

Enhanced expression of IFI16 and RIG-I in human third-trimester placentas following HSV-1 infection

A. Jabłońska ^{*}, M. Studzińska,^{*}
P. Suski,^{*} J. Kalinka[†] and
E. Paradowska ^{*}

^{*}Laboratory of Molecular Virology and
Biological Chemistry, Institute of Medical
Biology, Polish Academy of Sciences, and

[†]Department of Perinatology, First Chair of
Gynecology and Obstetrics, Medical University
of Lodz, Lodz, Poland

Summary

The innate immune response in the placenta depends on the ability of maternal immune cells and fetal trophoblast cells to detect and eliminate invading pathogens through germline-encoded pattern recognition receptors (PRRs). In the present study, we analysed the transcripts and protein expression of interferon (IFN)-inducible protein (IFI)16, melanoma differentiation-associated protein 5 (MDA5), RIG-I-like receptor (RIG-I) and Toll-like receptor (TLR)-3 in third-trimester human placentas and investigated cytokine profiles generated during herpes simplex type 1 (HSV-1) infection. Decidual and chorionic villous biopsies (38–42 weeks of gestation) were obtained from healthy women immediately after a caesarean section. The expression of the *DDX58* (RIG-I), *IFIH1* (MDA5), *IFI16* and *TLR3* transcripts was measured using quantitative real-time polymerase chain reaction (qRT-PCR). Extracellular cytokine and PRRs levels were then quantified by enzyme-linked immunosorbent assays (ELISAs). All examined PRRs genes, including *DDX58*, *IFIH1*, *IFI16* and *TLR3*, were expressed constitutively at the mRNA and protein levels in the placental biopsies. The concentration of the IFI16 protein was increased in HSV-1-infected decidual and chorionic villous explants compared to those of mock-infected tissues ($P = 0.029$). Higher protein expression levels of RIG-I in both the maternal and fetal parts of the placenta were found ($P = 0.009$ and $P = 0.004$, respectively). In addition, increased production of IFN- β by HSV-1-infected tissues was noticed ($P = 0.004$ for decidua, $P = 0.032$ for chorionic villi). No significant differences in the IFN- α , interleukin (IL)-6 and IL-8 levels were found. These results showed that HSV-1 infection can enhance the expression of IFI16 and RIG-I proteins in the human term placenta.

Keywords: cytokines, HSV-1, IFI16, pattern-recognition receptors, placenta

Accepted for publication 20 April 2018

Correspondence: E. Paradowska, Laboratory
of Molecular Virology and Biological
Chemistry, Institute of Medical Biology,
Polish Academy of Sciences, 106 Lodowa
Street, 93–232 Lodz, Poland.

E-mail: eparadowska@cbm.pan.pl

Introduction

Herpes simplex virus type 1 (HSV-1) is a member of the *Herpesviridae* family and is one of the most common sexually transmitted viruses among adult women [1,2]. HSV-1 has been associated traditionally with oral–facial infections, whereas HSV-2 is associated with anogenital infection transmitted through sexual contact. In recent decades, the number of cases of genital herpes caused by HSV-1 has increased [3,4]. A reduced seroprevalence of HSV-1 and an increased frequency of oral sex have been suggested to be responsible for this trend. Peña *et al.* [4] found that genital HSV-1 positivity rates were high in young women (47%)

in the United States. The increasing acquisition rate of genital HSV-1 among women of childbearing age suggests that more neonates may be exposed to the virus than in past decades. Although the mother-to-child transmission of HSV-1 infection is rare [5,6], it can result in severe disseminated neonatal disease and may lead to fetal abortion, congenital malformation and stillbirth [7,8]. The vast majority (85%) of mother-to-child HSV-1 transmissions occur during the peripartum period. An additional 10% of infected newborns acquire the virus postnatally and the final 5% are infected with HSV-1 *in utero* [9]. The most common and serious HSV-1 infection is associated with the first or second trimester of pregnancy [6,8]. Newborns with

intrauterine HSV-1 infection typically have a triad of clinical findings consisting of cutaneous manifestations (scarring, active lesions, aplasia cutis, hyper- or hypopigmentation and/or erythematous macular exanthem), neurological involvement (microcephaly, encephalomalacia, hydranencephaly and/or intracranial calcifications) and ophthalmological findings (chorioretinitis, microphthalmia and/or retinal dysplasia) [9–12]. An HSV-1 infection acquired in either the peripartum or postpartum periods can be categorized as the most common disease of the skin, eyes and/or mouth (SEM) disease (45% of cases), central nervous system (CNS) disease, including meningitis or encephalitis (30% of cases) and disseminated disease that involves multiple organs (25% of cases) [11,13]. It is therefore critically important to identify the host anti-viral factors that recognize and counteract viral infection in the placenta and to develop novel therapeutic strategies for improving the comfort and quality of life of affected children.

The interferon (IFN)-mediated response is a first line of host defence against viral infections and is important for the activation of both innate and adaptive immunity [14,15]. The innate immune response is initiated by the activation of a broad range of sensors termed pattern recognition receptors (PRRs) by invading pathogens [16,17]. It was shown that HSV-1 DNA and double-stranded RNA (dsRNA), a by-product of viral replication, are sensed by many types of PRRs, including RIG-I-like receptors (RLRs), IFN-inducible protein 16 (IFI16), DNA-dependent protein kinase (DNA-PKR), DNA-dependent activator of IFN-regulatory factor (DAI), DDX41 (DEAD-box helicase 41), cyclic guanosine monophosphate–adenosine monophosphate synthase (cGAS) and Toll-like receptors 3 and 9 (TLR-3 and TLR-9) [18–25]. PRRs that specifically recognize DNA as a potential marker of viral infection trigger the IFN-regulatory factor 3 (IRF-3) pathway, leading to the induction of type I IFN [20,26] and IFN-stimulated genes (ISGs). Importantly, it was shown that adaptor protein stimulator of interferon genes (STING) is also essential for IFN- β induction by DNA and by HSV-1 [27]. However, little is known about the pattern of PRR expression during HSV-1 infection in the human term placenta.

In this paper, we sought to identify and compare the expression patterns of the selected PRRs [TLR-3, RIG-I, melanoma differentiation-associated protein 5 (MDA5) and IFI16] in term placentas in order to understand their capacity to recognize HSV-1 infection as well as assess the cytokine levels after HSV-1 stimulation *ex vivo*.

Materials and methods

Tissue collection

Placental biopsies were collected anonymously from healthy women (median age = 31.7; range = 27.3–36.9

years) undergoing elective caesarean section (38–42 weeks of gestation). Pregnancies complicated by maternal autoimmune or infectious diseases, diabetes and fetal abnormalities were excluded from the study. This study was conducted under a protocol approved by the institutional ethics committee of the Medical University of Lodz (RNN/120/09/KE) and was run in accordance with the Declaration of Helsinki. Written informed consent was obtained from all donors prior to their inclusion in the trial.

Decidual and chorionic villous explants cultures were performed and tested separately in each organ. Briefly, placental tissues were separated from surrounding membranes, washed several times in ice-cold phosphate-buffered saline (PBS; Sigma-Aldrich Co. Ltd, Ayrshire, UK) until the supernatant was nearly free of blood, and minced into small pieces (approximately 2 mm³). The explants were either collected immediately at time zero or transferred into tissue culture plates and incubated in 2 ml of Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich) supplemented with 10% inactivated fetal bovine serum [fetal bovine serum (FBS); Sigma-Aldrich], 2 mM L-glutamine and 100 μ g streptomycin–100 U penicillin (Sigma-Aldrich). Cultures were incubated for 48 h at 37°C in 5% CO₂. After 24 and 48 h post-infection (h.p.i.), the placental explants were harvested and tissue-free supernatants were collected by centrifugation at 13 100 g for 10 min at 4°C and stored at –80°C until use.

Cell line and virus strain

The Vero cell line (ATCC CCL-81; Manassas, VA, USA) was grown in Eagle's minimum essential medium (EMEM; Sigma-Aldrich) containing 10% inactivated FBS and 2 mM L-glutamine and 100 μ g streptomycin–100 U penicillin at 37°C in 5% CO₂ until confluence. HSV-1 strain MacIntyre (McIntyre, ATCC VR-539) was cultured in the Vero cells with EMEM supplemented with 2% inactivated FBS and antibiotics. At 48 h.p.i., supernatants from infected Vero cells approaching > 80% cytopathic effect (CPE) were collected and used to infect the placental explants. The laboratory-adapted strain of HSV-1 was used for the inoculation of all placental explants and Vero cells. HSV-1 stocks were prepared and the virus was quantitated using a standard plaque assay on Vero cells [20]. Cellular debris was pelleted by centrifugation at 13 100 g for 10 min at 4°C, and the resulting supernatants were aliquot and stored at –80°C until use.

Viral infection

The explants were infected immediately with HSV-1 [1×10^5 plaque-forming units (PFU)/ml] in DMEM supplemented with 2% FBS and 2 mM L-glutamine and 100 μ g streptomycin–100 U penicillin. Following 1 h incubation at room temperature, the inoculum was removed and the explants were washed three times with PBS. After washing,

2 ml of fresh culture medium were added and the explants were cultured for 48 h at 37°C in 5% CO₂. Virus-infected explants that were kept at 4°C for 24 h.p.i. served as a control sample of the starting level of the virus, while mock-infected tissues served as a control. The tissue-free supernatants were harvested at 24 and 48 h.p.i., centrifuged at 13 100 g for 10 min and stored at -80°C until analysis by enzyme-linked immunosorbent assay (ELISA) and viral load measurement. As a positive control, permissive Vero cells inoculated with HSV-1 at a multiplicity of infection (MOI) of 1 were provided.

DNA isolation

Genomic DNA was extracted from the specimens using a QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's recommendations. The extracted DNA was diluted in elution buffer to the optimal concentration for use as a template for PCR amplification and stored at -20°C until use. The DNA concentration and purity was determined using a NanoDrop 2000c UV-Vis Spectrophotometer (Thermo Scientific, Waltham, MA, USA).

Assessment of HSV-1 load

HSV-1 DNA was detected and quantified in the placental tissues using the HSV-1 polymerase chain reaction (PCR) kit (GeneProof a.s., Brno, Czech Republic), according to the manufacturer's instructions. The quantitative real-time (qRT)-PCR was performed on the 7900HT Fast Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). A negative control without template DNA was included in every of amplification run.

Extraction of total RNA and cDNA synthesis

The decidual and chorionic villous tissues and the Vero cells were preserved in RNAlater (Ambion, Austin, TX, USA) at -80°C until further analysis. The total RNA was extracted using Tri Reagent (Ambion). First-strand cDNA synthesis was performed using the high-capacity cDNA reverse transcription kit (Applied Biosystems, according to the manufacturer's recommendations. The reverse transcription reaction was performed in a Veriti[®] 96-well thermal cycler (Applied Biosystems). The concentration and purity of the RNA and cDNA were assessed using a NanoDrop 2000c UV-Vis Spectrophotometer.

qRT-PCR analysis

Human *DDX58*, *IFIH1*, *IFI16* and *TLR3* mRNA expression was quantified by RT-PCR. The tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide (*YWHAZ*) was selected for the normalization of target gene expression, as its expression levels were both highly stable and detectable in the human placenta [28]. For each qRT-PCR, 50 ng cDNA was added to a mix

containing gene-specific primers [23,29,30], power SYBR Green PCR Master Mix (Applied Biosystems) and nuclease-free water in a final volume of 25 µl. Amplification was performed in a 7900HT fast RT-PCR system under the following conditions: one cycle at 95°C for 10 min, followed by 50 cycles of 15 s at 95°C and 60 s at 60°C. All reactions were run in triplicate with a non-template control. The qRT-PCR data were analysed using Data Assist version 3.0 software (Life Technologies, Grand Island, NY, USA), and the comparative C_t (ΔC_t) method was used to calculate the relative gene expression.

ELISA

The tissue homogenates were prepared using the Mammalian Cell Lysis Kit (Sigma-Aldrich), according to the manufacturer's instructions. The concentrations of IFI16, MDA5, RIG-I and TLR-3 proteins in tissue homogenates were determined using ELISA (EMELCA Biosciences, Breda, the Netherlands), according to the manufacturer's recommendations. The tissue culture supernatants were assayed for IFN- α , IFN- β (PBL Interferon Source, Piscataway, NJ, USA), IL-6, C-X-C motif chemokine ligand 8 (CXCL8)/IL-8 and tumour necrosis factor (TNF)- α (R&D Systems, Minneapolis, MN, USA). The absorbance values of the samples and standards were read using a Benchmark Plus microplate reader (Bio-Rad Laboratories, Hercules, CA, USA).

Statistical analysis

Statistical analyses were performed and graphs constructed using GraphPad Prism version 5.0 (GraphPad Software Inc., San Diego, CA, USA). All results are presented as the mean \pm standard error of the mean (s.e.m.). The Mann-Whitney *U*-test was used to assess any differences in the targeted genes and protein expression levels between the two groups (virus-infected and mock-infected tissues), while the Wilcoxon test was performed to compare two variables within the same group. A *P*-value < 0.05 was considered significant.

Results

HSV-1 replication in the placenta

To exclude the HSV-1 infection of the placental samples, extracted DNA was subjected to qRT-PCR. None of the analysed samples expresses the HSV-1 genome, indicating that the placentas were not infected with this virus *in vivo*. Hence, freshly obtained explants were infected with HSV-1 strain McIntyre, and the viral load in decidual and chorionic villous explants after *in-vitro* viral infection was studied. Isolated explants were relatively resistant to HSV-1 infection. Viral replication was observed in four of eight placentas (50.0%) (Fig. 1). No significant difference between HSV-1 replication in decidua and chorionic villi

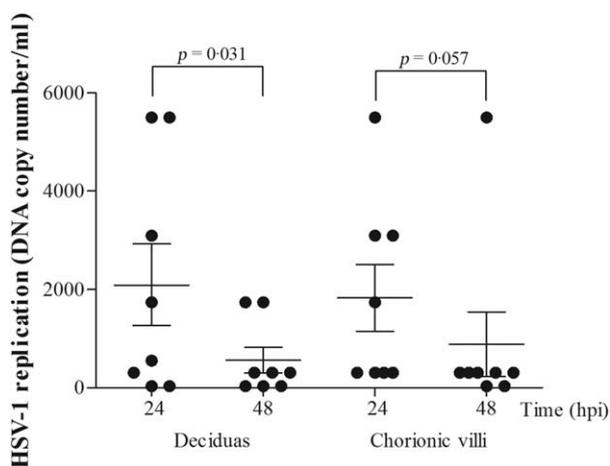


Fig. 1. Comparison of the levels of herpes simplex type 1 (HSV-1) replication in third-trimester human placentas. Villous and decidual explants were infected with laboratory strain of HSV-1 *in vitro*. HSV-1 DNA was detected and quantified in the placental tissues using the HSV-1 polymerase chain reaction (PCR) kit using quantitative real-time (qRT)-PCR under the following conditions: denaturation at 95°C for 10 min, followed by 45 cycles of 95°C for 5 s and 60°C for 40 s.

was found. All mock-infected placental explants remained free of virus throughout the experiment.

IFI16 and TLR-3 are expressed in term human chorionic villi and decidua

All examined PRRs genes, including *DDX58* (RIG-I), *IFIH1* (MDA5), *IFI16* (IFI16) and *TLR3* (TLR-3), were expressed constitutively at the mRNA and protein levels in the term decidual and chorionic villous tissues (Fig. 2a,b). A significantly higher level of *IFI16* expression in HSV-1-infected decidual and chorionic villous explants compared to mock-infected placentas was found at 48 h.p.i. ($P = 0.029$ and $P < 0.001$, respectively; Fig. 3a). Higher levels of *TLR3* mRNA expression were also observed at 24 h.p.i. ($P = 0.004$ and $P < 0.001$, respectively; Fig. 3b). The *DDX58* gene expression was lower in the virus-infected explants at 24 h.p.i. ($P = 0.028$ for chorionic villi; Fig. 3c), whereas the levels of *IFIH1* did not show any significant difference ($P > 0.05$; Fig. 3d).

IFI16 and RIG-I proteins are expressed during HSV-1 infection

The level of IFI16 protein after HSV-1 infection was higher than in mock-infected tissues ($P = 0.029$; Fig. 4a). An increase in the TLR-3 protein concentration was found in HSV-1-infected chorionic villi compared to mock-infected explants at 48 h.p.i. ($P = 0.028$; Fig. 4b). Higher protein expression levels of RIG-I upon virus infection were observed at 24 h.p.i. ($P = 0.009$ and $P = 0.004$ for decidua

and chorionic villi, respectively; Fig. 4c). Our results revealed that all PRRs were expressed at both mRNA and protein levels in term human placenta samples.

IFN- β is secreted in response to HSV-1 infection

To examine whether PRRs contribute to the host response to virus infection, we measured the concentrations of IFN- α , IFN- β , IL-6, IL-8 and TNF- α in supernatants from HSV-1-infected and mock-infected explants. HSV-1 induced IFN- β production in both decidua and chorionic villi at 48 h.p.i. ($P = 0.004$ and $P = 0.032$, respectively; Fig. 5a). The significant decrease in the circulating TNF- α levels was observed between mock-infected and HSV-1-infected explants ($P < 0.05$) (Fig. 5b). The release of IFN- α and ILs following HSV-1 infection was unchanged (data not shown).

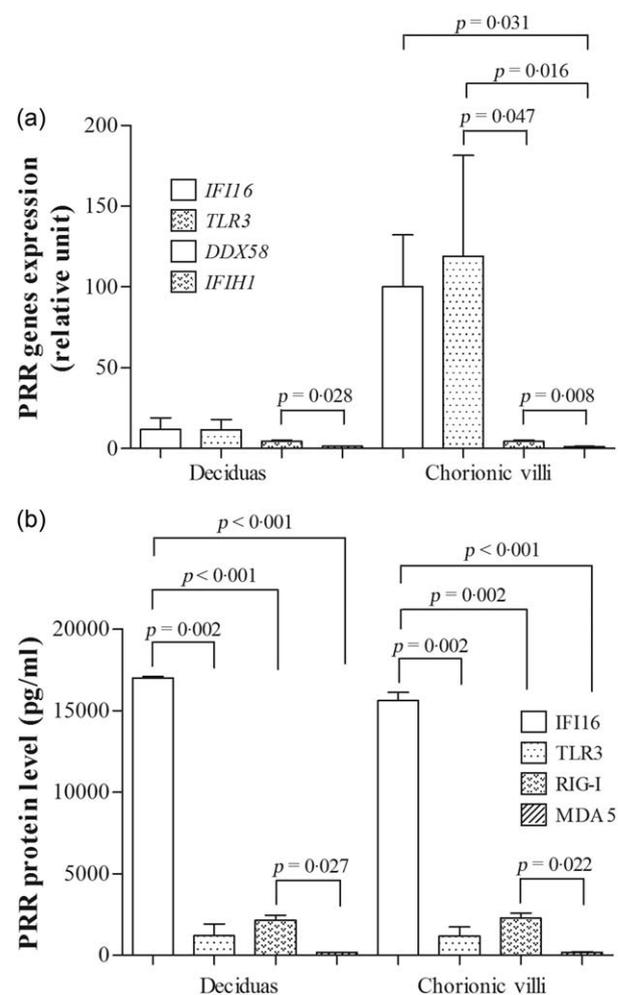


Fig. 2. The constitutive pattern recognition receptors (PRRs) (a) mRNA and (b) protein expression ($n = 8$). The data are reported as the mean values \pm standard error of the mean (s.e.m.). Statistically significant differences compared to mock-infected placental explants are shown.

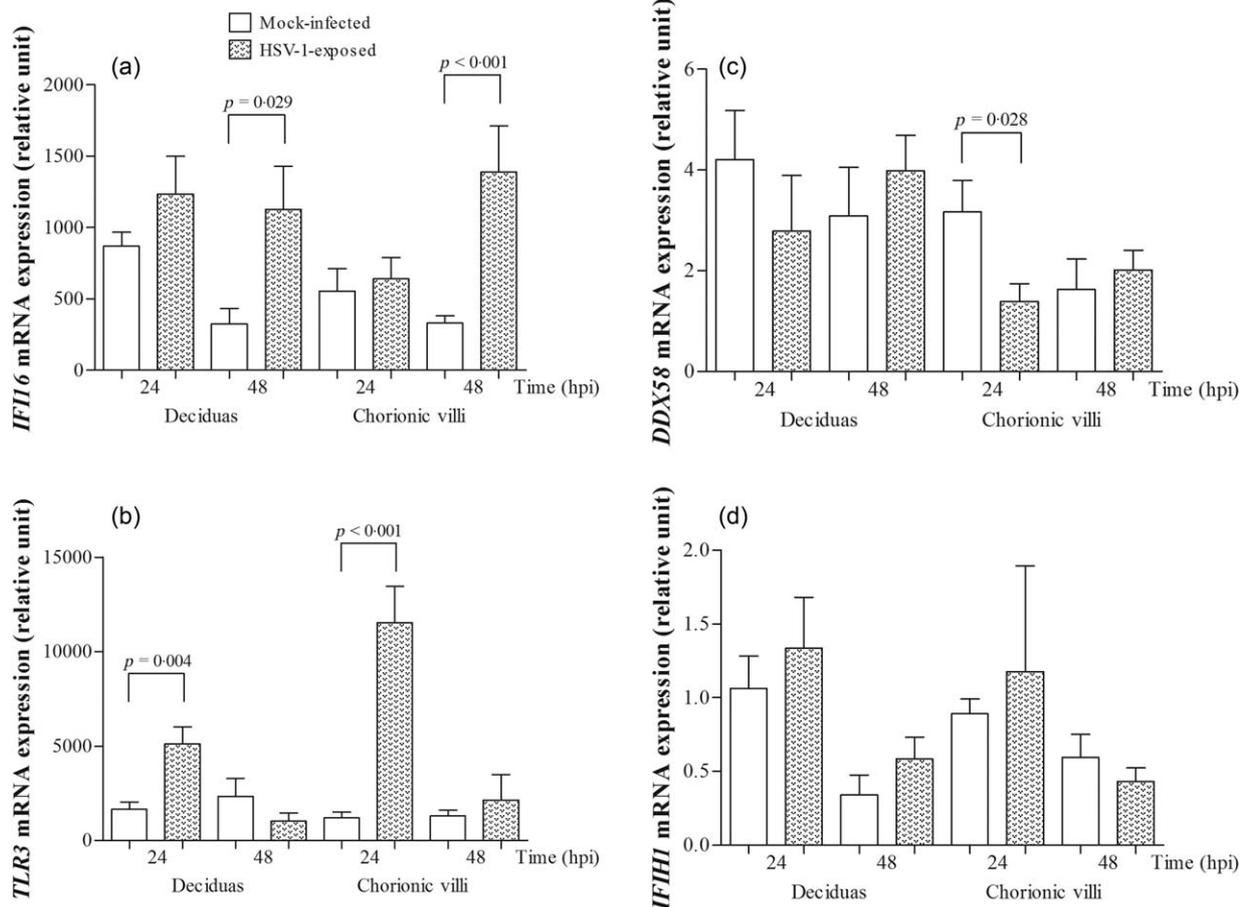


Fig. 3. The mRNA expression levels of (a) *IFI16*, (b) *TLR3*, (c) *DDX58* and (d) *IFI1* in third-trimester mock-infected and herpes simplex type 1 (HSV-1)-infected decidual and chorionic villi tissues ($n = 8$). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using *YWHAZ* as a control gene. The data are reported as the mean values \pm standard error of the mean (s.e.m.). Statistically significant differences compared to mock-infected placental explants are shown. The Mann-Whitney two-tailed test was used to assess statistically significant differences.

Transcription of PRR genes in Vero cells after HSV-1 infection

The next objective of this study was to determine which sensor is responsible for the HSV-1 recognition in susceptible Vero cells. We found that the mRNA expression of *TLR3*, *IFI16* and *DDX58* increased after virus infection ($P = 0.047$ for *TLR3*; Fig. 6), while that of *IFI1* was blocked. We did not observe the induction of IFN in response to HSV-1 infection in permissive Vero cells ($P > 0.05$; data not shown).

Discussion

PRRs are key components of the innate and adaptive immune responses and play a key role in the maternal-fetal interface [31-34]. We have shown that the *IFI16* expression at both mRNA and protein levels was elevated significantly in third-trimester human placentas infected with HSV-1 *ex*

vivo compared to mock-infected explants. A higher level of RIG-I protein expression in the tissues of fetal and maternal origin was observed. An enhanced IFN- β production upon HSV-1 infection was also noted, suggesting that these sensors activate downstream signalling in response to viral infection. To our knowledge, this is the first report that suggests that HSV-1 triggered enhanced *IFI16* and RIG-I expression in the human term placenta.

IFI16 is a member of the human PYHIN family, and is an important nuclear dsDNA sensor that mediates the IFN- β response [23,35]. It contains two C-terminal DNA-binding HIN domains and an N-terminal pyrin domain that plays a role in regulating the inflammatory response and apoptosis [36,37]. DNA sensing by *IFI16* is length-dependent and GC content- and sequence-independent [23]. It was found that *IFI16* binds fragments of dsDNA preferentially, which are approximately 150 base pairs (bp) long [38]. Therefore, host DNA (10-20 bp long) is too short to activate this receptor. To date, the expression of *IFI16*

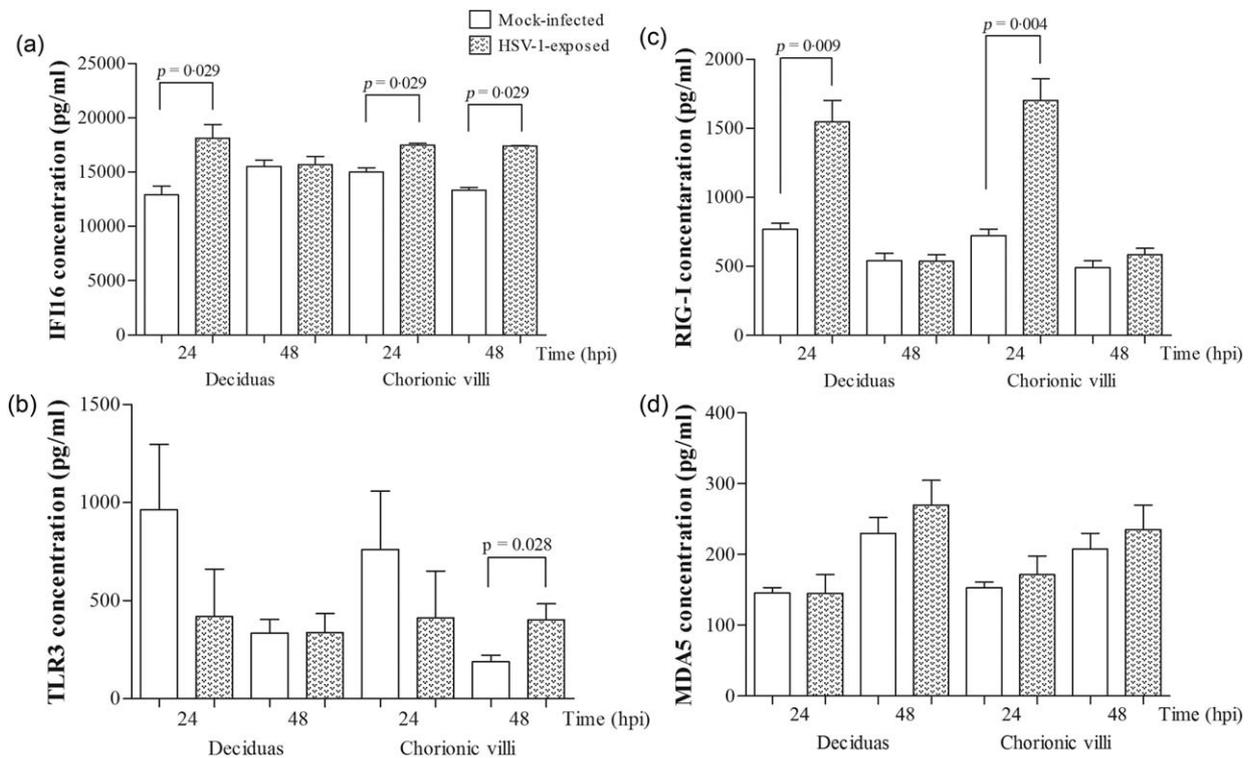


Fig. 4. Differential expression of (a) IFI16, (b) Toll-like receptor (TLR)-3, (c) RIG-I-like receptor (RIG-I) and (d) melanoma differentiation-associated protein 5 (MDA5) in the placental tissue samples from mock-infected and herpes simplex type 1 (HSV-1)-infected decidua and chorionic villi ($n = 8$). Data are reported as the mean values \pm standard error of the mean (s.e.m.). The Mann–Whitney two-tailed test was used to assess statistically significant differences.

during HSV-1 infection has been studied mainly in association with the innate immune response in other cells, including the corneal epithelium, human monocytic THP-1 cells, primary human macrophages, neurones and primary human foreskin fibroblasts [23,39–44]. We have shown that expression of IFI16 was induced during HSV-1 infection in the tissues of fetal and maternal origin. An elevated IFN- β production in supernatants of decidual and placental tissues following HSV-1 treatment was also observed. It is well known that type I IFNs are important for mounting an antiviral response [43,45]. Hence, an increase in IFN- β production in HSV-1-infected cultures suggests that the human term placenta initiate a classical anti-viral response upon recognition of HSV-1. As described previously, IFI16 can recognize HSV-1 DNA both in the nucleus and in the cytoplasm to mediate type I IFNs via the STING/TBK1/IRF3 pathway and inflammatory cytokine responses via the nuclear factor kappa B (NF- κ B) pathway. We observed a significant decrease in TNF- α production between supernatants of mock-infected and HSV-1 infected tissues that may be explained by the ability of HSV-1 to suppress expression of proinflammatory cytokines by decreasing the stability of mRNA transcripts [46]. It is known that proinflammatory immune responses are regulated tightly in the placenta to prevent immunological rejection of the fetal allograft and to

decrease the incidence of stillbirth and preterm birth. We supposed that upon infection of placental explants with HSV-1, IFI16 induces type I IFN through a pathway involving IFN-stimulated genes activation. It also appears reasonable that endogenous cytokine restricts viral replication or there is an additional as-yet undefined factor/s which blocks the replication machinery and proinflammatory responses.

Furthermore, we demonstrated that the viral infection of the placenta may also enhance the expression of RNA sensors, such as RIG-I. It was described previously that RIG-I acts in parallel with DAI in an RNA polymerase III-dependent manner to initiate inflammatory and antiviral glial responses to HSV-1 infection [47]. An increase in the *RIG-I* mRNA concentration and a trend towards an increased level of *MDA5* mRNA in the blood of persistently infected with the bovine viral diarrhoea virus (BVDV) fetuses *versus* control fetuses were also described [48]. These results showed that BVDV has the ability to cross the placenta and infect the fetus due to insufficient development of the fetal immune system and that RNA helicases RIG-I and MDA5 can be induced in infected fetuses. RIG-I, as well as MDA5, TLR-3, TLR-7 and TLR-8, were also found to be expressed by the term placenta, choriondecidua and amnion in a second study [31]. Our results may indicate that RIG-I activate in chorionic villi and decidua the

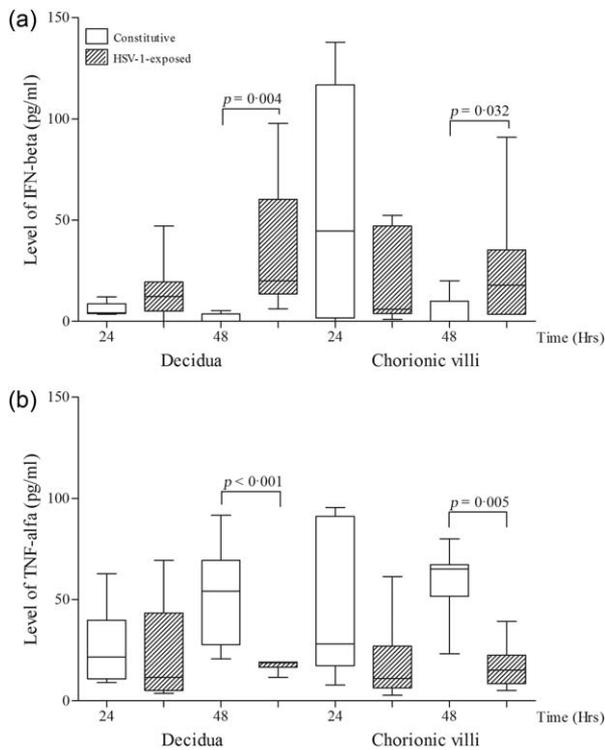


Fig. 5. Analysis of the supernatants concentrations of (a) interferon (IFN)- β and (b) tumour necrosis factor (TNF)- α in decidua and chorionic villous tissue after herpes simplex type 1 (HSV-1) infection. Decidua and chorionic villi explants were infected with 1×10^5 plaque-forming units (PFU)/ml HSV-1 ($n = 8$). Culture supernatants were harvested at 24 and 48 h post-infection. The boxes indicate the 25th and 75th percentiles, while the bands near the middle indicate the median values. Data are expressed as the mean \pm standard error of the mean (s.e.m.). The Mann–Whitney two-tailed test was used to assess statistically significant differences.

same intracellular signalling pathway as IFI16. We suggest that placental tissues possess a functional IFI16 as well as RIG-I signalling systems, the activation of which can lead to IFN-mediated anti-HSV-1 responses. In addition, the anti-viral response was determined in Vero cells, known as deficient in production of type I IFNs. The results showed that HSV-1 infection specifically enhanced expression of TLR-3, IFI16 and DDX58 in Vero cells, whereas no IFN type I production was detected. This may indicate that recognition of HSV-1 infection involves both TLR-dependent and -independent mechanisms.

Expression levels between transcript and protein are not always correlated. Post-transcriptional or post-translational modifications, such as phosphorylation and ubiquitination, may be the reasons for differences in protein production with the corresponding mRNA level in tissue. Post-translational management of proteins due to protein half-life, experimental condition, protein degradation, etc. may also contribute more for the reverse result in response to the above. Moreover, the proteins may be long-lived

proteins which accumulate over time, while the mRNA turnover is speedy. In the present study, a low expression of *IFI16* transcript and high expression of the protein in freshly isolated villous and decidua explants was found. We supposed that *IFI16* mRNA becomes degraded, while its protein has a higher half-life and remains in the cellular pool. Another possibility is that its mRNA is translated more preferentially to the protein. It is also possible that elevated expression of IFI16 in term placentas was associated with non-necroptotic programmed cell death [49].

Some potential limitations of the present study must be acknowledged. First, the main limitation is that this study has a small number of placental biopsies. Secondly, this study does not include the biopsies from intrauterine HSV-1-infected human placental tissues, as the availability of placental tissues with *in-utero* HSV-1 infection from pregnant women is limited. Thirdly, we did not isolate the specialized cells of the placenta (e.g. trophoblast), although we analysed the decidua and chorionic villi explants separately. Because the total concentration of proteins determined with the bicinchoninic acid (BCA) assay was very low, and we wanted to quantify the PRR proteins, we decided to use the ELISA assay. In addition, other putative DNA sensors, e.g. DAI, DDX41, cGAS and adaptor protein STING, were not included in this preliminary study. Hence, further studies will be needed to verify the importance of our observations.

In conclusion, the results of this study shed light upon the possible role of the PRR system in the detection of HSV-1 infection and the activation of innate immunity in the human placenta. These findings suggest a possible role of IFI16 in the DNA-induced immune response in the

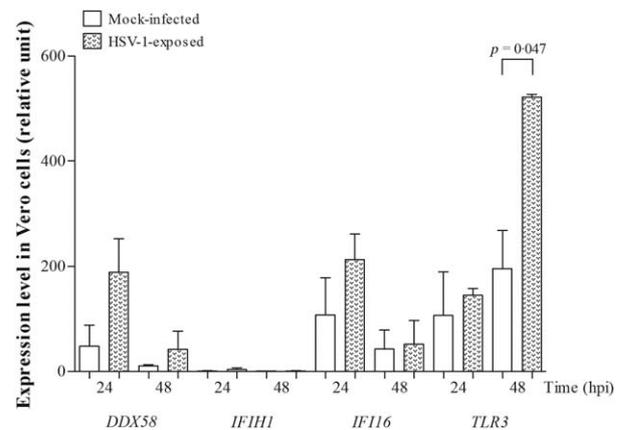


Fig. 6. The relative expression of *DDX58*, *IFI1*, *IFI16* and *TLR3* in mock-infected and herpes simplex type 1 (HSV-1)-infected Vero cells ($n = 4$ independent experiments; passages 134–135). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using *YWHAZ* as a housekeeping gene. The data are reported as the mean values \pm standard error of the mean (s.e.m.). The Mann–Whitney two-tailed test was used to assess statistically significant differences.

placenta, as well as RIG-I as a cytoplasmic sensor for non-self RNA. However, to confirm these hypotheses, future in-depth and more advanced investigations will be needed to define the precise mechanisms by which PRRs modulate HSV-1 pathogenesis in the placenta.

Acknowledgements

The authors are grateful all pregnant women who participating in this study. The authors thank to Edyta Jaworska for the technical support. This work was supported by the National Science Centre of Poland (Grant no. 2015/17/N/NZ6/02015) and the European Union Developmental Fund under Operational Programme Innovative Economy (Grant no. POIG.01.01.02–10-107/09).

Disclosure

All authors state that they have nothing to disclose.

References

- Bernstein DI, Bellamy AR, Hook EW III *et al.* Epidemiology, clinical presentation, and antibody response to primary infection with herpes simplex virus type 1 and type 2 in young women. *Clin Infect Dis* 2013; **56**:344–51.
- Webber BJ, Pawlak MT, Jones NM, Tchandja JN, Foster GA. Sexually transmitted infections in U.S. Air Force recruits in basic military training. *Med Surveill Monthly Rep* 2016; **23**:16–9.
- Gupta R, Warren T, Wald A. Genital herpes. *Lancet* 2007; **370**: 2127–37.
- Peña KC, Adelson ME, Mordechai E, Blaho JA. Genital herpes simplex virus type 1 infection in women: detection in cervicovaginal specimens from gynecological practices in the United States. *J Clin Microbiol* 2010; **48**:150–3.
- Anzivino E, Fioriti D, Mischitelli M *et al.* Herpes simplex virus infection in pregnancy and in neonate: status of art of epidemiology, diagnosis, therapy and prevention. *Virology* 2009; **6**:40.
- Brown ZA, Wald A, Morrow RA, Selke S, Zeh J, Corey L. Effect of serologic status and cesarean delivery on transmission rates of herpes simplex virus from mother to infant. *JAMA* 2003; **289**:203–9.
- Mercolini F, Verdi F, Eisendle K, Messner H, Staffler A. Congenital disseminated HSV-1 infection in preterm twins after primary gingivostomatitis of the mother: case report and review of the literature. *Z Geburtshilfe Neonatol* 2014; **218**:261–4.
- Syridou G, Spanakis N, Konstantinidou A *et al.* Detection of cytomegalovirus, parvovirus B19 and herpes simplex viruses in cases of intrauterine fetal death: association with pathological findings. *J Med Virol* 2008; **80**:1776–82.
- Kimberlin DW. Neonatal herpes simplex infection. *Clin Microbiol Rev* 2004; **17**:1–13.
- Allen UD, Robinson JL. Prevention and management of neonatal herpes simplex virus infections. *Paediatr Child Health* 2014; **19**:201–12.
- James SH, Sheffield JS, Kimberlin DW. Mother-to-child transmission of herpes simplex virus. *J Pediatric Infect Dis Soc* 2014; **3**:S19–23.
- Straface G, Selmin A, Zanardo V, De Santis M, Ercoli A, Scambia G. Herpes simplex virus infection in pregnancy. *Infect Dis Obstet Gynecol* 2012; **2012**:385697.
- Corey L, Wald A. Maternal and neonatal HSV infections. *N Engl J Med* 2009; **361**:1376–85.
- Gantt S, Muller WJ. The immunologic basis for severe neonatal herpes disease and potential strategies for therapeutic intervention. *Clin Dev Immunol* 2013; **2013**:369172.
- Su C, Zhan G, Zheng C. Evasion of host antiviral innate immunity by HSV-1, an update. *Virology* 2016; **13**:38.
- Paludan SR, Bowie AG, Horan KA, Fitzgerald KA. Recognition of herpesviruses by the innate immune system. *Nat Rev Immunol* 2011; **11**:143–54.
- Piret J, Boivin G. Innate immune response during herpes simplex virus encephalitis and development of immunomodulatory strategies. *Rev Med Virol* 2015; **25**:300–19.
- Ferguson BJ, Mansur DS, Peters NE, Ren H, Smith GL. DNA-PK is a DNA sensor for IRF-3-dependent innate immunity. *Elife* 2012; **1**:e00047.
- Melchjorsen J, Rintahaka J, Søbø S *et al.* Early innate recognition of herpes simplex virus in human primary macrophages is mediated via the MDA5/MAVS-dependent and MDA5/MAVS/RNA polymerase III-independent pathways. *J Virol* 2010; **84**:11350–8.
- Rasmussen SB, Sørensen LN, Malmgaard L *et al.* Type I interferon production during herpes simplex virus infection is controlled by cell-type-specific viral recognition through Toll-like receptor 9, the mitochondrial antiviral signaling protein pathway, and novel recognition systems. *J Virol* 2007; **81**:13315–24.
- Sun L, Wu J, Du F, Chen X, Chen ZJ. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. *Science* 2013; **339**:786–91.
- Takaoka A, Wang Z, Choi MK *et al.* DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. *Nature* 2007; **448**:501–5.
- Unterholzner L, Keating SE, Baran M *et al.* IFI16 is an innate immune sensor for intracellular DNA. *Nat Immunol* 2010; **11**: 997–1004.
- Weber F, Wagner V, Rasmussen SB, Hartmann R, Paludan SR. Double-stranded RNA is produced by positive-strand RNA viruses and DNA viruses but not in detectable amounts by negative-strand RNA viruses. *J Virol* 2006; **80**:5059–64.
- Zhang Z, Yuan B, Bao M, Lu N, Kim T, Liu YJ. The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells. *Nat Immunol* 2011; **12**:959–65.
- Almine JE, O'Hare CA, Dunphy G *et al.* IFI16 and cGAS cooperate in the activation of STING during DNA sensing in human keratinocytes. *Nat Commun* 2017; **8**:14392.
- Ishikawa H, Ma Z, Barber GN. STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. *Nature* 2009; **461**:788–92.
- Meller M, Vadachkoria S, Luthy DA, Williams MA. Evaluation of housekeeping genes in placental comparative expression studies. *Placenta* 2005; **26**:601–7.
- Komuro A, Horvath CM. RNA- and virus-independent inhibition of antiviral signaling by RNA helicase LGP2. *J Virol* 2006; **80**:12332–42.
- Patni S, Wynen LP, Seager AL, Morgan G, White JO, Thornton CA. Expression and activity of Toll-like receptors 1–9 in the human term placenta and changes associated with labor at term. *Biol Reprod* 2009; **80**:243–8.

- 31 Bryant AH, Menzies GE, Scott LM *et al.* Human gestation-associated tissues express functional cytosolic nucleic acid sensing pattern recognition receptors. *Clin Exp Immunol* 2017; **189**:36–46.
- 32 Cardenas I, Mulla MJ, Myrtolli K *et al.* Nod1 activation by bacterial iE-DAP induces maternal-fetal inflammation and preterm labor. *J Immunol* 2011; **187**:980–6.
- 33 Holmlund U, Cebers G, Dahlfors AR *et al.* Expression and regulation of the pattern recognition receptors Toll-like receptor-2 and Toll-like receptor-4 in the human placenta. *Immunology* 2002; **107**:145–51.
- 34 Zhang YY, Chen H, Sun C *et al.* Expression and functional characterization of NOD2 in decidual stromal cells isolated during the first trimester of pregnancy. *PLOS ONE* 2014; **9**:e99612.
- 35 Connolly DJ, Bowie AG. The emerging role of human PYHIN proteins in innate immunity: implications for health and disease. *Biochem Pharmacol* 2014; **92**:405–14.
- 36 Fairbrother WJ, Gordon NC, Humke EW *et al.* The PYRIN domain: a member of the death domain-fold superfamily. *Protein Sci* 2001; **10**:1911–8.
- 37 Jin T, Perry A, Jiang J *et al.* Structures of the HIN domain: DNA complexes reveal ligand binding and activation mechanisms of the AIM2 inflammasome and IFI16 receptor. *Immunity* 2012; **36**:561–71.
- 38 Gallucci S. An overview of the innate immune response to infectious and noninfectious stressors. In: Amadori M, ed. *The innate immune response to noninfectious stressors. Human and Animal Models*. Brescia, Italy: Academic Press, 2016:1–24.
- 39 Conrady CD, Zheng M, Fitzgerald KA, Liu C, Carr DJ. Resistance to HSV-1 infection in the epithelium resides with the novel innate sensor, IFI-16. *Mucosal Immunol* 2012; **5**: 173–83.
- 40 Diner BA, Lum KK, Toettcher JE, Cristea IM. Viral DNA sensors IFI16 and cyclic GMP-AMP synthase possess distinct functions in regulating viral gene expression, immune defenses, and apoptotic responses during herpesvirus infection. *MBio* 2016; **7**: e01553–16.
- 41 Horan KA, Hansen K, Jakobsen MR *et al.* Proteasomal degradation of herpes simplex virus capsids in macrophages releases DNA to the cytosol for recognition by DNA sensors. *J Immunol* 2013; **190**:2311–9.
- 42 Orzalli MH, DeLuca NA, Knipe DM. Nuclear IFI16 induction of IRF-3 signaling during herpesviral infection and degradation of IFI16 by the viral ICP0 protein. *Proc Natl Acad Sci USA* 2012; **109**:E3008–17.
- 43 Rosato PC, Leib DA. Neuronal interferon signaling is required for protection against herpes simplex virus replication and pathogenesis. *PLOS Pathog* 2015; **11**:e1005028.
- 44 Soby S, Laursen RR, Ostergaard L, Melchjorsen J. HSV-1-induced chemokine expression via IFI16-dependent and IFI16-independent pathways in human monocyte-derived macrophages. *Herpesviridae* 2012; **3**:6.
- 45 Goodbourn S, Didcock L, Randall RE. Interferons: cell signaling, immune modulation, antiviral responses and virus countermeasures. *J Gen Virol* 2000; **81**:2341–64.
- 46 Mogensen TH, Melchjorsen J, Malmgaard L, Casola A, Paludan SR. Suppression of proinflammatory cytokine expression by herpes simplex virus type 1. *J Virol* 2004; **78**:5883–90.
- 47 Crill EK, Furr-Rogers SR, Marriott I. RIG-I is required for VSV-induced cytokine production by murine glia and acts in combination with DAI to initiate responses to HSV-1. *Glia* 2015; **63**: 2168–80.
- 48 Smirnova NP, Webb BT, Bielefeldt-Ohmann H *et al.* Development of fetal and placental innate immune responses during establishment of persistent infection with bovine viral diarrhoea virus. *Virus Res* 2012; **167**:329–36.
- 49 Chu X, Chen W, Li N *et al.* Cytosolic double-stranded DNA induces nonnecroptotic programmed cell death in trophoblasts via IFI16. *J Infect Dis* 2014; **210**:1476–86.