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Variable responsiveness to agonists for TLR2 and TLR7 in myometrial cells from different sources: correlation with receptor expression

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Abstract

Myometrium plays a vital role in maintenance of pregnancy. Disruption of myometrial sensitivity to pro-contractile stimuli might lead to preterm labor. Inflammation and/or infection are common precursors to preterm birth, in part by initiating pro-contractile stimuli through toll like receptor (TLRs) activation

In this study we investigated the responses specific to inflammatory stimuli for both human primary myometrial cells (HPMCs), and PHM1-41 cells, a human immortalized myometrial cell line. Both these types of cells are commonly used to study labor and pregnancy. Both cell lines were treated with lipopolysaccharide (LPS), peptidoglycan (PGN) or imiquimod (IQ) (ligands for TLR 4, 2, and 7, respectively). We demonstrate that inflammatory cytokines increase significantly with LPS treatment, however no change occurs with PGN and IQ, suggesting lack of TLR2- and TLR7-specific signaling in both HPMCs and in the PHM1-41 cell line. Absence of TLR2- and TLR7-specific protein bands on western blots confirmed the lack of these receptors in both HPMCs maintained in long-term culture and PHM1-41 cells. However, TLR2 expression was present in freshly collected matched human myometrial tissue (i.e. the tissues used to create the HPMCs cell cultures), showing loss of TLR2 receptors by HPMCs during the cell culturing process. TLR7 protein expression was lacking both in myometrial tissue and in cultured cells. These results demonstrate the limited applicability and reliability of cellular models to investigate the role of myometrium during pregnancy and labor.

Keywords

Myometrium; TLRs; HPMCs; PHM1-41; Protein expression

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Declaration of conflicting interests

Authors declares that there is no conflict of interest.

Introduction

Myometrium plays a vital role in maintenance of pregnancy in humans. It is the middle layer of uterine wall between perimetrium (the outer uterine layer, also known as serosa) and endometrium (inner uterine lining).[1] It mainly consists of smooth muscle derived from mesoderm of urogenital ridge.[1] The primary function of the myometrium during labor is to contract, leading to expulsion of the fetus. During pregnancy several myometrial proteins are up- or down-regulated to support either quiescence or contractility depending on gestational age.[2–7] Previous studies done in animal models and tissues obtained from laboring women show increased myometrial expression of oxytocin receptors, cyclooxygenase-2, prostaglandin receptors and myometrial gap junction proteins compared to non-laboring tissues.[8,9] Several studies in human myometrium have identified gene transcripts differentially expressed in non-pregnant and pregnant human myometrium.[2–7] These include genes involved in growth and differentiation, such as those related to signaling by insulin like growth factor, prolactin, Wnt, plasminogen activator inhibitors, structural and contractile genes, genes involved in cell adhesion, molecular recognition and immunity, and others. [2] These and other adaptable changes in myometrium during pregnancy show the essential role of molecular modification in maintenance of pregnancy and the transition to a laboring phenotype. Whether these adaptations persist in long-term tissue culture is often been assumed but not well studied.[10]

Intraamniotic infection is thought to be caused most commonly by ascending infection from the vagina. Such infections may produce myometrial contractions leading to preterm birth, a major cause of neonatal morbidity and mortality. [11,12] Toll like receptors (TLRs) are a family of pattern recognition receptors (PRR) consisting of 13 members, of which 10 have been identified in human tissues. [13–17] They primarily mediate changes in gene expression induced by bacterial and viral infection. TLRs detect the presence of microbial or viral invaders by recognizing their pathogen-associated molecular patterns (PAMPs) or endogenous danger-associated molecular patterns (DAMPs) and inducing both inflammatory cascades and autophagy as an innate defense mechanism against harm. [18] TLRs are expressed in gestational tissues, including placenta, fetal membranes and myometrium, and expression of these TLRs is upregulated with advancing gestational age. [19–21] Activation of TLRs is one route to initiating the signaling cascades that result in myometrial contractions.[22]

Our aim was to validate the use of cell lines to study myometrial expression of toll-like receptors. Because inflammation-induced preterm labor is thought to involve activation of the innate immune system, we chose the most common TLRs involved in bacterial and viral infection i.e. TLRs 2, 4 and 7. We used two common sources of myometrial cells to study responsiveness to inflammatory stimuli: 1) human primary myometrial cells (HPMCs) collected from women undergoing non laboring cesarean section and maintained in long-term culture; and 2) PHM1-41, a human myometrial cell line originally obtained from a pregnant woman not in labor at 39 weeks and immortalized using an adenoviral vector expressing E6/E7 protein of human papilloma virus 16

Method

Reagents:

LPS (5ng/μl, Sigma, St. Louis, MO) dissolved in water, PGN (1μg/μl, Sigma) dissolved in water and IQ (5ug/μl, Invivogen, San Diego, CA) dissolved in water were frozen in aliquots and thawed and diluted before use.

Tissue collection and primary myometrial cell culture:

The protocol to collect human myometrial tissue samples was approved by the institutional review board of NorthShore University HealthSystem. Written informed consent was obtained from participating women. All myometrial samples were obtained from women undergoing non-laboring elective cesarean section either at term or preterm. Myometrial biopsies were obtained from the upper margin of the lower uterine segment incision during cesarean section and brought promptly to the research laboratory for processing according to the methods of Mosher et.al.,[23] Phaneuf et al.,[24] and Tribe et al.,.[25] Briefly, cells were isolated by placing the tissue in digestive media containing collagenase XI (1mg/ml) and collagenase IA (1mg/ml) for 60 minutes at 37°C and cultured in smooth muscle cell medium (PromoCell, Heidelberg, Germany). Cells were allowed to grow for two weeks after isolation and then sub-cultured as passage number one. As HPMCs are myofibroblast in nature, the purity of primary cultures was validated by identifying the expression of calponin (a marker for smooth muscle cells) and vimentin (a marker for fibroblasts) using immunocytochemistry (ICC) (Supplementary Figure 1).

Cell Culture:

HPMCs were maintained in smooth muscle cell medium. PHM1-41 (American Type Culture Collection (ATCC) CRL-3046, Manassas, Virginia) were maintained in Dulbecco's Modified Eagle's Medium (DMEM high glucose; 11965–092; Gibco, Waltham, MA) supplemented with 10% fetal bovine serum and 10% non-essential amino acids (11140–50; Gibco). Both cell lines were maintained at 37°C in 5% CO_2/95% air and were passaged every 2 – 3 days to maintain logarithmic growth. Cells were plated at a density of $4*10^5$/ml in twelve-well plates and allowed to attach overnight. Medium was discarded and wells were washed with PBS before incubation. Cells were then incubated for 6 hours with either LPS (5ng/ml), PGN (1ug/ml) or IQ (5μg/ml), each condition in triplicate. Total cellular protein and RNA was extracted using radioimmunoprecipitation lysis buffer (RIPA, Santa Cruz Biotechnology, Dallas, TX) and Trizol (Thermo Fisher, Waltham, MA), respectively.

RT-PCR:

Concentration and quality of RNA was analyzed using NanoDrop (Thermo Fisher). Samples were stored at −80°C until further use. One microliter of RNA was used as a template for cDNA synthesis (qScript cDNA SuperMix, Quanta Biosciences, Gaithersburg, MD). Duplex RT-PCR was performed using two primer-probe sets, one specific to GAPDH (a housekeeping gene used as an internal control) and the other for the gene of interest using the StepOnePlus Real Time PCR system (Applied Biosystems, Waltham, MA). For amplification of each gene of interest, prevalidated TaqMan gene expression assay primer-

probe mixes were used (Invitrogen, Waltham, MA): chemokine (C-C motif) ligand 5 (CCL5, Hs00982282_m1), interleukin (IL-) 1β (Hs01555410_m1), tumor necrosis factor (TNF, Hs00174128_m1), and IL-6 (Hs00174131_m1). Real time PCR was done with TaqMan Universal PCR Master Mix (Applied Biosystems) using the manufacturer's protocol in a 10μl reaction volume. Thermocycler parameters for RT-PCR included two minutes at 50° C followed by 10 minutes incubation at 95°C and then 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Semiquantitative analysis of gene expression was done using the comparative CT (ΔΔCT) method, normalizing expression of the gene of interest to that of GAPDH. Each PCR reaction was run in duplicate for each sample.

Protein extraction and western blot:

For protein extractions HPMCs and matched myometrial tissues (from which HPMCs were isolated) and PHM1-41 cells were lysed via sonication in ice cold 1X RIPA buffer containing protease and phosphatase inhibitors (Thermo Scientific, Waltham, MA). Lysates were incubated on ice for 30 minutes and centrifuged at 10,000 x g for 10 minutes at 4°C. Supernatants were collected and quantified using a bicinchoninic acid (BCA) protein analysis kit (Thermo Fisher) and a plate reader (Molecular Devices, Sunnyvale, CA). Proteins extracted from a human monocytic cell line (THP-1) (Santa Cruz Biotechnology, sc-2238) and a mouse macrophage line (RAW264.7), known to harbor TLR2, TLR4 and TLR7, were used as positive controls. 50μg of total protein was loaded per lane on a 4–12% Bis Tris protein gel (NuPAGE, Thermo Fisher). Protein was transferred onto PVDF membranes and blocked for 1 hour in 5% nonfat dry milk in TBST. The membranes were then incubated with primary antibody to TLR2 (human-specific-#12276 and mouse-specific-#13744, Cell Signaling, Danvers, MA), TLR4 (sc-293072, Santa Cruz, detects both mouse and human protein), TLR7 (human-specific-sc-57463, Santa Cruz and mouse-specific-ab205134, Abcam), IL-1β (sc-32294, Santa Cruz, specific for human protein) and GAPDH (3683, Cell Signaling, detects both mouse and human protein) overnight at 4°C. Secondary antibodies used were as follows: goat anti-mouse IgG-HRP (sc-2031), goat anti-rat IgG-HRP (sc-2032) and goat anti-rabbit IgG-HRP (sc-2030) all from Santa Cruz. Blots were developed using Pierce ECL plus western blotting substrates (32132X3, Thermo Fisher Scientific). Blots were imaged using a Storm Scanner (Molecular Dynamics) and quantified using ImageQuant software (Molecular Dynamics).

Statistical analysis:

Differences in gene expression between groups are calculated using ANOVA and Sidak test (for comparison of multiple variables). Differences between control and experimental groups were considered to be statistically significant when the P value was <0.05. GraphPad Prism was used for all analyses.

Results

HPMCs and PHM1-41 cells respond to lipopolysaccharide (LPS) but not to peptidoglycan (PGN) or imiquimod (IQ)

Both HPMCs and PHM1-41 cells were incubated *in vitro* with the TLR4 ligand LPS (5ng/ml), the TLR2 ligand PGN (1μg/ml), and the TLR7 ligand IQ (5μg/ml). Dose studies were

performed to identify the optimum concentration and duration of incubation for LPS, PGN, and IQ (not shown). Total cellular RNA was collected at 6 hours. Stimulation with LPS but not PGN or IQ resulted in the upregulation of mRNA for the inflammatory cytokines IL-6 and IL-1β-for HPMCs (Figure 1) and CCL5, TNF, IL-6 and IL-1β for PHM1-41 (Figure 2). These inflammatory markers were chosen to represent the activation of the MyD88-dependent pathway (IL-1β), the MyD88-independent pathway (CCLS) and both pathways (TNF and IL-6), and because the mRNA expression of these genes is known to correlate closely with the active proteins.[26]

HPMCs and the PHM1-41 cell line lack TLR2 and TLR7

Published work demonstrated either up or downregulation of TLR expression levels in cell lines upon treatment with TLR ligands [27]. Therefore, total cellular protein was extracted from the PHM1-41 cell line (both LPS-stimulated and unstimulated) and from HPMCs. Whole cell lysates of THP-1 (a human monocyte cell line) and RAW 264.7 (a mouse macrophage line) were used as positive controls. Absence of 98kDa and 140kDa protein bands specific to TLR2 and TLR7, respectively, in HPMCs and PHM1-41 cells demonstrated lack of TLR2 and 7 receptors (Figure 3), while the positive controls showed the presence of specific bands. In contrast, a TLR4-specific band was present in all lanes, confirming TLR4 expression in both PHM1-41 cells and HPMCs.

To examine whether native myometrium similarly lacks TLR2 and TLR7, we performed western blots on protein extracts from freshly frozen matched myometrium (i.e. the same tissue samples from which HPMCs were isolated) obtained from pregnant women undergoing cesarean section. TLR2 was present but not TLR7 (Figure 4). Again HPMCs lacked both receptors. These results suggest that TLR2 is lost during long-term culture and immortalization of human myometrial cells, and that TLR7 is not present in detectable and/or functional quantities even in fresh-frozen myometrial tissue.

To verify that m-RNA expression correlates with protein levels of inflammatory cytokines, we further validated our results using western blot for IL-1β in PHM1-41 and HPMC. Presence of a specific 17KDa IL-1β band in LPS-treated PHM1-41 and HPMC cells and absence of this band in PGN- and IQ-treated cells (Figure 5) confirm observations made at the mRNA level.

Discussion

Myometrium plays a major role in the process of birth, whether at term or preterm, due to its contractile activity during labor.[28] These contractions can be caused by TLR activation, especially during preterm labor in which the innate immune system is triggered in response to infection or danger signals. [29,30]

In this study we examined responses to TLR2, 4, and 7 ligands in two types of myometrial cell cultures (HPMCs and an immortalized myometrial cell line). We observed significant increases in the levels of m-RNAs for inflammatory cytokines in both cell types upon incubation with LPS (Figure 1 and 2). No change was observed with PGN or IQ for both

HPMCs and the PHM1-41 cell line. Therefore we examined the expression of TLR2, 4, and 7 receptors in both cell lines and demonstrated that these are lacking (Figure 3).

TLRs have been found previously in the female reproductive tract.[31] Expression of TLR2 has been shown in vagina, ecto- and endocervix, endometrium, myometrium and fallopian tubes.[20,21,31–34] We identified TLR2 in intact human myometrium (Figure 4A), which is consistent with a prior report of expression and upregulation of TLR2 in laboring human myometrium. [20,21] However TLR2 was not present in HPMCs isolated from matched myometrial tissue samples after long-term cell culture (Figure 4A), suggesting the loss of TLR2 receptors over time. This finding is consistent with a prior report showing variability in levels of gene expression among fresh tissues, explants, primary cells and immortalized cells and that even explants resemble more closely but are not identical to fresh tissues.[10] There is a body of evidence demonstrating that both immortalized and primary cells in prolonged culture may lose or gain expression of various genes, through either mutation, transcriptional or post-transcriptional changes.[35,36]

To our knowledge this is the first study to examine TLR7 protein expression in human myometrium. Previous studies showed constitutive expression of TLR7 in fallopian tubes, cervix and ectocervix, however for uterine endometrium there were contradictory results, with some studies demonstrating the expression of TLR7 [37,38] and others showing lack of expression. [39] We found no expression of TLR7 in either human myometrium or HPMCs (Figure 4B).

This is the first comparative analysis of TLR2 and 7 in matched myometrial tissue and primary cell samples, and the first to show loss of TLR2 receptors over the process of long-term culture or immortalization. This represents a potentially important distinction between native myometrial tissues and primary and immortalized myometrial cell lines commonly used to study pregnancy. Therefore, such cell cultures may have limited applicability to studying mechanisms of parturition and preterm labor. This observation needs to be further explored for other types of proteins and in other tissues of the female reproductive tract.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Blanks AM, Shmygol A, Thornton S. Preterm labour. Myometrial function in prematurity. Best Pract Res Clin Obstet Gynaecol. 2007;21(5):807–819. [PubMed: 17446138]
2. Rehman KS, Yin S, Mayhew BA, Word RA, Rainey WE. Human myometrial adaptation to pregnancy: cDNA microarray gene expression profiling of myometrium from non-pregnant and pregnant women. Mol Hum Reprod. 2003;9(11):681–700. [PubMed: 14561811]

3. Breuiller-Fouche M, Charpigny G, Germain G. Functional genomics of the pregnant uterus: from expectations to reality, a compilation of studies in the myometrium. BMC Pregnancy Childbirth. 2007;7 Suppl 1:S4. [PubMed: 17570164]
4. Havelock JC, Keller P, Muleba N, et al. Human myometrial gene expression before and during parturition. Biol Reprod. 2005;72(3):707–719. [PubMed: 15509731]
5. Charpigny G, Leroy MJ, Breuiller-Fouche M, et al. A functional genomic study to identify differential gene expression in the preterm and term human myometrium. Biol Reprod. 2003;68(6):2289–2296. [PubMed: 12606369]
6. Sharp GC, Hutchinson JL, Hibbert N, Freeman TC, Saunders PT, Norman JE. Transcription Analysis of the Myometrium of Labouring and Non-Labouring Women. PLoS One. 2016;11(5):e0155413. [PubMed: 27176052]
7. Breuiller-Fouche M, Germain G. Gene and protein expression in the myometrium in pregnancy and labor. Reproduction. 2006;131(5):837–850. [PubMed: 16672349]
8. Kimura T, Takemura M, Nomura S, et al. Expression of oxytocin receptor in human pregnant myometrium. Endocrinology. 1996;137(2):780–785. [PubMed: 8593830]
9. Martin C, Chapman KE, Thornton S, Ashley RH. Changes in the expression of myometrial ryanodine receptor mRNAs during human pregnancy. Biochim Biophys Acta. 1999;1451(2–3):343–352. [PubMed: 10556588]
10. Georgiou EX, Lei K, Lai PF, et al. The study of progesterone action in human myometrial explants. Mol Hum Reprod. 2016;22(8):877–889. [PubMed: 27235325]
11. Bastek JA, Gomez LM, Elovitz MA. The role of inflammation and infection in preterm birth. Clin Perinatol. 2011;38(3):385–406. [PubMed: 21890015]
12. Kim YM, Romero R, Oh SY, et al. Toll-like receptor 4: a potential link between “danger signals,” the innate immune system, and preeclampsia? Am J Obstet Gynecol. 2005;193(3 Pt 2):921–927. [PubMed: 16157088]
13. Thompson MR, Kaminski JJ, Kurt-Jones EA, Fitzgerald KA. Pattern recognition receptors and the innate immune response to viral infection. Viruses. 2011;3(6):920–940. [PubMed: 21994762]
14. Backhed F, Hornef M. Toll-like receptor 4-mediated signaling by epithelial surfaces: necessity or threat? Microbes Infect. 2003;5(11):951–959. [PubMed: 12941387]
15. Basu S, Fenton MJ. Toll-like receptors: function and roles in lung disease. Am J Physiol Lung Cell Mol Physiol. 2004;286(5):L887–892. [PubMed: 15064235]
16. Bsibsi M, Ravid R, Gveric D, van Noort JM. Broad expression of Toll-like receptors in the human central nervous system. J Neuropathol Exp Neurol. 2002;61(11):1013–1021. [PubMed: 12430718]
17. Zarember 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(2):554–561. [PubMed: 11777946]
18. Kumar H, Kawai T, Akira S. Pathogen recognition by the innate immune system. Int Rev Immunol. 2011;30(1):16–34. [PubMed: 21235323]
19. Kim YM, Romero R, Chaiworapongsa T, et al. Toll-like receptor-2 and -4 in the chorioamniotic membranes in spontaneous labor at term and in preterm parturition that are associated with chorioamnionitis. Am J Obstet Gynecol. 2004;191(4):1346–1355. [PubMed: 15507964]
20. O’Brien M, Morrison JJ, Smith TJ. Upregulation of PSCDBP, TLR2, TWIST1, FLJ35382, EDNRB, and RGS12 gene expression in human myometrium at labor. Reprod Sci. 2008;15(4):382–393. [PubMed: 18497345]
21. Youssef RE, Ledingham MA, Bollapragada SS, et al. The role of toll-like receptors (TLR-2 and -4) and triggering receptor expressed on myeloid cells 1 (TREM-1) in human term and preterm labor. Reprod Sci. 2009;16(9):843–856. [PubMed: 19564644]
22. Patni S, Flynn P, Wynen LP, et al. An introduction to Toll-like receptors and their possible role in the initiation of labour. BJOG. 2007;114(11):1326–1334. [PubMed: 17903232]
23. Mosher AA, Rainey KJ, Bolstad SS, et al. Development and validation of primary human myometrial cell culture models to study pregnancy and labour. BMC Pregnancy Childbirth. 2013;13 Suppl 1:S7. [PubMed: 23445904]

24. Phaneuf S, Europe-Finner GN, Varney M, MacKenzie IZ, Watson SP, Lopez Bernal A. Oxytocin-stimulated phosphoinositide hydrolysis in human myometrial cells: involvement of pertussis toxin-sensitive and -insensitive G-proteins. J Endocrinol. 1993;136(3):497–509. [PubMed: 8386215]
25. Tribe RM, Moriarty P, Poston L. Calcium homeostatic pathways change with gestation in human myometrium. Biol Reprod. 2000;63(3):748–755. [PubMed: 10952916]
26. Zhao TZ, Xia YZ, Li L, et al. Bovine serum albumin promotes IL-1beta and TNF-alpha secretion by N9 microglial cells. Neurol Sci. 2009;30(5):379–383. [PubMed: 19696964]
27. Oshikawa K, Sugiyama Y. Gene expression of Toll-like receptors and associated molecules induced by inflammatory stimuli in the primary alveolar macrophage. Biochem Biophys Res Commun. 2003;305(3):649–655. [PubMed: 12763043]
28. Taggart MJ, Blanks A, Kharche S, Holden A, Wang B, Zhang H. Towards understanding the myometrial physiome: approaches for the construction of a virtual physiological uterus. BMC Pregnancy Childbirth. 2007;7 Suppl 1:S3. [PubMed: 17570163]
29. Kemp MW. Preterm birth, intrauterine infection, and fetal inflammation. Front Immunol. 2014;5:574. [PubMed: 25520716]
30. Burdet J, Rubio AP, Salazar AI, Ribeiro ML, Ibarra C, Franchi AM. Inflammation, infection and preterm birth. Curr Pharm Des. 2014;20(29):4741–4748. [PubMed: 24588830]
31. Fazeli A, Bruce C, Anumba DO. Characterization of Toll-like receptors in the female reproductive tract in humans. Hum Reprod. 2005;20(5):1372–1378. [PubMed: 15695310]
32. Watari M, Watari H, Nachamkin I, Strauss JF. Lipopolysaccharide induces expression of genes encoding pro-inflammatory cytokines and the elastin-degrading enzyme, cathepsin S, in human cervical smooth-muscle cells. J Soc Gynecol Investig. 2000;7(3):190–198.
33. Lim R, Barker G, Lappas M. TLR2, TLR3 and TLR5 regulation of pro-inflammatory and pro-labour mediators in human primary myometrial cells. J Reprod Immunol. 2017;122:28–36. [PubMed: 28844021]
34. Pioli PA, Amiel E, Schaefer TM, Connolly JE, Wira CR, Guyre PM. Differential expression of Toll-like receptors 2 and 4 in tissues of the human female reproductive tract. Infect Immun. 2004;72(10):5799–5806. [PubMed: 15385480]
35. Milyavsky M, Shats I, Erez N, et al. Prolonged culture of telomerase-immortalized human fibroblasts leads to a premalignant phenotype. Cancer Res. 2003;63(21):7147–7157. [PubMed: 14612508]
36. Piao CQ, Liu L, Zhao YL, Balajee AS, Suzuki M, Hei TK. Immortalization of human small airway epithelial cells by ectopic expression of telomerase. Carcinogenesis. 2005;26(4):725–731. [PubMed: 15677631]
37. Hart KM, Murphy AJ, Barrett KT, Wira CR, Guyre PM, Pioli PA. Functional expression of pattern recognition receptors in tissues of the human female reproductive tract. J Reprod Immunol. 2009;80(1–2):33–40. [PubMed: 19406482]
38. Ghasemi N, Amjadi F, Salehi E, Shakeri M, Aflatoonian A, Aflatoonian R. Expression of Toll-like receptors 7–10 in human fallopian tubes. Iran J Reprod Med. 2014;12(6):389–394. [PubMed: 25071846]
39. 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(1):67–73. [PubMed: 15214945]

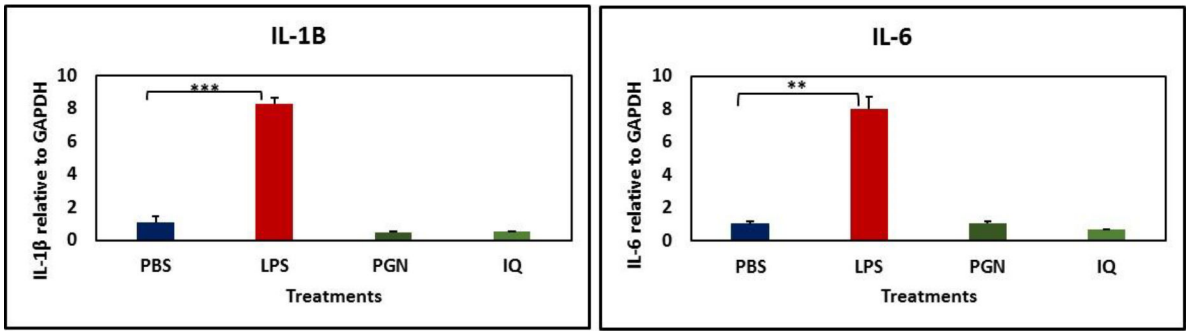

Figure 1: RT-PCR for IL-1β and IL-6 in HPMCs after 6 hours of incubation with LPS, PGN and IQ.

RT-PCR was performed on total cellular RNA. PBS: control. Each bar is an average of three replicates, Error bars = ±SD. One-way ANOVA with Sidak multiple comparison test was performed. LPS, PGN and IQ treatments were compared to control, PBS. The only statistically significant difference was between PBS and LPS treatments. *** $P < 0.001$, ** $P < 0.01$

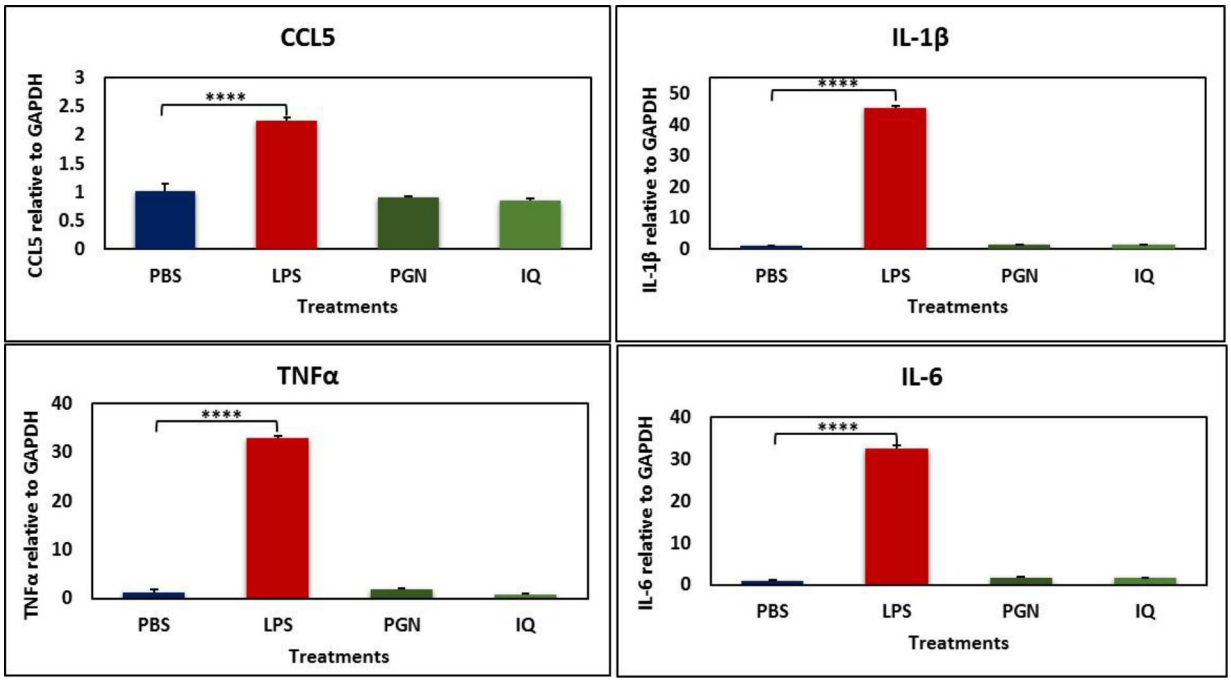

Figure 2: RT-PCR for CCL5, IL-1β, TNFα and IL-6 in the PHM1-41 cell line after 6 hours of incubation with LPS, PGN and IQ.

RT-PCR was performed on total cellular RNA. PBS: control. Each bar is an average of three replicates, Error bars = ±-SD. One-way ANOVA with Sidak multiple comparison test was performed. LPS, PGN and IQ treatments were compared to control PBS. The only statistically significant difference was between PBS and LPS treatments. **** $P < 0.0001$.

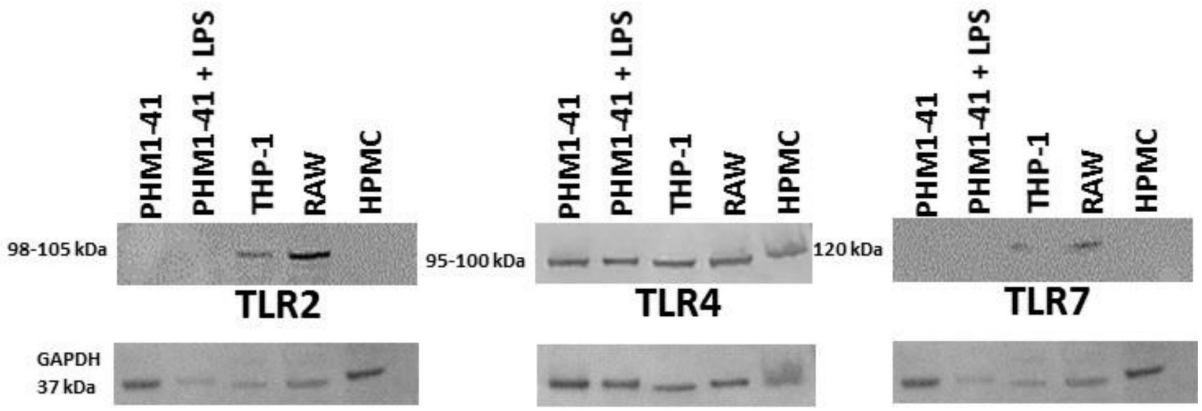

Figure 3: Western blots for TLR2, TLR4, and TLR7 in HPMCs and the PHM1-41 cell line.
PHM1-41 + LPS: PHM1-41 cells pre-treated with LPS (5ng/ml) for 6 hours, THP-1 and RAW: positive controls, GAPDH: Loading control.

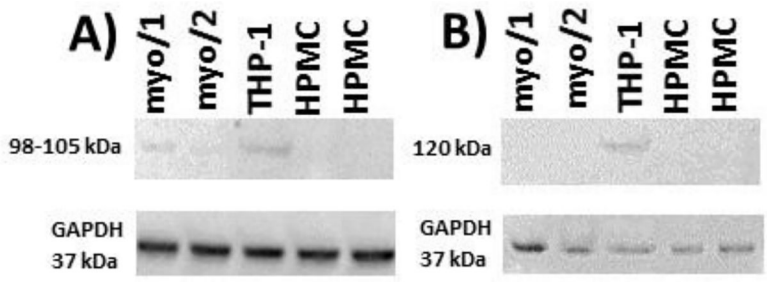

Figure 4: Western blot for TLR2 and TLR7 in human myometrium tissue.

myo/1 and 2: fresh-frozen myometrium tissue samples, THP-1 - Positive control, GAPDH-Loading control

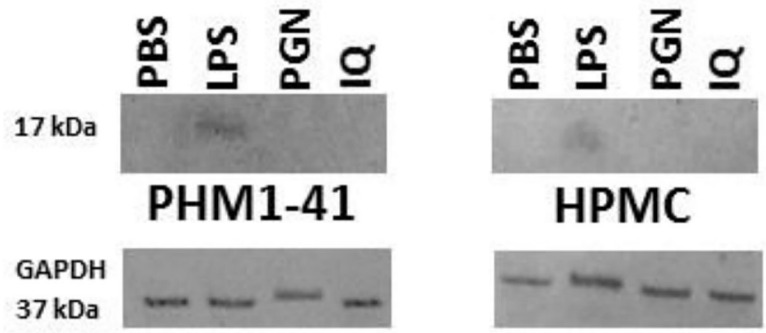

Figure 5: Western blot for IL-1β in HPMC and PHM1-41 cells treated with either LPS, PGN or IQ for 6 hours.

GAPDH- Loading control