Published in final edited form as:

Am J Reprod Immunol. 2013 September ; 70(3): 190–198. doi:10.1111/aji.12128.

Human Endometrial Endothelial Cells Generate Distinct Inflammatory and Anti-viral Responses to the TLR3 agonist, Poly(I:C) and the TLR8 agonist, viral ssRNA

Graciela Krikun¹, Julie A. Potter¹, and Vikki M. Abrahams^{1,2}

¹Department of Obstetrics, Gynecology & Reproductive Sciences, Yale University School of Medicine, New Haven, CT, 06510, USA

Abstract

Problem—Human endometrial endothelial cell (HEEC) innate immunity remains poorly characterized. Based on their direct contact with the circulation, HEECs are uniquely positioned to be exposed to viral infections. The present study evaluated the innate immune response generated by HEECs after exposure to the TLR3 agonist, Poly(I:C) and the TLR8 agonist, viral ssRNA.

Method of Study—HEECs were treated with or without Poly(I:C) or ssRNA. Culture supernatants were measured for cytokines by multiplex analysis. RNA was analyzed by qRT-PCR for type I interferons and anti-viral factors.

Results—Treatment of HEECs with Poly(I:C) rapidly upregulated the secretion of IL-2, IL-6, IL-8, IFN γ , G-CSF, GM-CSF, MCP-1, MIP-1 β , RANTES and GRO- α after 12 hrs, while ssRNA treatment induced the slower secretion of IL-6, IL-8, IFN γ , G-CSF, VEGF and GRO- α after 24 hrs. Both viral components induced HEEC IFN α and IFN β expression. While treatment with Poly(I:C) induced APOBEC3G and OAS expression, treatment with ssRNA upregulated APOBEC3G and MxA mRNA.

Conclusion—Our findings demonstrate that HEECs can differentially sense and respond to viral components by generating distinct inflammatory and anti-viral immune responses, indicating that these cells likely play an active role in the immune protection of the uterus towards viral infections.

Keywords

Inflammation; endometrium; endothelial; microvascular; Toll-like receptor; viral infection

INTRODUCTION

The endometrium is a complex mucosal system that must be able to allow implantation of a semi-allogeneic fetus, while maintaining host defense against potential pathogens^{1,2}. The upper female reproductive tract is not a sterile environment, and although the maintenance of a commensal microorganism population may be important³, a pathological infection within the upper tract can have serious consequences, such as chronic pelvic inflammation, infertility and pregnancy complications^{4–6}. Therefore, such infectious agents must be quickly removed. Indeed, cells of the upper female reproductive tract play an important role in host defense. The columnar epithelial cells that line the endometrium form a tight barrier

²Address all correspondence to: Vikki M. Abrahams, PhD, Yale University School of Medicine, Department of Obstetrics, Gynecology & Reproductive Sciences, 333 Cedar Street, LSOG 305C, New Haven, CT, 06510, USA. Tel: 203 785 2175; Fax: 203 785 4883; vikki.abrahams@yale.edu.

against invading infections¹. These cells also express pattern recognition receptors, such as the Toll-like receptors (TLRs), and when activated can generate specific innate immune cytokine, chemokine, anti-microbial, and anti-viral responses^{7–12}. In addition, within the endometrium there is a strong presence of innate and adaptive immune cells^{1, 2}. These, along with the endometrial stromal cells^{10–12}, also express functional TLRs^{13–16} and provide immune protection.

Another potential site of infection within the uterus is the microvasculature. Based on their direct contact with the circulation, human endometrial endothelial cells (HEECs) are uniquely positioned to become exposed to pathogens, in particular viral infections, that disseminate via the hematogenous route¹⁷. One of the most well characterized viral infections that can target the uterine microvascular endothelium is human cytomegalovirus (HCMV)^{17–19}. A HCMV infection of endometrial endothelial cells can not only spread to other uterine cell populations, but during pregnancy can infect the placenta and subsequently the fetus, leading to birth defects and adverse pregnancy outcomes^{17, 19, 20}. While HEECs are known to be permissive to viral infections, like HCMV^{17–19}, little is known about their abilities to sense and respond to pathogens. Using HEECs derived from the uterine microvasculature^{21–23}, we have previously demonstrated that these cells can respond to bacterial lipopolysaccharide by generating a specific cytokine/chemokine response in a TLR4-dependent manner²⁴. However, our knowledge about TLR expression and function in these cells, and specifically how HEECs respond to viral infections, is limited. Therefore, the objective of the present study was to evaluate the innate immune response generated by HEECs after exposure to the TLR3 agonist, Poly(I:C), a compound that mimics viral dsRNA, and the TLR8 agonist, viral ssRNA. Herein we report that HEECs can differentially sense and respond to viral dsRNA and ssRNA by generating distinct inflammatory, type I interferon (IFN), and anti-viral responses, indicating that HEECs likely play an active role in the immune protection of the uterus towards viral infections.

MATERIALS AND METHODS

Human endometrial endothelial cell culture

The human microvascular endometrial endothelial cells (HEECs) used in this study were isolated from the cycling endometrium of multiple women, and all stages of cycles were pooled. These primary cells were frozen from an original stock and have been previously characterized to express a range of adhesion molecules and endothelial markers, and to form endothelial tubes when seeded in Matrigel^{21–23}. HEECs were cultured in flasks coated with 2% gelatin in EGM-2 MV Singlequote Medium with 5% stripped fetal calf serum (Cambrex Bio Science, Inc., Walkersville, MD)²¹. For treatment experiments, cells were plated into 30 mm tissue culture dishes coated with 2% gelatin and allowed to grow to 70% confluency. Media was then replaced with serum-free OptiMEM (Invitrogen, Grand Island, NY), and the cells treated with or without the viral dsRNA analogue, Poly(I:C), at 1 µg/ml, or viral ssRNA (ssRNA40/Lyovec) at 5 µg/ml (Invivogen, San Diego, CA). The optimum time points to detect significant changes in mRNA expression and cytokine secretion were 12 hr for treatment with Poly(I:C) and 24 hr for treatment with ssRNA.

Cytokine analysis

Cell-free culture supernatants were analyzed for levels of IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-17, G-CSF, GM-CSF, IFN-γ, MCP-1, MIP-1α, MIP-1β, RANTES, VEGF, TNF-α and GRO-α using the Bio-Plex assay (Bio-Rad, CA, USA). Measurements were performed in duplicate on the Luminex multiplex 200 system (Upstate Biotechnology, Lake Placid NY) as previously described²⁵.

Quantitative Real Time RT-PCR

Total RNA was extracted using TRIzol (Life Technologies, Grand Island, NY). Briefly, 1 ml of TRIzol was added to adherent cells for 5 min. The cells were collected and 0.2 ml of chloroform was added, vortexed and incubated for 5 min. The suspension was then centrifuged at 13000×g for 15 min. The aqueous phase was collected, RNA was precipitated with 0.5 ml 2-propanol overnight at −20°C, after which the RNA was washed in 70% ethanol and then resuspended in nuclease free water. The total RNA concentration was measured at A260 using a NanoDrop and stored at −80°C. 1 µg of total RNA was then reverse transcribed using the Superscript II RT kit (Invitrogen) after which quantitative real-time PCR was performed using the KAPA SYBR Fast qPCR kit (Kapa Biosystems, Woburn, MA), and PCR amplification performed on the BioRad CFX Connect Real Time System (BioRad, Hercules, CA)²⁶ using the primer sequences shown in Table I. All primers sequences were obtained from Real Time Primers (Elkins Park, PA33 unless specified otherwise^{27–32}. Data was normalized to the housekeeping gene, GAPDH, analyzed using the $\Delta\text{-}\Delta\text{CT}$ method and presented as fold change (FC) in the expression of gene of interest relative to the no treatment (NT) control, as previously described²⁶. For relative abundance of TLR mRNA levels, expression was normalized to an internal positive control and GAPDH (Invivogen).

Statistical Analysis

Experiments were performed at least three times and data presented as mean $\pm$ SD. Prism from Graphpad Software Inc (La Jolla, CA) was used to calculate significance ($p < 0.05$). Statistical analysis was performed using either the paired t-test, or for multiple comparisons, one-way ANOVA with the Bonferroni post-test.

RESULTS

HEECs express TLRs

While endothelial cells express TLRs³³, the first objective of this study was to establish the baseline expression of TLRs 1-10 in HEECs. As shown in Figure 1, HEECs constitutively expressed TLRs 1-10. HEEC basal TLR3 mRNA expression was 2.1-fold higher than TLR8 mRNA levels (Figure 1; $p < 0.05$).

HEECs generate distinct pro-inflammatory responses to Poly(I:C) and ssRNA

Having established expression of TLR3 and TLR8, we next sought to determine their function in HEECs in response to their agonists, Poly(I:C) (a viral dsRNA mimic) and viral ssRNA, respectively. Treatment of HEECs with Poly(I:C) induced a rapid and robust pro-inflammatory cytokine/chemokine response after only 12 hours. As shown in Figure 2, Poly(I:C) significantly upregulated the cell's secretion of IL-2, IL-6, IL-8, IFN γ , MCP-1, MIP-1 β , G-CSF, GM-CSF, RANTES and GRO- α . The secretion of IL-1 β , IL-4, IL-10, IL-12, IL-17, MIP-1 α , MIP-1 β , VEGF, or TNF- α were not significantly altered after exposure to Poly(I:C) (data not shown). After 12 hrs, ssRNA had no significant effect on HEEC cytokine/chemokine production (data not shown). However, after 24 hrs, viral ssRNA significantly upregulated HEEC secretion of IL-6, IL-8, IFN- γ , G-CSF, VEGF, and GRO- α (Figure 3). HEEC secretion of IL-2, IL-10, IL-12, IL-17, GM-CSF, MCP-1, MIP-1 α , MIP-1 β , and RANTES were not altered after treatment with ssRNA, and levels of IL-1 β , IL-4 and TNF α were below the detection limit of the assay (data not shown). In addition to the ssRNA-induced cytokine/chemokine response being produced in a slower manner than that triggered by Poly(I:C), the profile generated was different, although both stimuli upregulated IL-6, IL-8, IFN γ , G-CSF and GRO- α . Of these, with the exception of G-CSF, the magnitude of response generated by HEECs after ssRNA treatment was lower

than that triggered by Poly(I:C). Poly(I:C) upregulated HEEC IL-6 by 61-fold; IL-8 by 6.9-fold; IFN γ by 93.9-fold; G-CSF by 4.2 fold; and GRO- α by 14.2 fold (Figure 2). Viral ssRNA upregulated HEEC IL-6 by 1.6-fold; IL-8 by 1.8-fold; IFN γ by 2.2-fold; G-CSF by 5.4 fold; and GRO- α by 1.3 fold (Figure 3).

HEECs generate distinct type I interferon response and anti-viral responses to Poly(I:C) and ssRNA

An important aspect of anti-viral immunity is the induction of a type I interferon response, and sensors of viral products, including TLR3 and TLR8, have the ability to mediate production of these cytokines³⁴. As shown in Figure 4, treatment of HEECs with Poly(I:C) or ssRNA induced the expression of IFN α and IFN β , however, the levels of IFN α were higher in response to ssRNA, while Poly(I:C) induced a greater IFN β response. In addition, the rate of production also differed with Poly(I:C)-induced IFN α/β response being significant after 12 hrs of treatment, while ssRNA-induced IFN α/β was significant after 24 hrs. Since both Poly(I:C) and ssRNA induced the production of type I IFNs by the HEECs, we next sought to determine whether these viral components could also trigger the production of the IFN-inducible anti-viral factors, 2',5'-oligoadenylate synthetase (OAS), Myxovirus-resistance A (MxA) and apolipoprotein B mRNA-editing enzyme-catalytic polypeptidlike 3G (APOBEC3G)^{29, 35–37}. As shown in Figure 5, Poly(I:C) significantly induced the expression of APOBEC3G and OAS. In contrast, viral ssRNA induced the expression of APOBEC3G and MxA, however, for APOBEC3G, the magnitude of response was much lower than after Poly(I:C) treatment (Figure 5). Since TLR activation by viral agonists has also been shown to induce the production of anti-microbial peptides with known anti-viral properties, such as secretory leukocyte protease inhibitor (SLPI) and human beta defensins (HBD1 and HBD2)^{9, 29}, we also assessed their expression. Treatment of HEECs with Poly(I:C) significantly reduced basal HBD1 mRNA levels (Figure 5), but had no significant effect on SLPI or HBD2 mRNA levels (data not shown). In contrast, ssRNA had no significant effect on SLPI, HBD1 or HBD2 mRNA levels (data not shown).

DISCUSSION

Despite our growing understanding of mucosal immunity and the role of the female reproductive tract in host protection^{1, 2}, uterine microvascular endothelial cell immune function remains poorly characterized. Based on their direct contact with the circulation, human endometrial endothelial cells (HEECs) are uniquely positioned to regulate the trafficking of immune cells to the uterus^{20, 38}, and to become exposed to systemic pathogens¹⁷. Since the microvasculature can be the site of viral infections^{17–19}, the objective of this study was to determine the innate immune response of HEECs to viral TLR agonists.

In this current study we report that under resting conditions, HEECs expressed all ten TLRs, and that TLR3 expression was much higher than TLR8 mRNA levels. Upon evaluating the function of these receptors in the HEECs, our first observation was that Poly(I:C) and viral ssRNA both induced the secretion of a number of pro-inflammatory cytokines and chemokines. Although both viral stimuli triggered HEEC inflammation, there were differences in the quality and degree of the response. In addition to the cytokine/chemokine profile being distinct, Poly(I:C) induced a stronger and more rapid response than ssRNA. The common factors induced by both were the chemokines IL-6, IL-8 and GRO- α , and the cytokines IFN γ and G-CSF. The upregulation of chemokine production by the HEECs indicates the potential of the endothelium to recruit immune cells to the site of infection^{39–41}. The induction of IFN γ may also play a role in immune cell trafficking, but also the cell's anti-viral properties. A microarray study reported that treatment of human uterine microvascular endothelial cells with IFN γ upregulated anti-viral genes and genes associated with natural killer cell recruitment⁴².

Our second observation was that while Poly(I:C) and viral ssRNA both induced HEEC expression of type I interferons, the Poly(I:C) response was predominately IFN β , while the ssRNA response was predominantly IFN α . Finally we found that both viral products induced the HEECs to express IFN-inducible anti-viral factors. Exposure to Poly(I:C) or ssRNA upregulated APOBEC3G expression, while only Poly(I:C) upregulated the expression of OAS and only ssRNA induced MxA expression. This differential response may be a reflection of the levels of IFN α and IFN β produced by the cells, with OAS being more dependent upon IFN β ^{29, 35–37}. Although Poly(I:C) has been reported to induce the expression of HBD1 and HBD2 in uterine epithelial cells⁹, and SLPI in trophoblast cells²⁹, treatment of the HEECs with Poly(I:C) slightly, but significantly, downregulated HBD1 expression, while ssRNA had no effect on HBD1 levels, and neither viral signature altered HEEC SLPI or HBD2 levels. While human beta defensins and SLPI have anti-viral properties, these are broad spectrum anti-microbials functioning against multiple bacterial, viral, and fungal pathogens^{43, 44}. Therefore, this lack of induction or downregulation by viral stimuli may be a reflection of the HEECs differentiating from a more general anti-microbial phenotype towards a more anti-viral phenotype.

Treatment of HEECs with Poly(I:C) and viral ssRNA trigger an inflammatory, type I IFN and antiviral response, however, as described above, qualitatively and quantitatively they differed. The timing and magnitude of the cell's responses to the two viral TLR agonists may be related to the levels of TLR3 and TLR8 expressed. Since there was less constitutive TLR8 mRNA than TLR3 expressed by the HEECs this may explain why ssRNA triggered a slower and less robust response when compared to Poly(I:C). However this does not explain the differences in the cytokine, chemokine, and anti-viral profiles produced HEECs after exposure to the two viral stimuli. Instead this is likely to be a reflection of the different signaling pathways activated by TLR3 and TLR8. Upon activation, TLR3 recruits the adapter protein, TRIF, which via TRAF6, activates NF κ B to induce an inflammatory cytokine/chemokine response^{45, 46}. However, TRIF via TRAF3 can also activate interferon regulatory factor (IRF) 3, leading to IFN β production^{45, 46}, and this may explain why HEECs produce more IFN β than IFN α in response to Poly(I:C). In contrast, TLR8 signals through the adapter protein MyD88, which via IRAK4, IRAK1, and TRAF6 activates NF κ B to induce an inflammatory cytokine/chemokine response, and activates IRF7, leading to an IFN α response^{46, 47}, and this may explain why HEECs produce a stronger IFN α response after treatment with ssRNA.

In summary, human endometrial endothelial cells can sense and respond to viral dsRNA and ssRNA by generating distinct and specific inflammatory, type I interferon, and anti-viral innate immune responses. Therefore, cells of the uterine microvasculature likely play an active role in contributing to the immune protection of the endometrium towards viral infections.

Acknowledgments

This work was supported by grant PO1HD054713 from NICHD, NIH (to VMA).

References

1. Hickey DK, Patel MV, Fahey JV, Wira CR. Innate and adaptive immunity at mucosal surfaces of the female reproductive tract: stratification and integration of immune protection against the transmission of sexually transmitted infections. *Journal of reproductive immunology*. 2011; 88:185–194. [PubMed: 21353708]
2. Wira CR, Fahey JV, Sentman CL, Pioli PA, Shen L. Innate and adaptive immunity in female genital tract: cellular responses and interactions. *Immunological reviews*. 2005; 206:306–335. [PubMed: 16048557]

3. Espinoza J, Erez O, Romero R. Preconceptional antibiotic treatment to prevent preterm birth in women with a previous preterm delivery. *Am J Obstet Gynecol.* 2006; 194:630–637. [PubMed: 16522390]
4. Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. *The New England journal of medicine.* 2000; 342:1500–1507. [PubMed: 10816189]
5. McCormack WM. Pelvic inflammatory disease. *The New England journal of medicine.* 1994; 330:115–119. [PubMed: 8259168]
6. Witkin SS. Immunological aspects of genital chlamydia infections. *Best practice & research. Clinical obstetrics & gynaecology.* 2002; 16:865–874. [PubMed: 12473287]
7. Schaefer TM, Desouza K, Fahey JV, Beagley KW, Wira CR. Toll-like receptor (TLR) expression and TLR-mediated cytokine/chemokine production by human uterine epithelial cells. *Immunology.* 2004; 112:428–436. [PubMed: 15196211]
8. Young SL, Lyddon TD, Jorgenson RL, Misfeldt ML. Expression of Toll-like receptors in human endometrial epithelial cells and cell lines. *Am J Reprod Immunol.* 2004; 52:67–73. [PubMed: 15214945]
9. Schaefer TM, Fahey JV, Wright JA, Wira CR. Innate immunity in the human female reproductive tract: antiviral response of uterine epithelial cells to the TLR3 agonist poly(I:C). *J Immunol.* 2005; 174:992–1002. [PubMed: 15634923]
10. Hirata T, Osuga Y, Hirota Y, Koga K, Yoshino O, Harada M, Morimoto C, Yano T, Nishii O, Tsutsumi O, Taketani Y. Evidence for the presence of toll-like receptor 4 system in the human endometrium. *The Journal of clinical endocrinology and metabolism.* 2005; 90:548–556. [PubMed: 15509642]
11. Aflatoonian R, Tuckerman E, Elliott SL, Bruce C, Aflatoonian A, Li TC, Fazeli A. Menstrual cycle-dependent changes of Toll-like receptors in endometrium. *Hum Reprod.* 2007; 22:586–593. [PubMed: 17043100]
12. Hirata T, Osuga Y, Hamasaki K, Hirota Y, Nose E, Morimoto C, Harada M, Takemura Y, Koga K, Yoshino O, Tajima T, Hasegawa A, Yano T, Taketani Y. Expression of toll-like receptors 2, 3, 4, and 9 genes in the human endometrium during the menstrual cycle. *Journal of reproductive immunology.* 2007; 74:53–60. [PubMed: 17292969]
13. Basu S, Eriksson M, Pioli PA, Conejo-Garcia J, Mselle TF, Yamamoto S, Wira CR, Sentman CL. Human uterine NK cells interact with uterine macrophages via NKG2D upon stimulation with PAMPs. *Am J Reprod Immunol.* 2009; 61:52–61. [PubMed: 19086992]
14. Eriksson M, Meadows SK, Basu S, Mselle TF, Wira CR, Sentman CL. TLRs mediate IFN-gamma production by human uterine NK cells in endometrium. *J Immunol.* 2006; 176:6219–6224. [PubMed: 16670332]
15. Eriksson M, Meadows SK, Wira CR, Sentman CL. Endogenous transforming growth factor-beta inhibits toll-like receptor mediated activation of human uterine natural killer cells. *Am J Reprod Immunol.* 2006; 56:321–328. [PubMed: 17076676]
16. Pioli PA, Weaver LK, Schaefer TM, Wright JA, Wira CR, Guyre PM. Lipopolysaccharide-induced IL-1 beta production by human uterine macrophages up-regulates uterine epithelial cell expression of human beta-defensin 2. *J Immunol.* 2006; 176:6647–6655. [PubMed: 16709823]
17. Pereira L, Maidji E, McDonagh S, Tabata T. Insights into viral transmission at the uterine-placental interface. *Trends in microbiology.* 2005; 13:164–174. [PubMed: 15817386]
18. Yamamoto-Tabata T, McDonagh S, Chang HT, Fisher S, Pereira L. Human cytomegalovirus interleukin-10 downregulates metalloproteinase activity and impairs endothelial cell migration and placental cytotrophoblast invasiveness in vitro. *Journal of virology.* 2004; 78:2831–2840. [PubMed: 14990702]
19. Weisblum Y, Panet A, Zakay-Rones Z, Haimov-Kochman R, Goldman-Wohl D, Ariel I, Falk H, Natanson-Yaron S, Goldberg MD, Gilad R, Lurain NS, Greenfield C, Yagel S, Wolf DG. Modeling of human cytomegalovirus maternal-fetal transmission in a novel decidual organ culture. *Journal of virology.* 2011; 85:13204–13213. [PubMed: 21976654]
20. Maidji E, Percivalle E, Gerna G, Fisher S, Pereira L. Transmission of human cytomegalovirus from infected uterine microvascular endothelial cells to differentiating/invasive placental cytotrophoblasts. *Virology.* 2002; 304:53–69. [PubMed: 12490403]

21. Schatz F, Soderland C, Hendricks-Munoz KD, Gerrets RP, Lockwood CJ. Human endometrial endothelial cells: isolation, characterization, and inflammatory-mediated expression of tissue factor and type 1 plasminogen activator inhibitor. *Biology of reproduction*. 2000; 62:691–697. [PubMed: 10684811]
22. Krikun G, Mor G, Huang J, Schatz F, Lockwood CJ. Metalloproteinase expression by control and telomerase immortalized human endometrial endothelial cells. *Histology and histopathology*. 2005; 20:719–724. [PubMed: 15944920]
23. Aldo PB, Krikun G, Visintin I, Lockwood C, Romero R, Mor G. A novel three-dimensional in vitro system to study trophoblast-endothelium cell interactions. *Am J Reprod Immunol*. 2007; 58:98–110. [PubMed: 17631003]
24. Krikun G, Trezza J, Shaw J, Rahman M, Guller S, Abrahams VM, Lockwood CJ. Lipopolysaccharide appears to activate human endometrial endothelial cells through TLR-4-dependent and TLR-4-independent mechanisms. *Am J Reprod Immunol*. 2012; 68:233–237. [PubMed: 22672000]
25. Holder BS, Tower CL, Forbes K, Mulla MJ, Aplin JD, Abrahams VM. Immune cell activation by trophoblast-derived microvesicles is mediated by syncytin 1. *Immunology*. 2012
26. Abrahams VM, Potter JA, Bhat G, Peltier MR, Saade G, Menon R. Bacterial modulation of human fetal membrane Toll-like receptor expression. *Am J Reprod Immunol*. 2013; 69:33–40. [PubMed: 22967004]
27. Zarembek KA, Godowski PJ. Tissue expression of human Toll-like receptors and differential regulation of Toll-like receptor mRNAs in leukocytes in response to microbes, their products, and cytokines. *J Immunol*. 2002; 168:554–561. [PubMed: 11777946]
28. Nishimura M, Naito S. Tissue-specific mRNA expression profiles of human toll-like receptors and related genes. *Biological & pharmaceutical bulletin*. 2005; 28:886–892. [PubMed: 15863899]
29. Abrahams VM, Schaefer TM, Fahey JV, Visintin I, Wright JA, Bole-Aldo P, Romero R, Wira CR, Mor G. Expression and secretion of anti-viral factors by trophoblast cells following stimulation by the TLR-3 agonist, Poly(I:C). *Human Reproduction*. 2006; 21:2432–2439. [PubMed: 16751646]
30. Prinz N, Clemens N, Strand D, Putz I, Lorenz M, Daiber A, Stein P, Degreif A, Radsak M, Schild H, Bauer S, von Landenberg P, Lackner KJ. Antiphospholipid antibodies induce translocation of TLR7 and TLR8 to the endosome in human monocytes and plasmacytoid dendritic cells. *Blood*. 2011; 118:2322–2332. [PubMed: 21734241]
31. Yokota S, Yokosawa N, Okabayashi T, Suzutani T, Miura S, Jimbow K, Fujii N. Induction of suppressor of cytokine signaling-3 by herpes simplex virus type 1 contributes to inhibition of the interferon signaling pathway. *Journal of virology*. 2004; 78:6282–6286. [PubMed: 15163721]
32. Ronay V, Belibasakis GN, Schmidlin PR, Bostanci N. Infected periodontal granulation tissue contains cells expressing embryonic stem cell markers. *Schweizer Monatsschrift für Zahnmedizin = Revue mensuelle suisse d'odonto-stomatologie = Rivista mensile svizzera di odontologia e stomatologia/SSO*. 2013; 123:12–16.
33. de Kleijn D, Pasterkamp G. Toll-like receptors in cardiovascular diseases. *Cardiovascular research*. 2003; 60:58–67. [PubMed: 14522407]
34. Arpaia N, Barton GM. Toll-like receptors: key players in antiviral immunity. *Current opinion in virology*. 2011; 1:447–454. [PubMed: 22440908]
35. Samuel CE. Antiviral actions of interferons. *Clin Microbiol Rev*. 2001; 14:778–809. table of contents. [PubMed: 11585785]
36. Turelli P, Mangeat B, Jost S, Vianin S, Trono D. Inhibition of hepatitis B virus replication by APOBEC3G. *Science*. 2004; 303:1829. [PubMed: 15031497]
37. Bonvin M, Achermann F, Greeve I, Stroka D, Keogh A, Inderbitzin D, Candinas D, Sommer P, Wain-Hobson S, Vartanian JP, Greeve J. Interferon-inducible expression of APOBEC3 editing enzymes in human hepatocytes and inhibition of hepatitis B virus replication. *Hepatology*. 2006; 43:1364–1374. [PubMed: 16729314]
38. van den Heuvel MJ, Chantakru S, Xuemei X, Evans SS, Tekpetey F, Mote PA, Clarke CL, Croy BA. Trafficking of circulating pro-NK cells to the decidualizing uterus: regulatory mechanisms in the mouse and human. *Immunological investigations*. 2005; 34:273–293. [PubMed: 16136782]

39. Hannan NJ, Salamonsen LA. Role of chemokines in the endometrium and in embryo implantation. *Current opinion in obstetrics & gynecology*. 2007; 19:266–272. [PubMed: 17495644]
40. Red-Horse K, Drake PM, Fisher SJ. Human pregnancy: the role of chemokine networks at the fetal-maternal interface. *Expert reviews in molecular medicine*. 2004; 6:1–14. [PubMed: 15130179]
41. Abrahams VM, Visintin I, Aldo PB, Guller S, Romero R, Mor G. A role for TLRs in the regulation of immune cell migration by first trimester trophoblast cells. *J Immunol*. 2005; 175:8096–8104. [PubMed: 16339547]
42. Kitaya K, Yasuo T, Yamaguchi T, Fushiki S, Honjo H. Genes regulated by interferon-gamma in human uterine microvascular endothelial cells. *International journal of molecular medicine*. 2007; 20:689–697. [PubMed: 17912462]
43. McNeely TB, Dealy M, Dripps DJ, Orenstein JM, Eisenberg SP, Wahl SM. Secretory leukocyte protease inhibitor: a human saliva protein exhibiting anti-human immunodeficiency virus 1 activity in vitro. *The Journal of clinical investigation*. 1995; 96:456–464. [PubMed: 7615818]
44. Quinones-Mateu ME, Lederman MM, Feng Z, Chakraborty B, Weber J, Rangel HR, Marotta ML, Mirza M, Jiang B, Kiser P, Medvik K, Sieg SF, Weinberg A. Human epithelial beta-defensins 2 and 3 inhibit HIV-1 replication. *AIDS*. 2003; 17:F39–48. [PubMed: 14571200]
45. Gauzzi MC, Del Corno M, Gessani S. Dissecting TLR3 signalling in dendritic cells. *Immunobiology*. 2010; 215:713–723. [PubMed: 20561711]
46. Thompson AJ, Locarnini SA. Toll-like receptors, RIG-I-like RNA helicases and the antiviral innate immune response. *Immunology and cell biology*. 2007; 85:435–445. [PubMed: 17667934]
47. Jenkins KA, Mansell A. TIR-containing adaptors in Toll-like receptor signalling. *Cytokine*. 2010; 49:237–244. [PubMed: 19264502]

Figure 1. TLR expression by HEECs

TLR1-10 expression was measured by qRT-PCR on RNA collected from untreated HEECs (n=3) and expressed as relative abundance. HEECs express all 10 TLRs. HEEC TLR3 mRNA expression is significantly higher than TLR8 mRNA levels (* $p<0.05$).

Figure 2. Poly(I:C) triggers a robust and rapid HECC inflammatory response
 HECCs were incubated with either no treatment (NT) or Poly(I:C) at 1 µg/ml for 12 hours, after which culture supernatants were measured for cytokines and chemokines by multiplex analysis. Barcharts show that Poly(I:C) significantly increased the secretion of IL-2, IL-6, IL-8, IFN γ , MCP-1, MIP-1 β , G-CSF, GM-CSF, RANTES and GRO- α . Data are from 5 independent experiments. * $p < 0.05$; ** $p < 0.001$ relative to the NT control.

Figure 3. Viral ssRNA triggers a distinct and weaker HEEC inflammatory response
 HECCs were incubated with either no treatment (NT) or ssRNA at 5 µg/ml for 24 hours, after which culture supernatants were measured for cytokines and chemokines by multiplex analysis. Barcharts show that ssRNA significantly increased the secretion of IL-6, IL-8, IFN γ , G-CSF, VEGF and GRO- α . Data are from 3 independent experiments. * p <0.05; ** p <0.001 relative to the NT control.

Figure 4. Poly(I:C) and viral ssRNA induce a type I IFN response in HECCs

HECCs were incubated with no treatment (NT) or with either: (A) Poly(I:C) for 12 hours or (B) ssRNA for 24 hours, after which RNA was isolated and analyzed for IFN α and IFN β levels by qRT-PCR. Barcharts show IFN α and IFN β mRNA expression as fold change relative to the NT control (* $p < 0.05$). Data are from 4–5 independent experiments.

Figure 5. Poly(I:C) and viral ssRNA trigger distinct HECC anti-viral responses

HECCs were incubated with no treatment (NT) or with either: (A) Poly(I:C) for 12 hours or (B) ssRNA for 24 hours, after which RNA was isolated and analyzed for APOBEC3G, OAS, MxA, and HBD1 levels by qRT-PCR. Barcharts show mRNA expression as fold change relative to the NT control (* $p < 0.05$). Data are from 4–6 independent experiments.

Table 1

Primers used for quantitative real-time PCR

Gene	Forward	Reverse
TLR1	ATTCCGCAGTACTCCATTCC	TTTGCTTGCTCTGTCTCAGCTT
TLR2	TCCTCCAAATCAGGCTTCTCT	AATTCCATTGGATGTCAGCA
TLR3	GAAAGGCTAGCAGTCATCCA	CATCGGTACCTGAGTCAAC
TLR4 ²⁶	CAGAGTTTCCCTGCATGGATCA	GCTTATCTGAAGGTGTTGCACAT
TLR5	GGAAACCAGCTCCTAGTCTCT	AAGAGGAAAACCCCAGAGAA
TLR6 ²⁷	CCCATTCCACAGAACAGCAT	ATAAGTCCGCTGGGTCAATGA
TLR7 ²⁶	TTTACCTGGATGGAACCAAGCTA	TCAAGGCCTGAGAAAGCTGTAAGCTA
TLR8	AGAGCATCAACCAAGCAAG	GCCGTAGCTCAAAATACTGA
TLR9 ²⁶	GGACCTCTGGTACTGCTTCCA	AAGCTCGTTGTACACCCAGTCT
TLR10 ²⁶	TGTTATGACAGCAGAGGGTGATG	GAGTTGAAAAAAGGAGGTATAGGATAAATC
IFN α ²⁹	GCAAGCCAGAAATATCTGC	ACTGGTTGCCATCAAACTCC
IFN β ²⁸	GCTCTCTCTGTTGTGCTTCTCCACTACAGC	CTGACTATGGTCCAGGCACAGTGACTGTACTCC
APOBEC3G ²⁸	CGTCGGAATACCGTCTTGGCTGTGCTACG	GATCTTCATGGTGGCACGCGGACCGTTC
OAS ³⁰	CCACCCTCAGAGGCCGATCTGACGCTGACCTGG	CCAGGAAATTAGGAGACAGC
MXA ²⁸	GGTGAGAAAGCTGATCCGCCCTCCACTTCCAG	GCCGGCGCCGAGCCTGCCGTGAGCCGTGC
SLP1 ²⁸	CCTGCCTTACCCATGAAGTCCAGCGGCC	CAITTTGATGCCACAAAGTGTC
HBD1 ²⁸	GTCAGCTCAGCCTCCAAAGG	CTTCTGCGTCATTTCTTCTG
HBD2 ²⁸	CCTGATGCCCTCTTCCAGGTG	GAGGAGGCCCTTCTCTGAATC
GAPDH ³¹	CAGCCTCCCGCTTCGGCTCTC	CCAGCGCCCAATACGACCA