Published in final edited form as:

Am J Reprod Immunol. 2016 April ; 75(4): 451–460. doi:10.1111/aji.12501.

Type I Interferon Regulates the Placental Inflammatory Response to Bacteria and is Targeted by Virus: Mechanism of Polymicrobial Infection-Induced Preterm Birth

Karen Racicot[1,2], **Ja Young Kwon**[1,3], **Paulomi Aldo**[1], **Vikki Abrahams**[1], **Ayman El-Guindy**[4], **Roberto Romero**[5], and **Gil Mor**[1]

[1]Department of Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA

[2]Department of OB/GYN, College of Human Medicine, Michigan State University, Grand Rapids, MI, USA

[3]Department of OB/GYN, Institute of Women's Life Medical Science, Yonsei University College of Medicine, Seoul, Korea

[4]Department of Pediatrics, Yale School of Medicine, New Haven, CT, USA

[5]Perinatology Research Branch, Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, DHHS, Detroit, MI, USA

Abstract

Problem—Preterm birth (PTB) affects approximately 12% of pregnancies and at least 50% of cases have no known risk factors. We hypothesize that subclinical viral infections of the placenta are a factor sensitizing women to intrauterine bacterial infection. Specifically, we propose that viral-induced placental IFN-β inhibition results in a robust inflammatory response to low concentrations of bacteria.

Methods—Human trophoblast SW.71, C57BL/6, and interferon (IFN) receptor knockout animals were used to determine IFN function. Illumina and Bio-Rad microarrays identified pathways.

Results—Inhibiting the IFN-β pathway resulted in a significant increase in inflammatory cytokines such as IL-1B in response to LPS. Twist was positively correlated with IFN-β expression and STAT3 phosphorylation and over-expressing Twist reduced IL-1B. Treating IFNAR−/− mice with low-dose LPS at E15.5 caused preterm birth.

Conclusion—IFN-β was identified as a key modulator of placental inflammation and, importantly, is commonly affected by viruses. We propose dysregulation of IFN-β is a major determinant for preterm birth associated with polymicrobial infection.

Keywords

Bacteria; infection; interferon; polymicrobial; pregnancy; trophoblast; virus

Correspondence. Gil Mor, Department of OB/GYN, Yale School of Medicine, 333 Cedar St. LSOG 305A, New Haven, CT 06520, USA. gil.mor@yale.edu.

Introduction

Preterm birth (PTB), or birth before 37 weeks' gestation, affects approximately 12% of pregnancies in the United States[1–7] and approximately 70% of these cases are spontaneous PTB with intact membranes or premature rupture of the membranes (PPROM), often with unknown etiology.[2,4,5,7–9] Spontaneous PTB is a leading cause of neonatal mortality resulting in an estimated 1 million neonatal deaths every year.[4,6,8] Furthermore, surviving preterm children often suffer from fetal inflammatory response syndrome (FIRS) and sepsis during development, which may contribute to both short- and long-term sequelae, including intraventricular hemorrhage, lung disease, and abnormal neurodevelopment among other pathologies.[9–15] Despite the severity and frequency of PTB and its numerous consequences, at least 50% of cases have no known risk factors and the PTB rates have increased over the last 30 years.[2,5,7] A lack of progress in preventing and treating PTB is due, in part, to a lack of understanding of the underlying causes of the condition. For example, it is estimated that approximately 25% of spontaneous PTBs with intact membranes are associated with intra-amniotic bacterial infection, with even higher rates specifically, in very early PTB,[2,16] but these bacteria are often introduced months prior to birth during amniocentesis or by ascending from the lower reproductive tract over time; so, what finally triggers labor and why don't antibiotics prevent PTB in most women?[1]

It is our working hypothesis that subclinical viral infections of the placenta are a factor sensitizing women to intrauterine bacterial infection or even to the placental microflora itself.[17–19] Several types of viral genomes have been detected in the amnion of pregnant women, including cytomegalovirus (CMV),[20] human papillomavirus,[21] adenovirus, and enterovirus.[22] Positive titers in the amnion have been associated with preterm labor, preterm PROM, low birth weight, and intrauterine death.[22,23] Despite these associations, many term pregnancies are positive for viral genomes, just as normal pregnancies are positive for bacteria. Together, this suggests that bacteria or virus alone do not necessarily cause adverse pregnancy outcomes, which led us to develop the 'two-hit hypothesis'. We propose a viral infection of the placenta (hit 1) changes the placental response to local microorganisms (hit 2) and, therefore, increases the risk of an inflammatory response resulting in preterm birth.[18]

Our previous studies support this hypothesis; viral infection of the placenta in a murine model resulted in increased sensitivity to bacterial products.[18,19] Specifically, all animals receiving LPS following viral infection experienced preterm birth associated with a placental 'cytokine storm', while LPS alone caused preterm labor in only 20% of the animals.[18,19] But how is viral infection changing the placental response to bacteria? Microarray analysis of placentas with and without viral infection indicated that type I interferons (IFN) were significantly downregulated by virus. Interferons are potent antiviral proteins that are targeted by a number of viruses, such as members of the herpes virus family, as a mechanism to evade immune recognition and cellular targeting.[24–31] Interestingly, type I IFN also has important immunomodulatory functions: After binding its receptor, a signaling cascade is activated resulting in activation of inhibitory proteins.[27,28] These act at the level of signal transduction of multiple inflammatory pathways and result in suppression of the immune response. Interestingly, placental extracts containing high concentrations of

'trophoblast interferon' were used to treat inflammation historically, and presently purified type I IFN is used as an anti-inflammatory in patients with MS.[32]

These observations led us to propose type I IFN may be the protein/pathway targeted by viruses that could be affecting the placental inflammatory response to bacterial products. Specifically, we hypothesize that when a virus infects the placenta, it inhibits IFN; this results in a change in placental immune regulation resulting in a robust inflammatory response to even very low concentrations of bacteria. The objectives of this study were to define the mechanism of virus-induced placental sensitivity to bacteria via downregulation of type I IFN and evaluate whether loss of IFN results in sensitization to bacteria and preterm birth.

Materials and methods

MHV68 Production and Quantification

MHV68 was passaged in NIH3T3 cells with DMEM-10% FBS. Supernatants were harvested, filtered (0.45 uM pore), and tittered by twofold serial dilutions on NIH3T3. Viral titers in mouse tissues and cells were determined as previously described.[33] In short, tissues were homogenized and approximately 25 mg of tissue from the reproductive tract or 10 mg of spleen was cut into small pieces and added to lysis buffer supplemented with proteinase K. Samples were incubated at 56°C with shaking for 4–6 hr as recommended for the DNeasy blood and tissue kit (Qiagen, Valencia, CA, USA). Cells from culture were lysed with the same buffer and vortexed at room temperature. All samples were then processed according to the DNeasy protocol. DNA concentration and purity were assessed using spectrophotometric analyses. About 100 ng total DNA was then assayed using primers directed against MHV68 ORF53 and compared to a standard curve created using serial dilutions of purified virus. Results are reported as copies per 100 ng DNA.

Animals

All animals were maintained in the Yale University School of Medicine Animal Facility under specific pathogen-free conditions. All procedures were approved by the Yale University Institutional Animal Care and Use Committee and used between 6 and 10 mice. The IFNAR−/− and C57BL/6 mice were obtained from the Jackson Laboratory (Bar Harbor, ME). The IFNAR−/− (B6.129S2-Ifnar1^{tm1Agt}/Mmjax) were backcrossed onto C57bl/6J for 3 generations. The genotyping protocol is described for stock number 32045-JAX through Jackson Laboratories; primers = Common (9850): 5′-CGAGGCGAAGTGGTTAAAAG-3′; Wild-type reverse (9851): 5′-ACGGATCAACCTCATTCCAC-3′; Mutant (oIMR8963): 5′-AATTCGCCAATGACAAGACG-3′.

LPS Treatment (Animals)

Adult mice (8–12 wks of age) with vaginal plugs were injected with low-dose LPS (20 ug/kg, 0111: B4; Sigma, Saint Louis, MO) in sterile water, i.p. at E15.5 then monitored for 48 hr to determine whether preterm labor occurred. Preterm labor was defined as birth of 100% of fetuses before E18.5.

Cell Culture

Immortalized human trophoblast Sw.71 cells or primary first-trimester trophoblasts were cultured in DMEM/F12 with 10% FBS under 5% CO2 at 37°C. For viral infections, MHV68 (3×10^6 pfu/mL) was added for 30 min, washed, and then cells were maintained in DMEM/F12-10%FBS. For IRF3 blocking experiments, cells in DMEM/F12 1% FBS were treated with 100 nM BX795 (Selleckchem, Houston, TX) for 1 hr, followed by treatment with 1 ug/mL ultrapure LPS (0111:B4; Sigma) and collected 24 hr after LPS. For Twist overexpression, Sw.71 was treated with MHV68 followed by LPS as previously described, to induce inflammation. Twenty-four hours preceding LPS, 500 ng of the constitutive mammalian overexpression plasmid (Invitrogen, Grand Island, NY) containing human Twist, pcDNA3-h-Twist, was added in association with 2 uL Lipofectamine 2000 reagent in Opti-MEM (Invitrogen). After 4 hr, cells were replenished with DMEM/F12 10% overnight, followed by addition of LPS for 24 hr. Controls were treated with pcDNA3 empty plasmid replacing pcDNA3-h-Twist.

RNA Extraction, cDNA Synthesis, and qPCR

RNA was extracted using the RNeasy kit (Qiagen). RNA concentration and purity were assessed using spectrophotometric analyses of 260/280 ratios, and only samples with values of 1.8 or higher were used for PCR analysis. One microgram of RNA was reverse transcribed for each sample using oligo-(d)T priming and SuperScript II reverse transcriptase (Invitrogen). Syber (KAPA Biosystems, Wilmington, MA) and gene-specific primers were added to the RT reactions that were diluted 1:10 with nuclease-free water and run on the CFX96, C1000 system qPCR machine (Bio-Rad, Hercules, CA). No RT controls and no template controls were used to confirm specificity. Values were normalized to GAPDH and calculated with delta-delta C_t method: delta-delta C_t = delta C_t treated- delta C_t control; results expressed as fold differences are $2^{-(\text{average delta}-\text{delta } C_t)}$ for negative delta −delta C_t values or $-(2^{[\text{average delta}-\text{delta } C_t]})$ for positive delta-delta C_t values.

Cytokine Detection

Evaluation of cytokines and chemokines in tissue samples and cell culture supernatants was performed using a custom Bio-Plex kit (Bio-Rad). Wells of a 96-well plate were loaded with either 50 μL of prepared standard solution or 50 μL of cell-free supernatant and incubated with a custom Bio-Plex Pro™ human 17-plex (Bio-Rad) at ±800 rpm for 30 min in the dark at room temperature. Wells were then vacuum-washed three times with 100 μL wash buffer. Samples were then incubated with 25 μL of biotinylated detection antibody at ±800 rpm for 30 min at room temperature in the dark. After three washes, 50 μL of streptavidin-phycoerythrin was added to each well and incubated for 10 min at ±800 rpm at room temperature in the dark. After a final wash, the beads were resuspended in 125 μL of sheath buffer for measurement with the LUMINEX 200 (LUMINEX, Austin, TX, USA).

Western Blot Analysis

Thirty micrograms of total protein was separated using SDS–PAGE at a constant 100V for approximately 2 hr. Following transfer to nitrocellulose membranes (Protran, 0.2 μM, Schleicher & Schuell, Keene, NH, USA) at a constant 100 V for 90 min, non-fat milk

(NFM) (5%) was used to block (Fisher Scientific, Pittsburgh, PA), and immunoblotting was performed with a 1:1000 dilution of primary antibodies in 2% NFM at 4°C overnight. Antibodies were Twist1/2 (SC-81417; Santa Cruz, Dallas, TX), IRF3 (D83B9; Cell Signaling, Danvers, MA), IRF3-pSer396 (4D4G; Cell Signaling), STAT3 (124H6; Cell Signaling), STAT3-pY705 (9131S; Cell Signaling, Rockford, IL), and beta-actin (Cell Signaling). A 1:10,000 dilution of goat anti-rabbit or goat anti-mouse IgG-horseradish peroxidase conjugate (Pierce, Waltham, MA) was added for 1 hr, and Western Lighting Plus (PerkinElmer, Waltham, MA, USA) was used for detection of proteins.

Statistics

Differences between means (three groups or more) were determined using analysis of variance and differences between two groups were analyzed using independent *t*-test functions of GraphPad inSTAT statistical software (La Jolla, CA). A *P*-value of ≤ 0.05 was considered significant, and data are presented as mean ± standard error of the mean (S.E.M.).

Results

Interferon-Beta (IFN-β) is Downregulated in Trophoblast Cells Infected with MHV68

To determine the potential mechanism by which viral infection affects the trophoblast response to bacterial products, gene arrays were used to identify differentially expressed genes between human firsttrimester trophoblast Sw.71 cells with and without viral infection. Viral infection significantly decreases IFN-β mRNA and several IFN-regulated genes such as CXCL11, vascular cell adhesin molecule 1, and C-reactive protein (Fig. 1a). Viral inhibition of IFNβwas confirmed in independent experiments with Sw.71 and mouse placenta at E15.5. (Fig. 1b,c). Furthermore, phosphorylation of IRF3, the transcription factor responsible for IFN-β expression, was significantly decreased in trophoblast infected with MHV68 compared to non-infected cells (Fig. 1d).

Mice Lacking the Type I IFN Receptor have Increased Placental Pro-Inflammatory Cytokines in Response to Low-Dose LPS and Higher Rates of Preterm Birth

We previously determined that MHV68 infection increased the placental inflammatory response to LPS.[18] As interferon-β can function as an immune modulator,[28] we hypothesized that MHV68 enhanced placental inflammation in response to LPS by inhibiting IFN-β in these cells. Therefore, we evaluated the placental inflammatory response to LPS, *in vivo*, using pregnant mice lacking the type I IFN receptor (IFNAR−/−) compared to pregnant WT animals. The placentas from IFNAR−/− mice had increased IL-1β and TNF-α protein in response to LPS, while WT controls had no inflammatory response to the same concentration of endotoxin (Fig. 2a,b). Furthermore, over 60% IFNAR−/− mice suffered preterm birth in response to low-dose LPS, while 90% LPS-treated WT mice went to term (Fig. 2c).

Lipopolysaccharide Induces IFN-β in Trophoblast Cells

TLR4 ligation by LPS in the trophoblast was characterized by the production of IFN-related chemokines, including RANTES and IP-10 (Fig. 3a,b) but not NF-κB-related inflammatory

cytokines TNF-α and IL-12 (Fig. 3c). To determine whether the production of RANTES and IP-10 was associated with the expression of type I IFN-β, we treated trophoblast cells with increasing concentrations of LPS and observed a dose-dependent induction of IFN-β expression by LPS at 4 hr after treatment (Fig. 3c).

Type I IFN Affects TLR4 Regulation of Pro-Inflammatory Cytokines in Human Trophoblast Cells

To test whether IFN-β regulated the inflammatory response to LPS in trophoblast cells, Sw. 71 were treated with LPS alone or BX795, an inhibitor of IRF3 phosphorylation (pIRF3), followed by LPS. Treatment with BX795 resulted in loss of phosphorylated IRF3 (Fig. 4a). Interestingly, LPS induced a significant increase in secretion of pro-inflammatory cytokines IL-6 and IL-1β only in trophoblast cells pre-treated with the IRF3 inhibitor (Fig. 4b) suggesting IFN-β modulates the trophoblast inflammatory response to LPS.

The NF-κB Pathway is Functional in the Cytoplasm of the Trophoblast

We next investigated the potential mechanism of IFN-dependent regulation of these pro-inflammatory cytokines. The proteins we identified to be inhibited by IFN-β, IL-6, TNF-α, and IL-1β can all be regulated by NF-κB; therefore, we evaluated whether the NFκB pathway was functional in the trophoblast. Treatment of trophoblast cells with LPS or TNF-α was able to induce IKB-α degradation (Fig. 5a) and nuclear translocation of NF-κB effector, p65 (Fig. 5b); however, as shown above, we did not observe the expression of several pro-inflammatory cytokines typically regulated by these factors. These results demonstrated that the NF-κB pathway was functional; therefore, we postulated there must be inhibition at the level of gene expression.

MHV68 and Type I IFN Regulate STAT3 and Twist1

To determine how IFN regulates pro-inflammatory gene expression, we identified two transcription factors downregulated by MHV68 in the trophoblast, STAT3, and Twist1 (Fig. 6a). To test whether the downregulation was due to viral inhibition of IFN, we inhibited pIRF3 with BX795 in Sw.71 cells and confirmed that, again, pSTAT3 and Twist were downregulated (Fig. 6b). On the other hand, inhibiting pSTAT3 with Simvistatin did not affect pIRF3 showing pIRF3 is upstream of pSTAT3 (Fig. 6c).

Twist1 Overexpression Downregulates IL-1β in Sw.71 Trophoblast Cells

We next determined whether Twist1, which was regulated by viral infection and IFN, could indeed regulate inflammatory cytokines in the trophoblast. Sw.71 cells were transfected with a constitutive overexpression construct encoding the full-length sequence of human Twist1 (pcDNA-Twist1) and then infected with MHV68 followed by exposure to LPS (1 μg/mL) for 24 hr. The combination of MHV68 and LPS was previously shown to induce an inflammatory response in Sw.71 cells; therefore, we sought to determine whether Twist overexpression could decrease this response. The supernatants from the treatment and plasmid control cells were analyzed for IL-1β protein, and as shown in Fig. 6b, Twist overexpression significantly decreased IL-1β (Fig. 7).

Discussion

Here, we demonstrate that placental IFN-β is an immune-regulator during pregnancy controlling trophoblast responses to bacterial products. We found that viral inhibition of the trophoblast IFN pathway inadvertently disabled the tight regulation of TLR4-mediated inflammatory responses, promoting a pro-inflammatory response to bacteria and increased risk for preterm birth in a mouse model of pregnancy.

The immunomodulatory functions of type I IFNs have been previously defined.[26,28] Studies in the 1970s and 1980s first identified type I IFNs had anti-inflammatory activity when treatment reduced footpad swelling in murine models of inflammation.[34] Since then multiple animal models have demonstrated IFN's anti-inflammatory functions including LPS-induced sepsis,[26,35] colitis models[36] and other experimental models of autoimmune disease.[37] Furthermore, several anti-inflammatory mediators downstream of IFN have been identified, including STAT proteins,[38] TAM receptors,[39] and numerous histone modifiers.[40] Interestingly, only limited tissues benefit from the anti-inflammatory properties of type I IFNs. Here, we determined that IFN is downstream of TLR4 activation and it modulates the TLR4 pro-inflammatory response to LPS in human trophoblast cells. Furthermore, loss of IFN signaling in pregnant mice alone did not cause preterm labor, but these animals were more sensitive to low-dose LPS, which induced placental inflammation in all animals after 24 hr and preterm birth in 50% of the dams. This suggests that, alone, loss of IFN will not cause an inflammatory response, but will make the cells more responsive to bacterial challenge. This finding has increased relevance since recent work has confirmed that women have a complex network of commensal bacteria at the maternal–fetal interface. This means a woman with a placental viral infection that downregulates IFN could experience heightened inflammation in the presence of local microbes that normally do not elicit a response.

It has been postulated that the tissue-specific function of IFN depends on the ratio and/or expression of STAT1 versus STAT3,[38,41,42] expression of TAMs,[39,43] or the presence of cofactors required for transcription of anti- or pro-inflammatory cytokines. Interestingly, in the trophoblast, we observed a significant decrease in STAT3 phosphorylation as result of viral infection or inhibition of IRF3/IFN. We also found the basic helix–loop–helix transcription factor, Twist1, was constitutively expressed in the trophoblast and was inhibited by viral infection, inhibition of IFN-β, and inhibition of STAT3. Therefore, viral infection inhibits IFN, which causes decreased activation of STAT3, which is upstream of the transcriptional mediator, Twist1.

Twist1 regulates gastrulation and mesoderm differentiation during embryonic development,[44] epithelial–mesenchymal transition (EMT)[45] and regulates the expression of cytokine genes during inflammation.[46] Mice deficient for *Twist1* and *Twist2* succumb to severe systemic inflammation suggesting an important role for Twist1 in the regulation of the inflammatory process.[46] Twist1 is known to regulate the NF-κB pathway through either the induction of microRNAs[45] or by inhibiting at the transcriptional level.[46,47] Therefore, we postulated that virus-induced loss of Twist1 could result in the pro-inflammatory response to LPS. Indeed, when we overexpressed Twist1 in trophoblast cells, it was able to reduce the expression of IL1-β, an NF-κB-dependent pro-inflammatory cytokine. These data suggested

that Twist1 expression and function are associated with the modulation of TLR4 responses to LPS and are under the control of IFN-β; however, we cannot disregard the role of additional transcriptional factors such as HDACs, which may also influence the function of NF-κB in trophoblast cells.

In conclusion, our data reveal an active mechanism regulating the inflammatory response to bacteria by the trophoblast. Its complexity ensures the protection of the pregnancy by preventing an inflammatory response to non-pathogenic or low abundance bacterial colonization. However, viral infections target this pathway and consequently affect the normal homeostasis between the placenta and its normal flora, resulting in a switch to a pro-inflammatory response and, potentially, preterm labor.

Acknowledgments

This study is in part funded by grants P01HD054713 and 3N01 HD23342 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services. We are grateful to A. F. Parlow, Scientific Director at Los Angeles Biomedical Research Institute National Hormone & Peptide Program for the Pregnant Mare Serum Gonadotropin (PMSG) used for superovulation protocols for embryo transfer experiments. Also, we extend our gratitude to JoAnn Bilyard for the careful editing of this manuscript.

References

1. Iams JD, Romero R, Culhane JF, Goldenberg RL. Primary, secondary, and tertiary interventions to reduce the morbidity and mortality of preterm birth. Lancet. 2008; 371:164–175. [PubMed: 18191687]
2. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. Science. 2014; 345:760–765. [PubMed: 25124429]
3. Simmons LE, Rubens CE, Darmstadt GL, Gravett MG. Preventing preterm birth and neonatal mortality: exploring the epidemiology, causes, and interventions. Semin Perinatol. 2010; 34:408–415. [PubMed: 21094415]
4. Rubens CE, Sadovsky Y, Muglia L, Gravett M, Lackritz E, Gravett C. Prevention of preterm birth: harnessing science to address the global epidemic. Sci Transl Med. 2014; 6:262sr265.
5. Muglia L, Katz M. The enigma of spontaneous preterm birth. N Engl J Med. 2010; 362:529–535. [PubMed: 20147718]
6. Lawn J, Kinney M. Preterm birth: now the leading cause of child death worldwide. Sci Transl Med. 2014; 6:263ed221.
7. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008; 371:75–84. [PubMed: 18177778]
8. Howson, C.Kinney, M., Lawn, J., editors. Dimes Mo, PMNCH, Children, S, WHO: Preterm birth matters. Born Too Soon: The Global Action Report. Geneva, Switzerland: WHO; 2012. p. 1-8.
9. Romero R, Chaiworapongsa T, Espinoza J. Micronutrients and intrauterine infection, preterm birth and the fetal inflammatory response syndrome. J Nutr. 2003; 133:1668S–1673S. [PubMed: 12730483]
10. Gotsch F, Romero R, Kusanovic JP, Mazaki-Tovi S, Pineles BL, Erez O, Espinoza J, Hassan SS. The fetal inflammatory response syndrome. Clin Obstet Gynecol. 2007; 50:652–683. [PubMed: 17762416]
11. Pacora P, Chaiworapongsa T, Maymon E, Kim Y, Gomez R, Yoon BH, Ghezzi F, Berry S, Qureshi F, Jacques S, Kim J, Kadar N, Romero R. Funitis and chorionic vasculitis-the histological counterpart of the fetal inflammatory response syndrome. J Matern Fetal Neonatal Med. 2002; 11:18–25. [PubMed: 12380603]
12. Yoon BH, Romero R, Park JS, Kim M, Oh SY, Kim CJ, Jun JK. The relationship among inflammatory lesions of the umbilical cord (funisitis), umbilical cord plasma interleukin 6

concentration, amniotic fluid infection, and neonatal sepsis. Am J Obstet Gynecol. 2000; 183:1124–1129. [PubMed: 11084553]

13. Yoon BH, Romero R, Park JS, Kim CJ, Kim SH, Choi J-H, Han TR. Fetal exposure to an intra-amniotic inflammation and the development of cerebral palsy at the age of three years. Am J Obstet Gynecol. 2000; 182:675–681. [PubMed: 10739529]
14. Yoon BH, Jun J, Romero R, Park K, Gomez R, Choi J-H, Kim I-O. Amniotic fluid inflammatory cytokines (interleukin-6, interleukin-l [3, and tumor necrosis factor-s), neonatal brain white matter lesions, and cerebral palsy. Am J Obstet Gynecol. 1997; 177:19–26. [PubMed: 9240577]
15. Villar J, Papageorghiou AT, Pang R, Ohuma EO, Ismail LC, Barros FC, Lambert A, Carvalho M, Jaffer YA, Bertino E, Gravett MG, Altman DG, Purwar M, Frederick IO, Noble JA, Victora CG, Bhutta ZA, Kennedy SH. The likeness of fetal growth and newborn size across non-isolated populations in the INTERGROWTH-21st Project: the Fetal Growth Longitudinal Study and Newborn Cross-Sectional Study. Lancet Diabetes Endocrinol. 2014; 2:781–792. [PubMed: 25009082]
16. Romero R, Gomez R, Chaiworapongsa T, Conoscenti G, Kim J, Kim Y. The role of infection in preterm labour and delivery. Paediatr Perinat Epidemiol. 2001; 15:41–56. [PubMed: 11520399]
17. Takahashi T, Tanaka M, Brannan C, Jenkins N, Copeland N, Suda T, Nagata S. Generalized lymphoproliferative disease in mice, caused by point mutation in the Fas ligand. Cell. 1994; 76:969–976. [PubMed: 7511063]
18. Cardenas I, Mor G, Aldo P, Lang SM, Stabach P, Sharp A, Romero R, Mazaki-Tovi S, Gervasi M, Means RE. Placental viral infection sensitizes to endotoxin-induced pre-term labor: a double hit hypothesis. Am J Reprod Immunol. 2011; 65:110–117. [PubMed: 20712808]
19. Cardenas I, Means RE, Aldo P, Koga K, Lang SM, Booth CJ, Manzur A, Oyarzun E, Romero R, Mor G. Viral infection of the placenta leads to fetal inflammation and sensitization to bacterial products predisposing to preterm labor. J Immunol. 2010; 185:1248–1257. [PubMed: 20554966]
20. Fisher S, Genbacev O, Maidji E, Pereira AC. Human CMV infection of placental cytotrophoblasts in vitro and in utero: implications for transmission and pathogenesis. J Virol. 2000; 74:6808–6820. [PubMed: 10888620]
21. Gomez LM, Ma Y, Ho C, McGrath CM, Nelson DB, Parry S. Placental infection with human papillomavirus is associated with spontaneous preterm delivery. Hum Reprod. 2008; 23:709–715. [PubMed: 18184644]
22. Reddy U, Zlatnik M, Baschat A, Towbin J, Harman C, Weiner C. Detection of viral deoxyribonucleic acid in amniotic fluid: predictor of abnormal pregnancy. Fetal Diagn Ther. 2005; 20:203–207. [PubMed: 15824499]
23. Romero R, Espinoza J, Goncalves LF, Kusanovic JP, Friel L, Hassan S. The role of inflammation and infection in preterm birth. Semin Reprod Med. 2007; 25:21–39. [PubMed: 17205421]
24. McNab F, Mayer-Barber K, Sher A, Wack A, O'Garra A. Type I interferons in infectious disease. Nat Rev Immunol. 2015; 15:87–103. [PubMed: 25614319]
25. Odorizzi PM, Wherry EJ. Immunology. An interferon paradox. Science. 2013; 340:155–156. [PubMed: 23580520]
26. Trinchieri G. Type I interferon: friend or foe? J Exp Med. 2010; 207:2053–2063. [PubMed: 20837696]
27. Edwards MR, Slater L, Johnston SL. Signalling pathways mediating type I interferon gene expression. Microbes Infect. 2007; 9:1245–1251. [PubMed: 17904888]
28. Kovarik P, Sauer I, Schaljo B. Molecular mechanisms of the anti-inflammatory functions of interferons. Immunobiology. 2007; 212:895–901. [PubMed: 18086388]
29. Garcia-Sastre A. Inhibition of interferon-mediated antiviral responses by influenza A viruses and other negative-strand RNA viruses. Virology. 2001; 279:375–384. [PubMed: 11162793]
30. Haller O, Kochs G, Weber F. The interferon response circuit: induction and suppression by pathogenic viruses. Virology. 2006; 344:119–130. [PubMed: 16364743]
31. Katze MG, He Y, Gale M Jr. Viruses and interferon: a fight for supremacy. Nat Rev Immunol. 2002; 2:675–687. [PubMed: 12209136]
32. Johnson KP, Panitch HS. Interferon therapy for multiple sclerosis. Md Med J. 1992; 41:601–603. [PubMed: 1379317]

33. Racicot K, Cardenas I, Wunsche V, Aldo P, Guller S, Means RE, Romero R, Mor G. Viral infection of the pregnant cervix predisposes to ascending bacterial infection. J Immunol. 2013; 191:934–941. [PubMed: 23752614]
34. Maeyer E, Maeyer-Guignard J, Vandeputte M. Inhibition of interferon of delayed type hypersensitivity in the mouse. PNAS. 1975; 72:1753–1757. [PubMed: 168573]
35. Mahieu T, Libert C. Should we inhibit type I interferons in sepsis? Infect Immun. 2007; 75:22–29. [PubMed: 17000722]
36. Lee J, Rachmilewitz D, Raz E. Homeostatic effects of TLR9 signaling in experimental colitis. Ann N Y Acad Sci. 2006; 1072:351–355. [PubMed: 17057215]
37. Agrawal H, Jacob N, Carreras E, Bajana S, Putterman C, Turner S, Neas B, Mathian A, Koss MN, Stohl W, Kovats S, Jacob CO. Deficiency of type I IFN receptor in lupus-prone New Zealand mixed 2328 mice decreases dendritic cell numbers and activation and protects from disease. J Immunol. 2009; 183:6021–6029. [PubMed: 19812195]
38. Ho HH, Ivashkiv LB. Role of STAT3 in type I interferon responses. Negative regulation of STAT1-dependent inflammatory gene activation. J Biol Chem. 2006; 281:14111–14118. [PubMed: 16571725]
39. Rothlin CV, Ghosh S, Zuniga EI, Oldstone MB, Lemke G. TAM receptors are pleiotropic inhibitors of the innate immune response. Cell. 2007; 131:1124–1136. [PubMed: 18083102]
40. Ivashkiv LB, Donlin LT. Regulation of type I interferon responses. Nat Rev Immunol. 2014; 14:36–49. [PubMed: 24362405]
41. Luu K, Greenhill CJ, Majoros A, Decker T, Jenkins BJ, Mansell A. STAT1 plays a role in TLR signal transduction and inflammatory responses. Immunol Cell Biol. 2014; 92:761–769. [PubMed: 25027037]
42. Miller AM, Wang H, Park O, Horiguchi N, Lafdil F, Mukhopadhyay P, Moh A, Fu XY, Kunos G, Pacher P, Gao B. Anti-inflammatory and anti-apoptotic roles of endothelial cell STAT3 in alcoholic liver injury. Alcohol Clin Exp Res. 2010; 34:719–725. [PubMed: 20102572]
43. Sharif MN, Sosic D, Rothlin CV, Kelly E, Lemke G, Olson EN, Ivashkiv LB. Twist mediates suppression of inflammation by type I IFNs and Axl. J Exp Med. 2006; 203:1891–1901. [PubMed: 16831897]
44. Bourgeois P, Stoetzel C, Bellemin A, Mattei M, Perrin-Schmitt P. Human h-twist gene is located at 7p21 and encodes a B-HLH [rpteom tjat is 96% similar to its murine M-twist counterpart. Mamm Genome. 1996; 7:915–917. [PubMed: 8995765]
45. Yin G, Chen R, Alvero AB, Fu HH, Holmberg J, Glackin C, Rutherford T, Mor G. TWISTing stemness, inflammation and proliferation of epithelial ovarian cancer cells through MIR199A2/214. Oncogene. 2010; 29:3545–3553. [PubMed: 20400975]
46. Sosic D, Richardson J, Yu K, Ornitz D, Olson EN. Twist regulates cytokine gene expression through a negative feedback loop that represses NF-kB activity. Cell. 2003; 112:169–180. [PubMed: 12553906]
47. Niesner U, Albrecht I, Janke M, Doebis C, Loddenkemper C, Lexberg MH, Eulenburg K, Kreher S, Koeck J, Baumgrass R, Bonhagen K, Kamradt T, Enghard P, Humrich JY, Rutz S, Schulze-Topphoff U, Aktas O, Bartfeld S, Radbruch H, Hegazy AN, Lohning M, Baumgart DC, Duchmann R, Rudwaleit M, Haupl T, Gitelman I, Krenn V, Gruen J, Sieper J, Zeitz M, Wiedenmann B, Zipp F, Hamann A, Janitz M, Scheffold A, Burmester GR, Chang HD, Radbruch A. Autoregulation of Th1-mediated inflammation by twist1. J Exp Med. 2008; 205:1889–1901. [PubMed: 18663125]

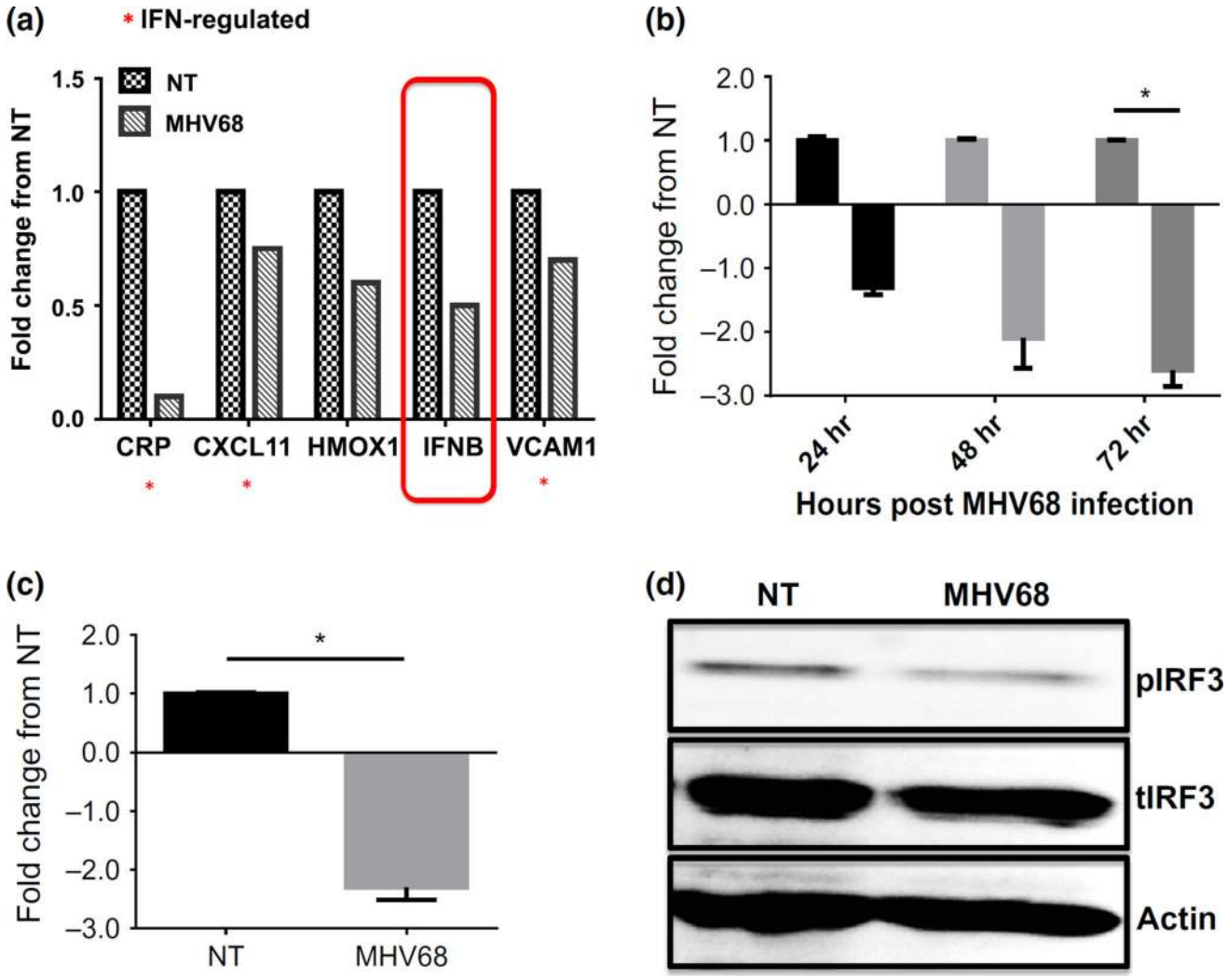

Fig. 1.

Herpes virus infection suppresses IFN-β in trophoblast cells and the murine placenta. IFN-β mRNA and several IFN-regulated genes were downregulated in Sw.71 infected with murine gammaherpesvirus 68 (MHV68, 3×10^6 pfu/mL) for 24 hr as measured by qPCR array (a). IFN-β was confirmed to be downregulated in Sw.71 at 24, 48, and 72 hr post-infection, and IFN-β mRNA expression was decreased in the placentas of C57BL/6J mice infected with MHV68 at E8.5 and collected at E15.5 (b, c). Viral infection decreased IRF3 phosphorylation in Sw.71 cells 24 hr post-infection (d). Bar graph presents mean ± S.E.M. (*$P < 0.05$).

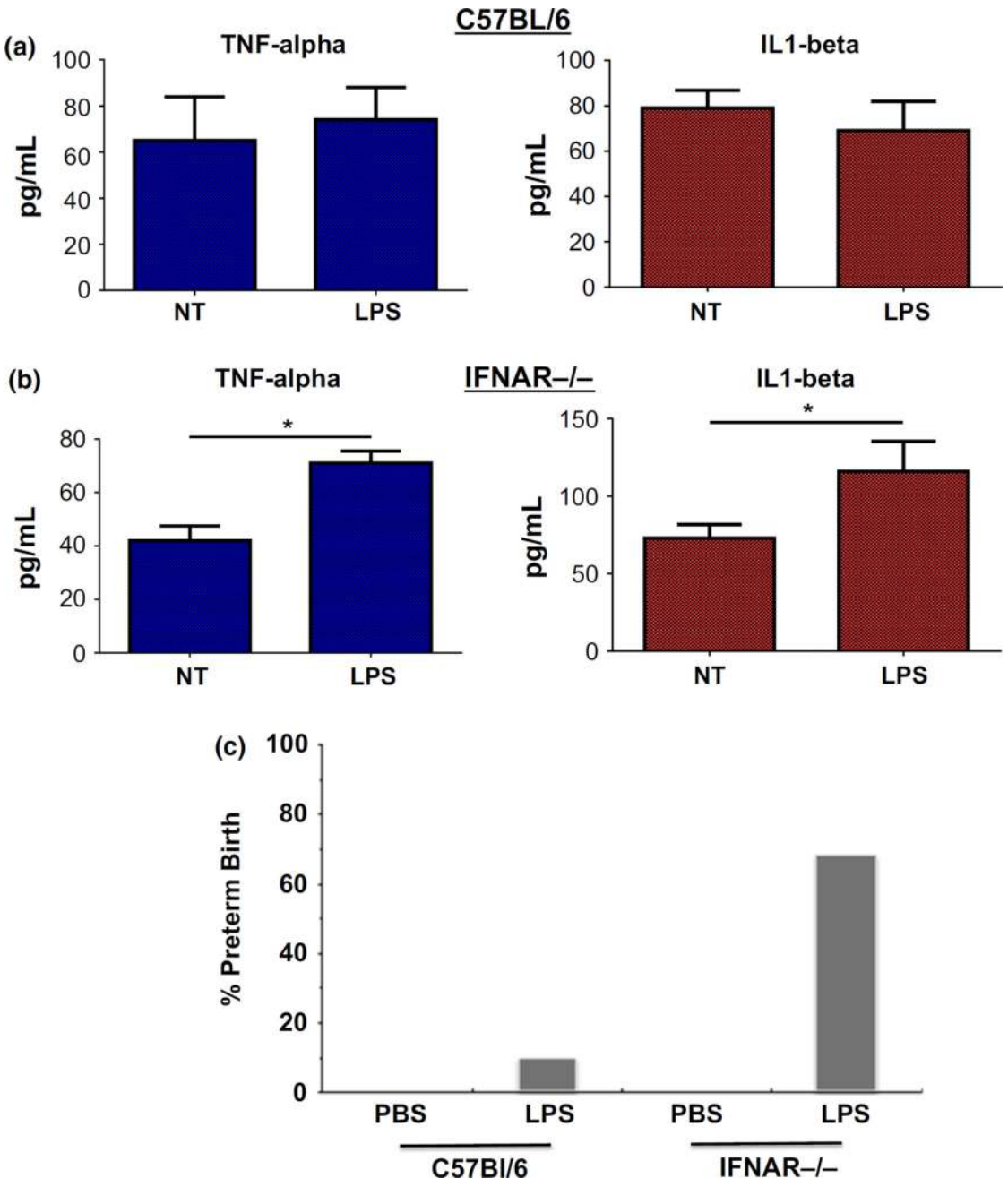

Fig. 2.

Loss of IFN signaling causes enhanced placental inflammation and preterm labor in a murine model of pregnancy. Wild-type (WT) and IFN receptor knockout (IFNAR–/–) mice were injected with low-dose LPS (20 μg/kg) intraperitoneally at E15.5. Placental TNF-α and IL-1β were significantly increased in IFNAR–/– mice 24 hr post-LPS (a, b) and over 60% of IFNAR–/– mice delivered preterm while there was less than 10% preterm birth following LPS in WT mothers (c). ($n = 6$). *$P < 0.05$

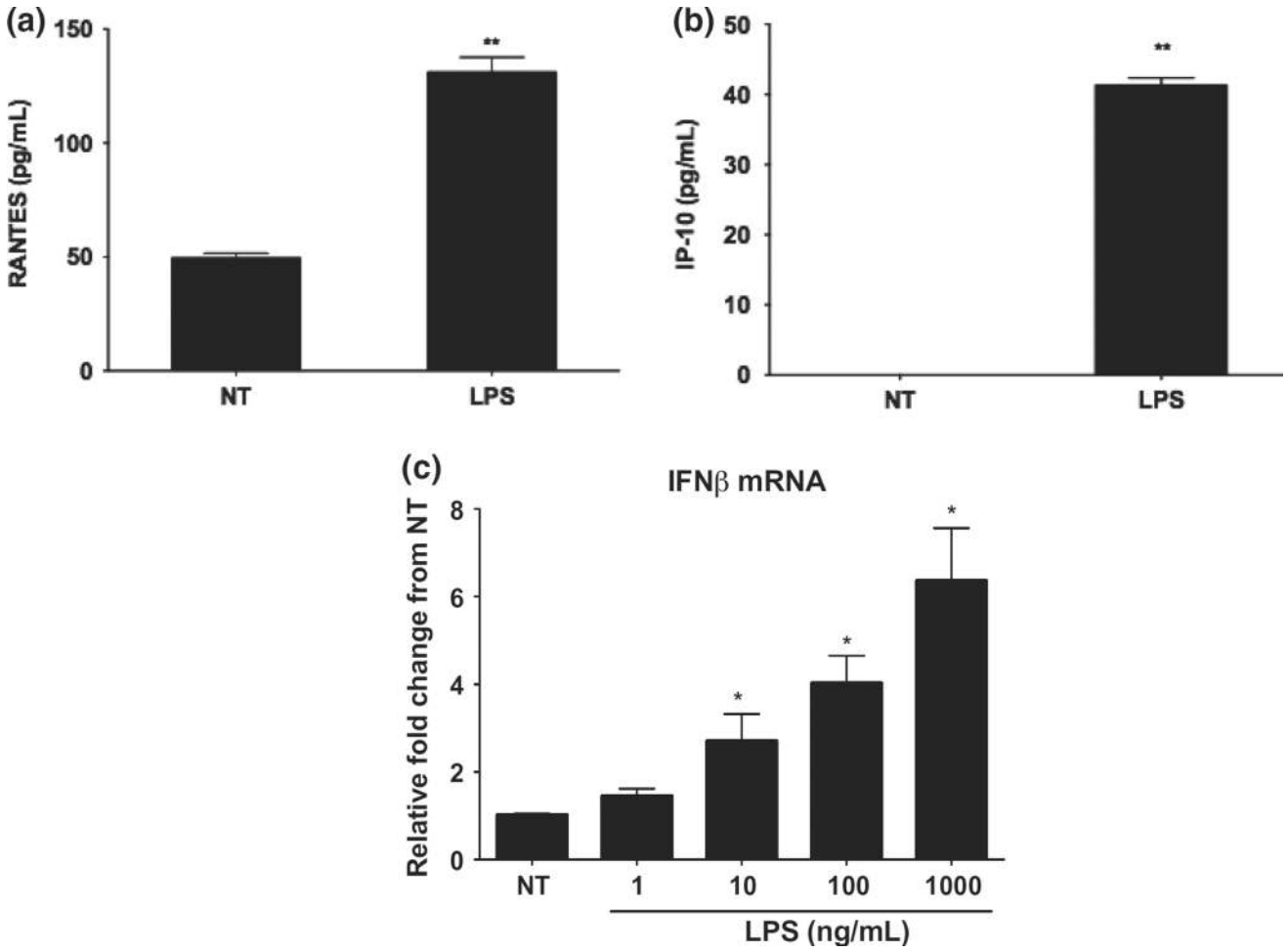

Fig. 3.

Lipopolysaccharide regulates IFN-β in Sw.71 trophoblast cells. Sw.71 cells were incubated with sterile PBS or LPS (1 μg/mL) for 48 hr, and RANTES and IP-10 secretions were quantified in conditioned medium (a, b). There was a dose-dependent increase in IFN-β mRNA 4 hr post-LPS treatment (c). This is representative of 3 independent experiments. Bar graph presents mean ± S.E.M. (*$P < 0.05$; **$P < 0.001$).

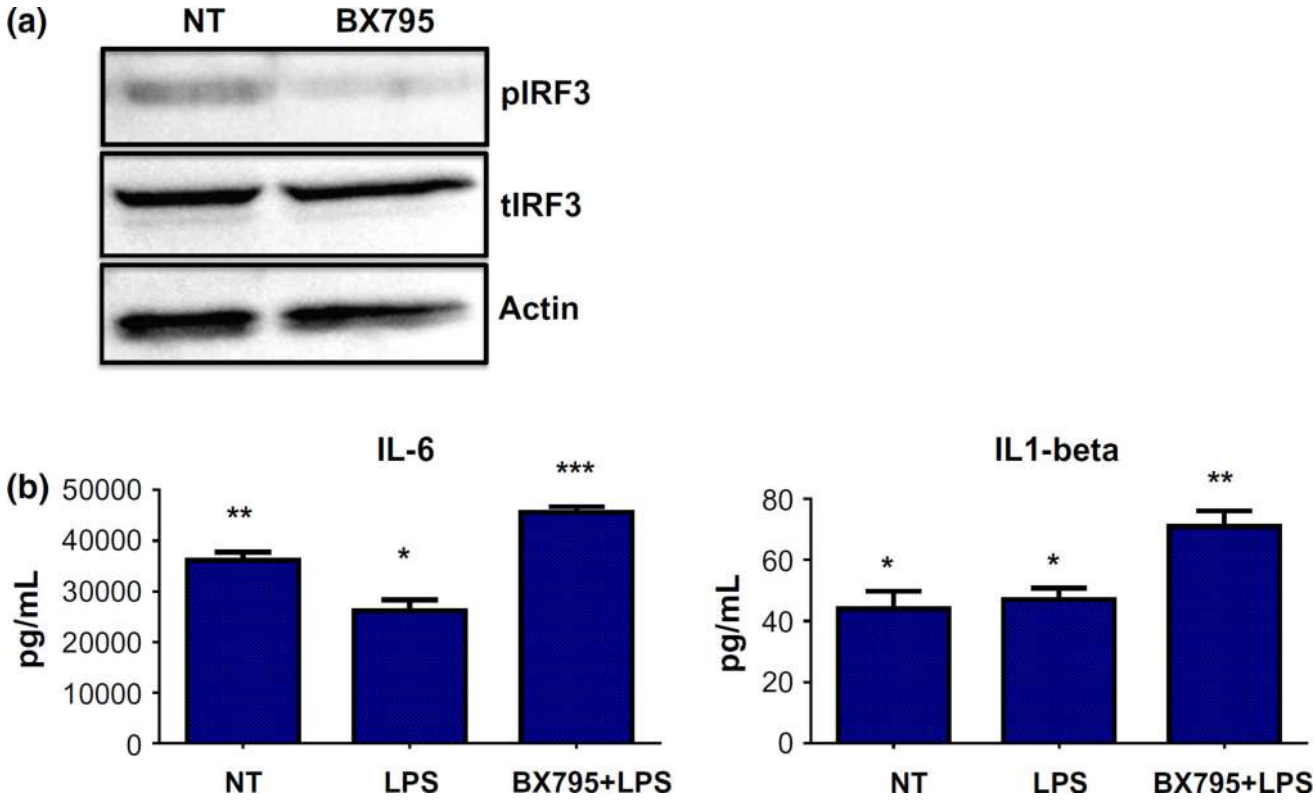

Fig. 4.

Inhibition of IFN-β sensitizes trophoblast cells to LPS. Sw.71 was pre-treated with sterile PBS or TBK1 inhibitor (BX-795) for 2 hr, followed by sterile PBS or LPS (1 μg/mL) for 48 hr. BX795 inhibited IRF3 phosphorylation (a) and removed inhibition of the cytokine response to LPS (b). This is representative of three independent experiments. Bar graph presents mean ± S.E.M. (*$P < 0.05$; **$P < 0.001$; ***$P > 0.05$).

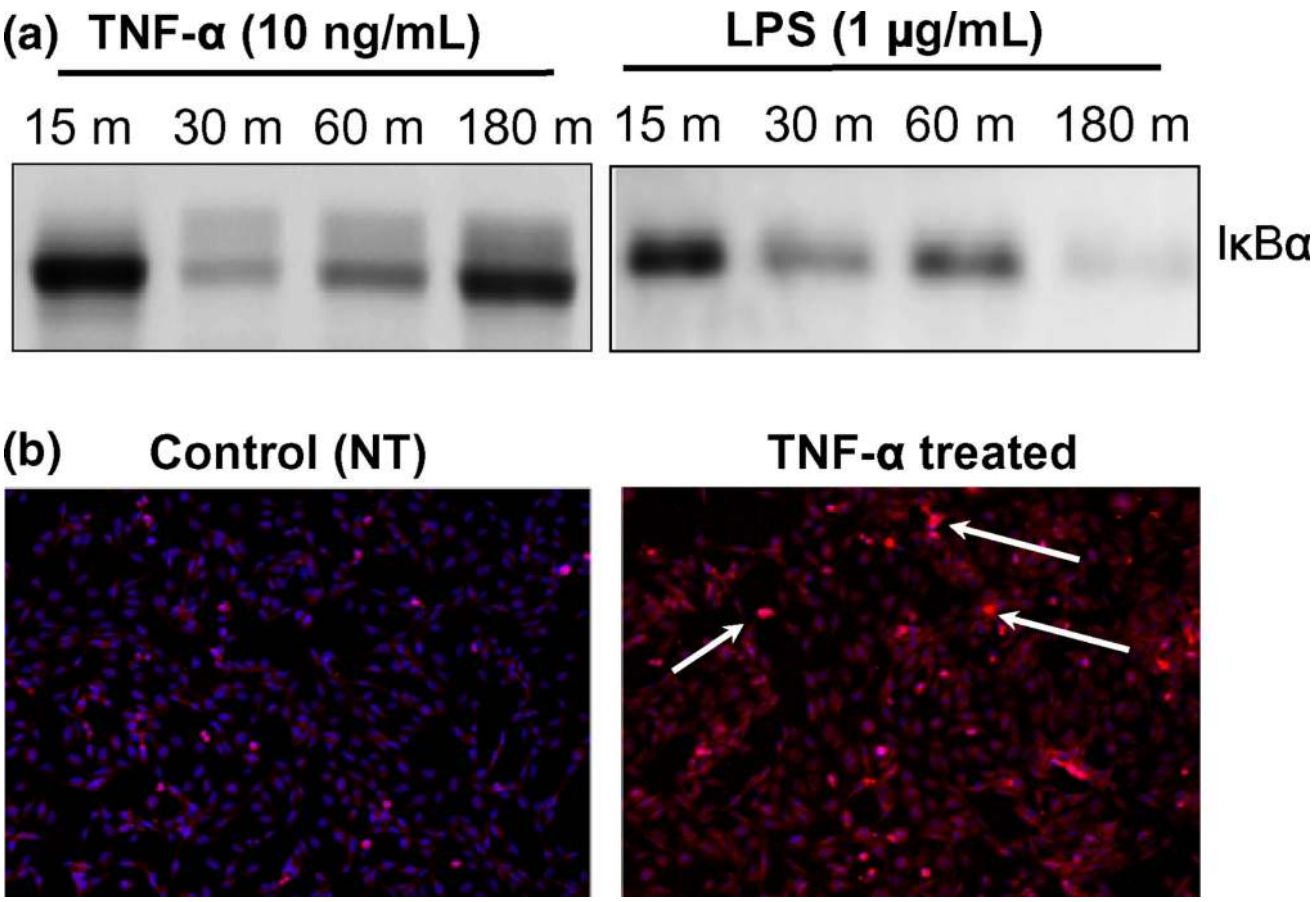

Fig. 5.

NF-κB active subunit can enter the nucleus of Sw.71 cells following TNF-α treatment. TNF-α or LPS treatment for 15, 30, 60, or 180 m caused a downregulation of the NF-κB inhibitor IκBα in Sw.71 cells (a). Treatment also induced nuclear translocation of the NF-κB active subunit, p65, in Sw.71 cells (arrows, b).

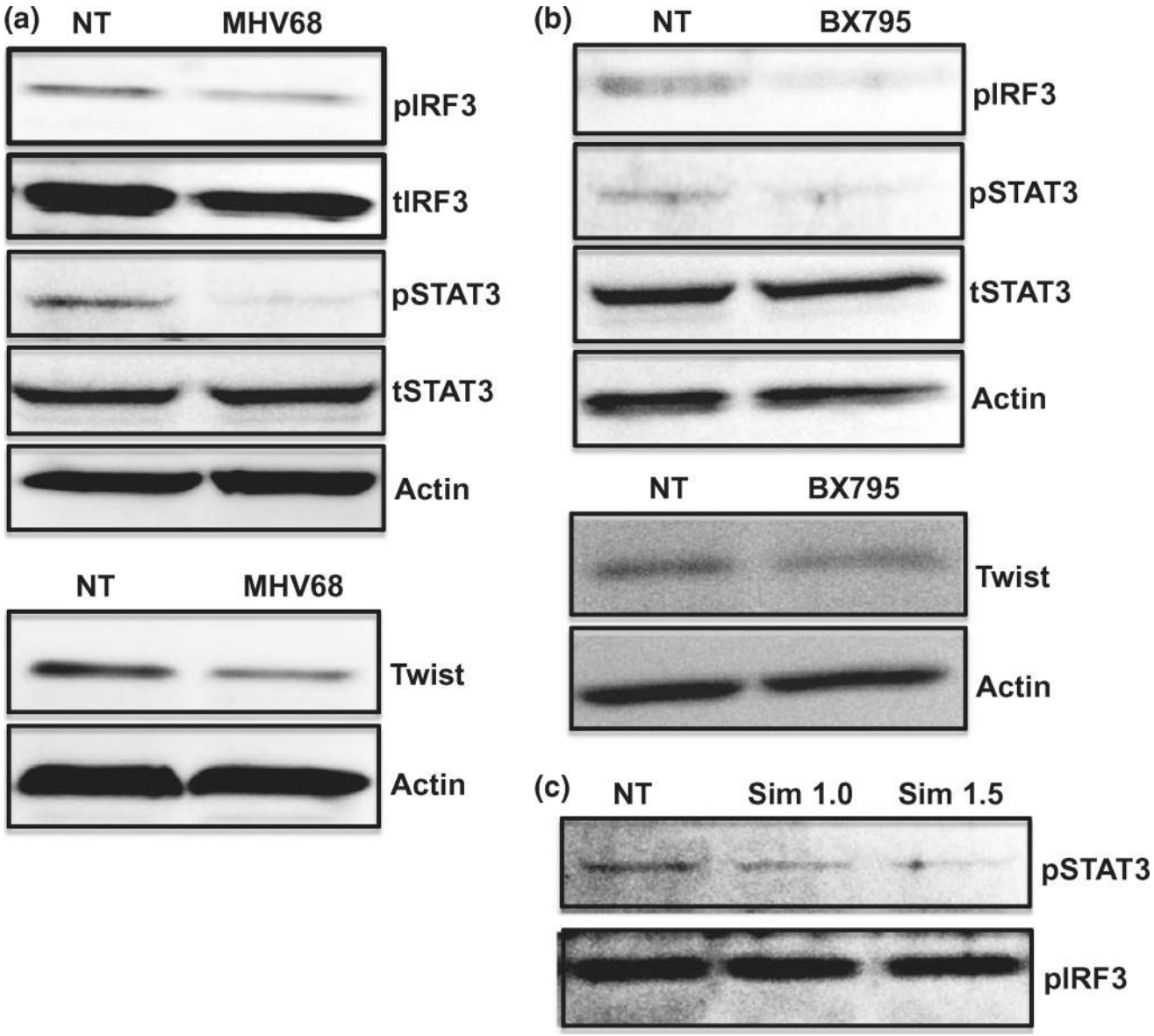

Fig. 6.

Herpes infection and type I IFN regulate the transcription factors STAT3 and Twist1 in trophoblast cells. MHV68 infection inhibits phosphorylation of IRF3 and STAT3, and expression of Twist1 in Sw.71 cells (a). BX795 inhibition of IRF3 also results in decreased phosphorylation of STAT3 and Twist1 expression, suggesting virus mediates these factors through inhibition of IRF3 (b). Inhibition of STAT3 phosphorylation with simvastatin does not affect IRF3 phosphorylation, suggesting STAT3 is regulated by IRF3, not vice versa (c).

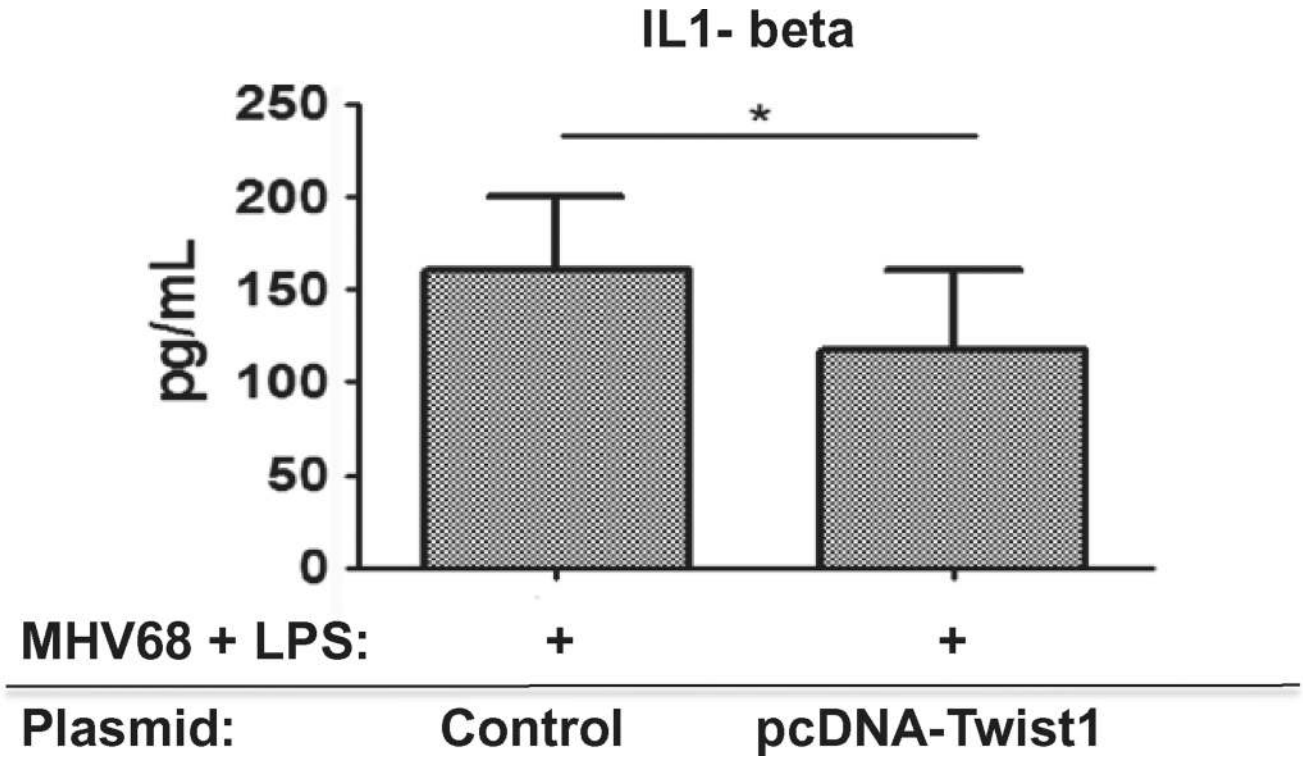

Fig. 7.

Twist1 regulates IL-1β in Sw.71 cells. Sw.71 cells were treated with a control plasmid or a plasmid overexpressing Twist1, followed by treatment with MHV68 and LPS, which together causes secretion of IL-1β. Overexpressing Twist1 in Sw.71 cells was able to decrease the secretion of IL-1β. $*P < 0.05$