b; Zhang et al., 1993; Franco et al., 1999; Uchide et al., 2002). However, the extent and regulatory mechanisms for such antiviral responses are largely unknown.

Recent studies have demonstrated that, through the expression of Toll-like receptors (TLRs), the placenta can recognize and respond to micro-organisms at the maternal–fetal interface (Abrahams and Mor, 2005). TLRs are a family of innate immune receptors that recognize pathogen-associated molecular patterns (PAMPs) expressed by micro-organisms and subsequently mediate an immune response. To date, 10 functional human TLRs have been characterized (Kawai and Akira, 2005), and their expression is not restricted to leukocytes but is also found in a wide range of non-immune cells (Zaremba and Godowski, 2002). Each TLR responds to a particular ligand, e.g., TLR-4 mediates responses towards Gram-negative bacterial lipopolysaccharide (LPS) (Hoshino et al., 1999), whereas TLR-3 mediates immune responses towards viral dsRNA (Alexopoulou et al., 2001). Following ligand recognition,
TLRs activate a common intracellular signaling pathway by recruiting the intracellular signaling adapter protein, MyD88. A subsequent kinase cascade triggers the activation of the nuclear factor κB (NFκB) pathway, which results in the generation of an inflammatory response (Akira and Takeda, 2004). Studies using MyD88-deficient mice have demonstrated that TLR-3 and TLR-4 can also signal in an MyD88-independent manner (Yamamoto et al., 2002). Such MyD88-independent signalling occurs through another adapter protein, TRIF, which while it can activate the NFκB pathway, also results in the phosphorylation of IRF-3. This pathway generates an antiviral response associated with the production of type I interferons and interferon-inducible genes (Takeuchi et al., 2004).

We have recently demonstrated that first-trimester trophoblast cells express TLR-3 and TLR-4 and that in these cells they signal through distinct pathways (Abrahams et al., 2004a, 2005). Chemokine production by first-trimester trophoblast cells induced by the TLR-4 agonist, LPS, appears to occur in an MyD88-dependent manner, whereas TLR-3 ligation by Poly(I : C), a synthetic analogue of viral dsRNA, occurs in an MyD88-independent manner (Abrahams et al., 2005). Therefore, the objective of this study was to determine whether the stimulation of TLR-3 with viral dsRNA would activate first-trimester trophoblast cells to generate an antiviral response. Herein, we report that first-trimester trophoblast cells constitutively express many antiviral factors, including beta interferon-β (IFNβ), 2′,5′-oligoadenylate synthetase (OAS), Myxovirus-resistance A (MxA), apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like 3G (APOBEC3G), human beta defensin 1 (HBD1) and secretory leukocyte protease inhibitor (SLPI). Moreover, following exposure to the TLR-3 ligand, Poly(I : C), first-trimester trophoblast cells generate a rapid, potent and highly specific antiviral response, characterized by the secretion of IFNβ and SLPI, as well as by the increased expression of OAS. These findings show that trophoblast cells are able to recognize and specifically respond to viral products in a highly regulated fashion and suggest that the placenta may be pivotal in the control of viral infections at the maternal–fetal interface.

Materials and methods

Reagents
Poly(I : C), the synthetic analogue of viral dsRNA and a TLR-3 specific agonist, was purchased from Invivogen (San Diego, CA, USA). The TLR-4 agonist, LPS isolated from Escherichia coli (0111:B4), was purchased from Sigma Aldrich (St Louis, MO, USA). Human recombinant IFNβ was obtained from R&D Systems (Minneapolis, MN). The SLPI ELISA kit was purchased from Caltag (Burlingame, CA, USA) and the IFNβ ELISA kit from Biosource (Camarillo, CA, USA). All immunoassays were performed according to the manufacturer’s instructions.

Patient samples
First-trimester placentas (8–12 weeks of gestation n = 9) were obtained from elective terminations of normal pregnancies performed at Yale-New Haven Hospital. Patients’ ages were between 18 and 28 years old. All patients signed consent forms approved by Yale University’s Human Investigations Committee (HIC).

Isolation and culture of trophoblast cells from first-trimester placenta
Primary trophoblast cells from first-trimester placentas were prepared as described previously (Abrahams et al., 2004b; Straszewski-Chavez et al., 2004). Briefly, tissue specimens were washed with cold Hank’s balanced salt solution (HBSS; Gibco-Invitrogen, Carlsbad, CA, USA) to remove excess blood. Placental villi were scraped from fetal membranes, transferred to trypsin-EDTA (Gibco-Invitrogen) digestion buffer and incubated at 37°C for 10 min with shaking. An equal volume of Dulbecco’s modified Eagle’s medium (DMEM; Gibco-Invitrogen) containing 10% fetal bovine serum (FBS) was added to inactivate the trypsin. This mixture was vortexed for 20 s and allowed to sediment, and the supernatant was collected. This was repeated twice, and the collected supernatant was centrifuged at 400 g for 10 min. Contaminating red blood cells were removed by resuspending the cellular pellet with HBSS, layering this over the same volume of Lymphocyte Separation Media (ICN Biomedicals, Aurora, OH, USA) and centrifuging at 500 g for 25 min. The cellular interface containing the trophoblast cells was collected and resuspended in DMEM supplemented with 10% normal human serum (Gemini Bio-Products, Woodland, CA, USA) and cultured at 37°C/5% CO₂ for no more than three passages. Purity of the trophoblast cells was >98% as determined by immunostaining for cytokeratin-7 and hCG production (Aschkenazi et al., 2002).

First-trimester trophoblast cell line
The human first-trimester extravillous trophoblast cell line, HTR8-SVneo (referred to from hereon as H8), was a kind gift from Dr. Charles Graham (Queens University, Kingston, ON, Canada) (Graham et al., 1993) and was cultured at 37°C/5% CO₂ in RPMI 1640 (Invitrogen, Carlsbad, CA, USA), supplemented with 10% FBS (Hyclone, South Logan, UT, USA), 10 mM Hepes, 0.1 mM MEM non-essential amino acids, 1 mM sodium pyruvate and 100 ng ml⁻¹ of penicillin/streptomycin (Gibco-Invitrogen).

Stimulation of first-trimester trophoblast cells
Trophoblast cells (1 x 10⁶) were seeded into 35 mm tissue culture dishes and cultured overnight in culture media, until 80% confluent. The trophoblast cells were then changed into serum-free OptiMEM (Gibco-Invitrogen, Carlsbad, CA) for 4 h after which they were treated with either OptiMEM for the no treatment control, Poly(I : C) or LPS. Following culture for various time points, total RNA was extracted from cells as described below, and the cell-free supernatants were collected by centrifugation at 400 g for 10 min and stored at –80°C until analysis by enzyme-linked immunosorbent assay (ELISA). For blocking experiments, trophoblast cells were pretreated with the anti-TLR-3 mAb (clone TLR3.7; Cell Sciences, Canton, MA, USA) or a mouse IgG1 isotype control (clone 107.3; BD Pharmingen, San Jose, CA, USA) at a final concentration of 25 μg ml⁻¹ for 1 h at 37°C and then stimulated with Poly(I : C) (25 μg ml⁻¹) for 24 h.

Real-time RT–PCR
Total RNA was isolated from cells and tissues using the RNeasy kit from Qiagen (Valencia, CA, USA). Real-time RT–PCR was performed as described previously (Schafer et al., 2005). For each specimen, 400 ng of total RNA was reverse transcribed using the iScript cDNA synthesis kit according to the manufacturer’s recommendations (BioRad, Hercules, CA, USA) in a 20 μl volume. Relative expression levels of IFNβ, SLPI, OAS, MxA and APOBEC3G were measured using the 5’ fluorogenic nuclease assay in real-time quantitative PCR using Taqman chemistry on the ABI 7300 Prism real-time PCR instrument (ABI, Foster City, CA, USA). The IFNβ, SLPI, OAS, MxA,
APOBEC3G, β-2-microglobulin and β-actin primer/MGB probe sets were obtained from ABI assays-on-demand (ID numbers Hs00277188, Hs00268206, Hs0024294, Hs00182073, Hs00222415, 4333766 and 4333762, respectively). PCR was conducted using the following cycle parameters: 95°C for 12 min for 1 cycle, 95°C for 20 s, 60°C for 1 min, for 40 cycles. Analysis was carried out using the sequence detection software supplied with the ABI 7300. The software calculates the threshold cycle (Ct) for each reaction, and this was used to determine the amount of starting template in the reaction. The Ct values for each set of duplicate reactions were averaged for all subsequent calculations. A difference in Ct values (∆Ct) was calculated for each gene by taking the mean Ct of gene of interest and subtracting the mean Ct for β-2-microglobulin or β-actin for each cDNA sample. Assuming that each reaction functions at 100% PCR efficiency, a difference of one Ct represents a two-fold difference in mRNA expression. Relative expression levels were expressed as a fold increase in mRNA expression.

**RT-PCR analysis**

One microgram of total RNA was reverse transcribed using the Superscript First-Strand Synthesis System for RT–PCR according to the manufacturer’s recommendations (Invitrogen, Austin, TX, USA). PCR amplification was performed using Platinum PCR Supermix (Invitrogen) on the PTC-100 Thermal Cycler (MJ Research, Waltham, MA, USA) on the PTC-100 Thermal Cycler (MJ Research, Waltham, MA, USA) for 35 cycles using the following cycling conditions: 94°C for 1 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min and then a final extension of 72°C for 2 min. Forward and reverse primer pairs were as follows: HBD1, forward (5′-GTCAGCT CAGCCTCTAAAGG-3′) and reverse (5′-CTTCTGCGTATCTTCT TCTG-3′); HBD2, forward (5′-CCCTAGTCCCTTCAGGGT-3′) and reverse (5′-GAGGGAGCCCTTTTCTGAATC-3′); MxA, forward (5′-CGTGAAGGCTATCCGGTCCTCCACCTCAG-3′) and reverse (5′-GCCGGCGCCGACGGCCGCAGGTGTCAGCCGTGC-3′); IFNβ, forward (5′-GCTCTCTCTGTTGCTTCTCCTCCTACAGC-3′) and reverse (5′-CTGACTATGGTCAGGCCAACGTGACTGTACTCC-3′); APO BEC3G, forward (5′-CGTCGGAAATACCCGTCGCTGTGCTACG-3′) and reverse (5′-GATCTTCATGTTGCGACGGCGACCGT-3′); OAS, forward (5′-CCACCTCTGACAGGGCTAGCCTGACCTG-3′); SLPI, forward (5′-CCTGCTCTTACCATGGGACCCGACCGC-3′) and reverse (5′-CATTTGATGCCACAAGTGTCA-3′). The lack of DNA contamination in the RNA preparations was verified by PCR amplification in the absence of reverse transcription.

**Statistical analysis**

Each experiment was performed in triplicates and repeated at least three times. Data are expressed as mean ± SD and statistical significance (P < 0.05) was determined using Student’s t-test.

**Results**

**First-trimester trophoblast cells generate a type I interferon response following exposure to Poly(I : C)**

The production of type I interferons is characteristic of an immune response to viral products following TLR-3 activation (Takeda and Akira, 2005). Therefore, the first objective of this study was to determine whether the ligation of TLR-3 by Poly(I : C) in first-trimester trophoblast cells was able to generate the production of the type I interferon, IFNβ. The first-trimester trophoblast cell line H8 constitutively expressed IFNβ mRNA (Figure 1), as demonstrated by both semi-quantitative and quantitative real-time RT–PCR. Furthermore, data were treated with Poly(I : C), IFNβ expression increased in a time-dependent manner, with mRNA levels peaking after 4 h of treatment (Figure 2A). Next, we evaluate whether these cells have the ability to secrete IFNβ. As shown in Figure 2B, IFNβ secretion was undetectable in the supernatants collected from unstimulated H8 cells, suggesting that these cells do not constitutively secrete IFNβ. However, following treatment with Poly(I : C), IFNβ secretion by H8 cells was increased in a dose-dependent manner, with Poly(I : C) at 25 μg ml⁻¹ inducing marked secretion. A maximum increase in IFNβ secretion was observed 48 h after treatment and remained high at 72 h after stimulation with Poly(I : C) (Figure 2C).

To determine the specificity of the IFNβ response to Poly(I : C), we treated first-trimester trophoblast H8 cells with bacterial LPS and IFNβ expression and secretion was evaluated. As shown in Figure 3A, although Poly(I : C) was able to induce a significant increase in IFNβ mRNA expression by H8 cells, LPS treatment resulted in no change in the baseline IFNβ expression. Similar results were found using primary first-trimester trophoblast cells. Thus, although IFNβ secretion by primary trophoblast cells was induced by Poly(I : C), IFNβ was undetectable in the supernatants collected from LPS-stimulated trophoblast cells (Figure 3B).

**First-trimester trophoblast cells express IFN-inducible genes**

TLR-3 stimulation by viral dsRNA, in addition to triggering the production of type I interferons, is also known to induce the
expression of intracellular antiviral factors, known as interferon-inducible genes (Samuel, 2001; Takeuchi et al., 2004). Therefore, the trophoblast expression of the interferon-stimulated factors, OAS and MxA, was evaluated. We also evaluated the trophoblast expression of APOBEC3G, an intracellular antiretroviral factor (Turelli et al., 2004). Semi-quantitative and real-time RT–PCR results demonstrated that H8 first-trimester trophoblast cell line constitutively expressed OAS, MxA and APOBEC3G mRNA (Figure 1). Furthermore, as shown in Figure 4A, OAS mRNA expression was markedly up-regulated in H8 cells following stimulation with Poly(I : C), and this occurred in a time-dependent manner with expression levels peaking after 10 h of treatment. This response was specific towards Poly(I : C), because the treatment of H8 trophoblast cells with LPS had no effect on OAS expression levels (Figure 4B). Although the treatment of H8 trophoblast cells with Poly(I : C) induced an increase in MxA and APOBEC3G expression, the magnitude of this response was substantially lower than that for OAS. Thus, after 10 h of treatment, a minor increase by 1.9-fold in MxA and a 1.6-fold in APOBEC3G mRNA expression was observed, whereas LPS had no such effect (data not shown).

**IFNβ induces OAS, MxA and APOBEC3G production by trophoblast cells**

Because the increase in OAS expression induced by Poly(I : C) peaked 6 h later than the peak in IFNβ expression, we postulated that OAS expression in trophoblast cells might be an indirect effect of TLR-3 ligation, mediated by the type I interferon response. Therefore, H8 trophoblast cells were treated with increasing concentrations of recombinant human IFNβ for 10 h, after which OAS mRNA levels were determined. As shown in Figure 5A, OAS expression was indeed up-regulated following...
Antiviral responses of trophoblast cells

the treatment of trophoblast cells with IFNβ, and this occurred in a dose-dependent manner. Similarly, the treatment of H8 trophoblast cells with IFNβ also induced the expression of MxA and APOBEC3G (Figure 5B).

First-trimester trophoblast cells secrete SLPI following exposure to Poly(I : C)

Recently, uterine epithelial cells, upon stimulation with Poly(I : C), have been shown to produce SLPI and HBDs (Schaefer et al., 2005). These factors, although usually associated with antibacterial properties, are also known to possess antiviral activities (McNeely et al., 1995; Quinones-Mateu et al., 2003). Therefore, the expression and secretion of SLPI, HBD1 and HBD2 by first-trimester trophoblast cells was evaluated. Using semi-quantitative and quantitative real-time RT–PCR performed for OAS. The figure is representative of at least three independent experiments.

secretion by H8 trophoblast cells occurred in a dose-dependent manner (Figure 6B; *P < 0.05; ***P < 0.001). Moreover, the stimulation of trophoblast cells with LPS had no effect on the levels of SLPI secretion, suggesting that the above observed effects with Poly(I : C) may be a specific TLR-3 response (Figure 6C). These similar results, as obtained using H8 cells, were also observed when primary cultures of first-trimester trophoblast cells were treated with either Poly(I : C) or LPS (Figure 6C).

Because the Poly(I : C)-induced OAS expression in trophoblast cells appeared to be mediated by type I interferons, we evaluated the effect of IFNβ on SLPI secretion. As shown in Figure 7A, although Poly(I : C) significantly increased SLPI secretion by trophoblast cells, IFNβ had no such effect.

SLPI expression following exposure to Poly(I : C) is TLR-3 dependent

Although Poly(I : C) has been shown to be a ligand for TLR-3 which is expressed by trophoblast cells, we evaluated the
specificity of TLR-3 stimulation by blocking Poly(I : C) binding using a competitive anti-TLR-3 monoclonal antibody. As shown in Figure 7B, the pretreatment of H8 trophoblast cells with a blocking anti-TLR-3 antibody decreased SLPI secretion following Poly(I : C) treatment.

Discussion

During pregnancy, the placenta may become exposed to microorganisms, such as viruses, which pose a significant threat to fetal well-being and to the success of the pregnancy. Originally, the placenta was viewed as merely a physical barrier; however, it is becoming increasingly apparent that this complex organ plays an active role in protecting the developing fetus from infection (Mor et al., 2005). In the present study, we demonstrate for the first time that trophoblast cells, following TLR-3 ligation, are capable of producing the antimicrobial factors IFNβ, OAS, MxA, APOBEC3G and SLPI, which may constitute a highly specific and potent antiviral response.

The innate immune response towards microbes consists of an assortment of soluble components including cytokines, chemokines and antimicrobial peptides (Tosi, 2005). The production of type I interferons is a critical part of antiviral immunity. IFNα and IFNβ are important for activating innate immune cells, such as macrophages and NK cells, and for up-regulating major histocompatibility complex class I expression, thus facilitating a cytotoxic T-cell response (Samuel, 2001). Type I interferons are also important for inducing the production of intracellular proteins with antiviral activities, such as OAS and MxA (Brierley and Fish, 2002). Several studies have reported the presence of antimicrobial peptides in...
human placental tissues. The expression of interferon-α and -β has been localized to the villous syncytiotrophoblast and extravillous trophoblast throughout gestation (Bulmer et al., 1990; Paulesu et al., 1991). In addition, OAS, an intracellular interferon-induced factor that inhibits viral replication, has been detected in both first- and third-trimester trophoblast cells (Zhang et al., 1993; Dalsgaard et al., 1995). Together, these data suggest that the trophoblast is able to recognize viral products and actively respond to them; however, the cellular mechanisms mediating such recognition and response has not been fully elucidated.

Recently, we have described the expression of TLR-3 and TLR-4 in first-trimester trophoblast cells (Abrahams et al., 2004a, 2005). Furthermore, we have demonstrated that following the ligation of TLR-3, by viral dsRNA, or TLR-4, by bacterial LPS, trophoblast cells are able to regulate immune cell migration through the production of inflammatory cytokines and chemokines (Abrahams et al., 2004a, 2005). In this study, we have evaluated whether the stimulation of TLR-3 with its specific agonist, Poly(I : C) generates the production of factor(s) characteristic of an antiviral response. Our first set of experiments confirmed that first-trimester trophoblast cells do indeed express IFNβ mRNA and that following ligation of TLR-3 a significant increase in both expression and secretion occurs. Under resting conditions, first-trimester trophoblast cells do not secrete IFNβ. However, following stimulation, IFNβ secretion rapidly occurs and may represent an acute mechanism of defence for the trophoblast towards a virus. The quick secretion of IFNβ and additional chemokines, such as RANTES (Abrahams et al., 2005), will promote immune cell migration to and activation at the site of infection, thus preventing viral dissemination towards the maternal or fetal circulation.

Using quantitative RT–PCR, we were also able to detect the presence of many intracellular antiviral factors in first-trimester trophoblast cells including OAS, MxA and APOBEC3G. Furthermore, Poly(I : C) triggers an increase in their production. Previous studies have also demonstrated that trophoblast exposure to viral products triggers IFN and OAS production (Toth et al., 1990a,b, 1991; Aboagye-Mathiesen et al., 1993; Uchide et al., 2002). However, this study provides additional evidence on the mechanisms regulating these responses. The increased production of OAS, MxA, APOBEC3G as well as IFNβ in trophoblast cells appears to be under the control of the TLR-3 signalling pathway. The specificity of the TLR-3 antiviral response by trophoblast cells is evidenced by the lack of an effect on IFNβ, OAS, MxA or APOBEC3G expression level in response to LPS, a TLR-4 specific ligand. When the expression patterns of IFNβ and OAS following Poly(I : C) stimulation were evaluated, IFNβ mRNA levels were found to peak as early as 4 h following stimulation, whereas OAS expression peaked after 10 h. A potential explanation for these findings could be that OAS production is the result of an autocrine and/or paracrine effect of IFNβ. Therefore, we evaluated the effect of IFNβ on OAS expression in trophoblast cells. As expected, IFNβ had a significant effect, not only on OAS mRNA levels, but also on MxA and APOBEC3G expression, suggesting the presence of a feedback mechanism mediated by IFNβ. Indeed, studies by Paulesu et al. (1997) have demonstrated the expression of type I interferon receptors by first-trimester villous cytotrophoblast and extravillous trophoblast cells. In addition, both OAS and MxA are known to be IFN-inducible factors (Samuel, 2001). Therefore, we propose that upon stimulation of TLR-3 by viral products, such as dsRNA, first-trimester trophoblast cells secrete IFN-β, which will not only act on immune cells at the maternal–fetal interface but will also induce the expression of antiviral products by the same and surrounding trophoblast, thus preventing the transmission of a viral infection to the fetus.

Antimicrobial peptides, such as the HBDs, and antiproteinases, such as the secretory leukocyte inhibitor, play key roles in innate immune defence and in controlling excessive inflammation. These peptides are generally produced at mucosal sites by epithelial cells and have broad antimicrobial activity including antiviral properties (Sallnave, 2002; Tosi, 2005). In this study, we hypothesized that the trophoblast functions as a ‘mucosal barrier’ and, therefore, might produce antimicrobial peptides following pathogen recognition through TLRs. Indeed, using quantitative RT–PCR, we were able to detect the presence of the antimicrobial peptides, HBD1 and SLPI, in first-trimester trophoblast cells. However, only the production of SLPI was found to be modulated following stimulation by the TLR-3 agonist, Poly(I : C). These findings concur with those by Svinarich et al. (1997) who found HBD1 and HBD3 mRNA to be expressed in placenta. However, the lack of an HBD reporter exposure was surprising, because a recent study has shown uterine epithelial cells to express increased HBD1 and HBD2 following TLR-3 ligation (Schaefer et al., 2005). Although the treatment of trophoblast cells with LPS or Poly(I : C) had no effect on SLPI mRNA expression, Poly(I : C), but not LPS, treatment increased the secretion of this antimicrobial factor. Furthermore, this suggests that exposure to viral products triggers the release of preformed SLPI and not de novo synthesis. Interestingly, Poly(I : C)-induced SLPI secretion appears not to be regulated by IFNβ, suggesting that the release of this factor may be a direct effect of ligand–receptor interactions as evidenced by a decrease in SLPI secretion in the presence of a blocking anti-TLR-3 antibody. These findings are of interest in view of a report from Ding et al. (2005) showing that macrophages produce SLPI in response to Mycobacterium tuberculosis in a TLR-2-dependent but MyD88-independent manner and of our recent findings showing that TLR-3 effects on trophoblast cells are MyD88 independent (Abrahams et al., 2005).

In summary, we have demonstrated that first-trimester trophoblast cells are able to produce major antiviral factors necessary to mount a specific and potent immune response towards a viral infection. We have shown that, in the absence of any stimulation, first-trimester trophoblast cells constitutively express a host of antiviral factors, which become differentially up-regulated and secreted following exposure to the TLR-3 ligand, Poly(I : C). TLR-3, the receptor for viral dsRNA, and TLR-4, the receptor for bacterial LPS, both have the ability to signal a common MyD88-independent pathway, which results in a type I interferon response and the up-regulation of interferon-stimulated gene expression. However, such a response is only generated by trophoblast cells following stimulation with
Poly(1 : C). In addition, the exposure of trophoblast cells to Poly(1 : C) triggers the production of SLPI, an antimicrobial peptide with antiviral properties. Together, these findings support the notion that the placenta can specifically recognize and differentially respond to micro-organisms. Moreover, the trophoblast can mount a potent and tightly regulated antiviral response, and thus, the placenta may play an active role in defence against viral infection at the maternal–fetal interface.

Acknowledgements

This study was supported in part by grants RO1HD049446-01 (VMA) from the NICHD, 2NO1HD23342 from the Intramural Research Program of the NICHD (GM and RR) and AI51877 (CRW) from NIH.

References


Abrahams VM, Straszewski-Chavez SL, Guller S and Mor G (2004b) First trimester trophoblast cells secrete Fas ligand which induces immune cell apoptosis. Mol Hum Reprod 10,55–63.


