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Original article

The effect of lipopolysaccharide on anti-inflammatory and pro-inflammatory cytokines production of human amniotic epithelial cells

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ABSTRACT

Intrauterine infection is a major cause of immune imbalance at the maternal-fetal interface, which leads to spontaneous abortion, premature rupture of the fetal membranes, and preterm birth. Human amniotic epithelial cells (hAECs) play a fundamental role in the maintenance of pregnancy. We hypothesize that bacteria influence the immunomodulatory effects of hAECs through stimulation of Toll-like receptors (TLRs). Here, we investigated how lipopolysaccharide (LPS) as a bacterial component affects anti-inflammatory and pro-inflammatory cytokines production of hAECs. Human placentas were obtained from six healthy pregnant women and hAECs were isolated. The phenotypic characteristics of hAECs were determined by flow cytometry. The hAECs (4×10^5 cells/ml) were cultured in the presence or absence of LPS (5 μg/ml). The viability of the cells was assessed and culture supernatants of hAECs were collected after 24, 48 and 72 h of incubation. The levels of transforming growth factor-beta1 (TGF-β1), interleukin-4 (IL-4), tumor necrosis factor-alpha (TNF-α), interleukin-17 A (IL-17A), and interferon-gamma (IFN-γ) were measured by ELISA. Our data showed that LPS treatment did not affect the viability of hAECs, while had a stimulatory effect on TGF-β1 production of hAECs ($p < 0.001$). A significant reduction in IL-4 production of LPS-stimulated hAECs was observed ($p < 0.05$). LPS enhanced the production of TNF-α and IL-17 A of hAECs ($p < 0.05$–0.0001). The IFN-γ level was only detectable in two culture supernatants of hAECs, and the level was unchanged after stimulation with LPS. Based on these findings, LPS may play a pivotal role in immune imbalance at the feto-maternal interface through affecting anti-inflammatory and pro-inflammatory cytokines production of hAECs.

1. Introduction

Maternal immunological tolerance to the fetus plays a key role in the successful pregnancy [1]. During pregnancy, there is a bias of Th1 immunity toward Th2 responses [2,3]. Th2 cells secrete anti-inflammatory cytokines such as IL-4, IL-5, and IL-10 that redirect maternal immunity away from cellular immunity towards enhanced humoral responsiveness which is critical for the implantation and maintenance of pregnancy [2,4]. Thus, pregnant women are more susceptible to intracellular infections [5]. In this notion, intrauterine infection is considered to be an important risk factor for spontaneous abortion, premature rupture of the fetal membranes (PROM), and preterm birth (PTB) [6]. Extensive data from the literature have demonstrated that intrauterine infections contribute to the weakening and the rupture of the fetal membranes through inducing pro-inflammatory cytokines production of different immune cells at the feto-maternal interface. These cytokines stimulate prostaglandins production and thereby lead to uterine contractions, enhance matrix metalloproteinase production and programmed cell death (apoptosis) which cause PROM and PTB [6,7].

The female genital tract is a suitable place for colonizing many microorganisms which are thought to be a major cause of spontaneous abortion and PTB [6]. Intrauterine infections are mainly polybacterial and typically representative of the normal microbial flora of the cervicovaginal tract, such as *Ureaplasma urealyticum*, *Streptococcus agalactiae* and other Gram-negative and -positive bacteria [8]. Recognition of microorganisms colonized in the cervicovaginal tract is mediated by pattern recognition receptors (PRRs) of the innate immune system which one of these is Toll-like receptors (TLRs) that detect conserved pathogen associated molecular patterns (PAMPs). To date, several

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Table 1
Primary and matched isotype control antibodies used to determine the purity and immunophenotyping of hAECs by flow cytometry.

Fluorochrome/Antibody	Isotype	Working volume	Source of Primary Antibodies
Alexa Fluor® 488 anti-Cytokeratin (pan reactive)	Mouse IgG1, κ	5 µl/test	Biolegend, San Diego, CA, USA
FITC anti-human CD105	Mouse IgG1, κ	5 µl/test	Biolegend, San Diego, CA, USA
FITC anti-human CD90	Mouse IgG1, κ	5 µl/test	Biolegend, San Diego, CA, USA
FITC anti-human CD45	Mouse IgG1, κ	5 µl/test	Biolegend, San Diego, CA, USA
FITC anti-human CD86	Mouse IgG1, κ	5 µl/test	Biolegend, San Diego, CA, USA
FITC anti-human CD44	Mouse IgG1, κ	5 µl/test	Biolegend, San Diego, CA, USA
PE anti-human SSEA-4	Mouse IgG3, κ	20 µl/test	Biolegend, San Diego, CA, USA
PE anti-human CD133	Mouse IgG1, κ	20 µl/test	Biolegend, San Diego, CA, USA
FITC anti-human CD64	Mouse IgG1, κ	20 µl/test	Biolegend, San Diego, CA, USA
FITC anti-human HLA-DR	Mouse IgG2a, κ	20 µl/test	BD Biosciences, San Jose, CA, USA
FITC anti-human CD34	Mouse IgG1, κ	20 µl/test	BD Biosciences, San Jose, CA, USA
PE anti-human CD73	Mouse IgG1, κ	5 µl/test	BD Biosciences, San Jose, CA, USA

functional TLRs have been identified in human in which sense different ligands and have a distinct specificity for pathogen detection [9]. TLR4 recognizes lipopolysaccaride (LPS), a constituent of the cell wall of Gram–negative bacteria, and thereby triggers intracellular signaling cascades [6]. In turn, intracellular signaling pathways activate nuclear factor-kappa B (NF-κB) and activation protein-1(AP-1), which are known as key transcription factors responsible for the expression of many genes involved in inflammation [10].

Numerous studies have been carried out on the expression and function of TLRs in the female genital tract [11–13]. Several lines of evidence indicate that human placenta expresses different TLRs and participates in maternal immune responses through the production of pro-inflammatory cytokines [6,14]. Traditionally, human placenta is comprised of three layers including amnion, chorion, and decidua. The amnion is the closest layer to the fetus, in direct contact with the amniotic fluid, and consists of epithelial cells and avascular stroma [15]. Human amniotic epithelial cells (hAECs) possess immunomodulatory properties, which are of considerable importance for the successful pregnancy. Previous studies have provided convincing evidence that the immunomodulatory effects of hAECs are mainly mediated by the secretion of immunosuppressive compounds such as transforming growth factor-beta (TGF-β), prostaglandin E2 (PGE2) and interleukin-4 (IL-4) [16,17]. Besides having pregnancy-friendly immunomodulatory effects, hAECs provide a physical barrier to protect the developing fetus against invading pathogens [6]. Others have reported that hAECs express functional TLRs and respond to invading pathogens by enhancing the production of antimicrobial β-defensins and key pro-inflammatory mediators involved in pregnancy failure [6,18,19]. These findings suggest that hAECs activation by bacteria or bacterial productions may have an important role in modulating immunomodulatory effects of hAECs, which may lead to the failure of feto-maternal immunologic tolerance.

Although previous studies have provided convening evidence to show that intrauterine infections play an important role in pregnancy failure [6], the role of intra-amniotic infections and molecular mechanisms involved in the effects of pathogenic agents on hAECs are not fully recognized so far. In this study, we investigated whether LPS, as a known bacterial product, may be a risk factor for immune imbalance at the maternal-fetal interface through affecting the production of interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), interleukin-17 A (IL-17 A), TGF-β1, and IL-4 cytokines of hAECs.

2. Materials and methods

2.1. Isolation of hAECs

Human placentas (n = 6) were obtained from term normal singleton pregnancies during uncomplicated elective cesarean deliveries before the onset of labor. hAECs were isolated using a method described previously [15]. Briefly, the amnion membrane was manually stripped from the chorion and divided into small pieces. In order to remove blood and cellular debris, the amnion layer was washed several times with phosphate-buffered saline (PBS, pH 7.2) and then incubated at 37 °C for 10 min with pre-digestion buffer (calcium and magnesium-free hank's balanced salt solution containing 0.53 mM EDTA4Na). Afterwards, the amnion was digested at 37 °C for 30 min with 0.05% EDTA/trypsin (Gibco, USA). The digestion of the amnion was followed twice at 37 °C for 30 min with 0.05% EDTA/trypsin. The cells obtained from the second and third digests were pooled and washed with ice-cold Roswell Park Memorial Institute (RPMI) medium. Cell viability was determined by trypan blue dye exclusion and cell counts were performed using a haemocytometer. To determine the purity of the cells, the fixation and permeabilization of the cells were performed using Intracellular Fixation and Permeabilization Buffer Set (eBiosciences, USA) according to the manufacturer's protocol. Next, intracellular staining of cytokeratin as an epithelial marker was carried on with primary or matched isotype control antibodies for 25 min at 4 °C (Table 1). The matched isotype control antibody was used as negative control. The percentage of the stained cells was measured by a FACSCalibur system (Becton Dickinson, San Jose, CA) and then analyzed using FlowJo software (v10.1, FlowJo, Ashland, OR, USA). The cells with the purity more that 97% were considered as hAECs.

2.2. hAECs characterization

To determine the phenotypic characteristics of freshly isolated hAECs, the cells were analyzed by flow cytometry. The hAECs ($6–10 \times 10^5$) were suspended in 100 µl PBS/1% bovine serum albumin (BSA) and incubated with different primary antibodies or isotype matched control IgG for 30 min at 4 °C (Table 1). The cells were then washed three times with cell staining buffer (BioLegend, USA) and centrifuged at 200 ×g for 5 min at 4 °C. The percentage of the stained cells was measured by a FACSCalibur flow cytometer and analyzed using FlowJo software.

2.3. hAECs culture

The isolated hAECs were suspended in Dulbecco's modified Eagle's medium/ Nutrient Mixture F-12 (DMEM/F12, Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin/streptomycin (Sigma-Alderich, USA) and then cultured in 75 cm^2 tissue culture flasks at a density of 2.5×10^5 cells/cm^2. After 24 h of incubation at 37 °C, adhered hAECs were dissociated by trypsin and used in the experiments described below.

2.4. hAECs stimulation and cell viability determination

In order to stimulate hAECs by LPS, the cells obtained from six healthy pregnant women were cultured in DMEM/F12 supplemented with 10% FBS and 1% penicillin/streptomycin at a density of 4×10^5

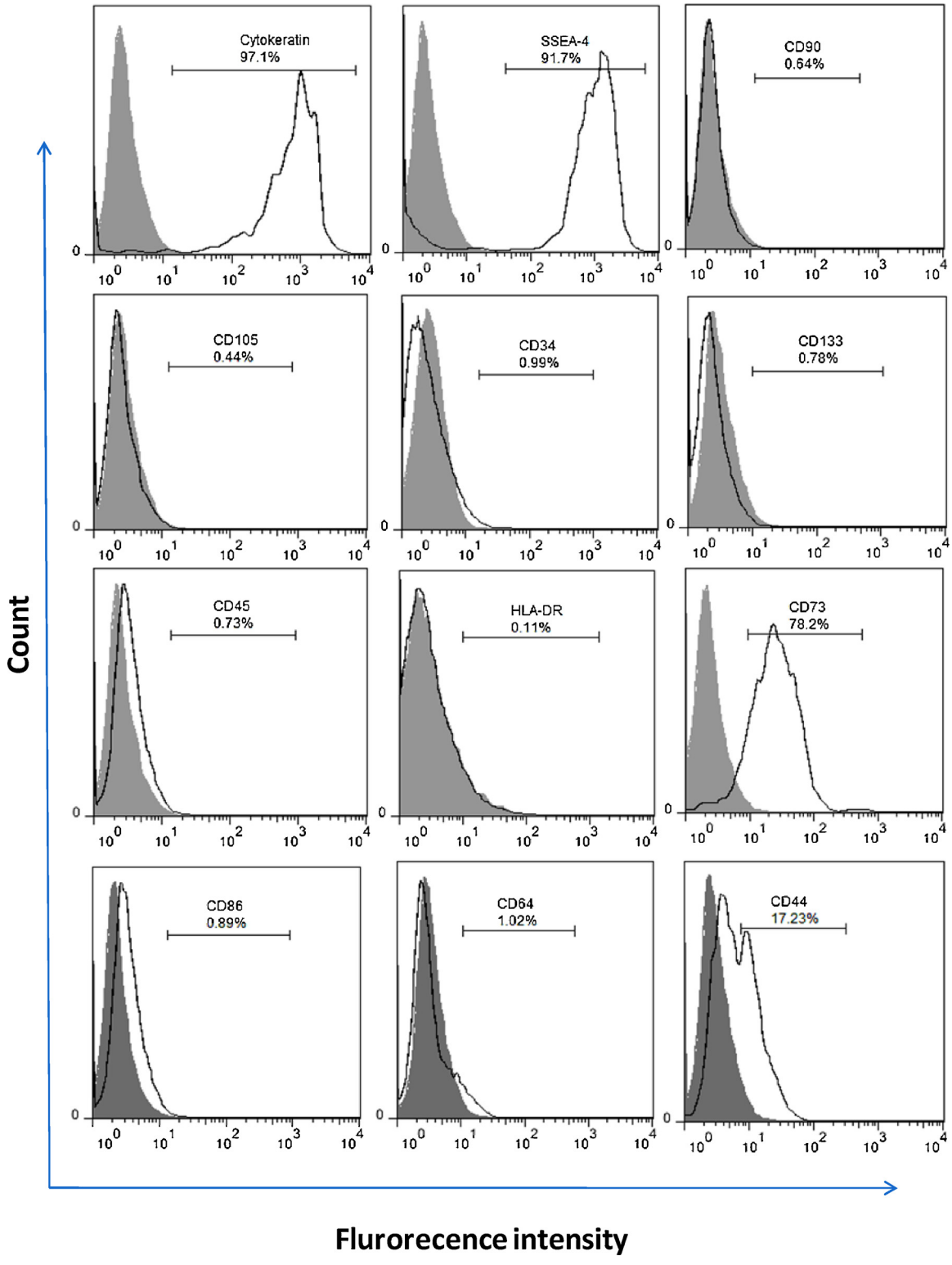

Fig. 1. Immunophenotyping of hAECs. Black line: hAECs were stained with different primary antibodies. Gray shaded histogram: the cells were stained with the matched isotype control antibodies as negative controls. Each histogram is representative of three independent experiments.

cells/ml in 24-well plates. The hAECs were then stimulated with LPS (5 μg/ml) and considered as test group, while hAECs cultured in the absence of LPS served as control group. The cells were then incubated at 37 °C with 5% CO_2. All cultures were done in duplicate. After 24, 48 and 72 h of incubation, the adhered cells were detached by trypsin and cell viability was determined by trypan blue dye exclusion.

2.5. Cytokine assay

To assess the effect of LPS on production of pro-inflammatory and anti-inflammatory cytokines of hAECs, the culture supernatants of hAECs cultured in the presence or absence of LPS were collected after 24, 48 and 72 h of culture and quantitative analysis of cytokines was performed by an Enzyme-linked immunoasorbent assay (ELISA) kit

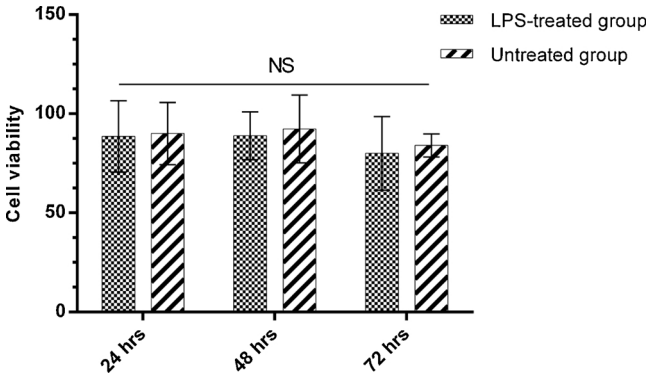

Fig. 2. LPS effects on the viability of hAECs. The cultured hAECs were detached by trypsin after 24, 48, and 72 h of incubation. The effect of LPS on cell viability was assessed by trypan blue dye. Each bar is representative of three independent experiments. Data are shown as mean ± SEM.

according to the manufacturer's instructions (Mabtech, Sweden). The following cytokines were measured: IFN-γ, TNF-α, IL-4, TGF-β1, and IL-17 A.

2.6. Statistical analysis

The results are expressed as mean ± standard error of mean (SEM). Statistical analyses were done using GraphPad Prism 6 (GraphPad software, San Diego, CA). Unpaired *t*-test was used to compare two groups with normal distribution, while Mann-Whitney test was performed in the case of non-normal distribution. P value < 0.05 was considered statistically significant.

3. Results

3.1. Phenotypic characteristics of hAECs

Having considered that hAECs are obtained from a primary tissue source and there are no specific selection criteria for these cells, we analyzed the expression of some identified markers of hAECs isolated from three separate amnion donors. As shown in Fig. 1, hAECs strongly expressed cytokeratin (an epithelial marker) and SSEA-4 (an embryonic stem cell marker), while these cells were negative for expression of mesenchymal stem cell (MSC) markers (CD90, CD105), some of tissue macrophage markers (CD86 and CD64), and hematopoietic stem cell markers including CD34, CD133, and CD45 (Fig. 1). Moreover, our data revealed that hAECs failed to express HLA-DR which is a polymorphic molecule and increase the risk of immune responses against the cells (Fig. 1). Nearly all hAECs expressed high level of CD73, a MSC marker (Fig. 1). It was also observed that all hAECs expressed low level of CD44 (hyaluronic acid receptor), which is consistent with previous reports [17,20,21].

3.2. The effect of LPS on the viability of hAECs

To determine LPS effect on cell viability, hAECs cultured in the presence or absence of LPS were collected and stained by trypan blue dye. Despite a numerical reduction in the viability of LPS-treated hAECs, our results indicated that LPS had not significant effect on the viability of the cells after 24, 48, and 72 h of culture (Fig. 2).

3.3. The effect of LPS on production of pregnancy-friendly cytokines of hAECs

Given that anti-inflammatory cytokines play an important role in the successful pregnancy, the effects of LPS on TGF-β1 and IL-4 production of hAECs were assessed. As shown in Fig. 3A, LPS treatment had a stimulatory effect on TGF-β1 production of hAECs. After 72 h of incubation, the production of TGF-β1 of LPS-stimulated hAECs was significantly higher than the level of TGF-β1 production of unstimulated hAECs ($p < 0.001$, Fig. 3A). In contrast, we observed that LPS reduced the IL-4 production of hAECs after 24, 48 and 72 h of culture (Fig. 3B). There was a significant reduction in IL-4 production of LPS-treated hAECs after 24 h ($p < 0.05$, Fig. 3B).

3.4. LPS effect on pro-inflammatory cytokines production of hAECs

Since pro-inflammatory cytokines have deleterious effects on pregnancy outcome, we assessed the effects of LPS on the production of IL-17 A, IFN-γ, and TNF-α of hAECs, which play a critical role in cell-mediated immunity. Our data indicated a significant increase in the production of TNF-α of hAECs stimulated with LPS after 24, 48, 72 h of incubation ($p < 0.0001$-0.01, Fig. 4A). The same trend was also observed in the IL-17 A production of LPS-treated hAECs (Fig. 4B). The level of IL-17 A in 72-hour culture supernatants of LPS-stimulated hAECs was significantly increased compared to control group ($p < 0.05$, Fig. 4B). In spite of measurement and assessment of the effects of LPS on TNF-α and IL-17 A production of hAECs, we were unable to determine LPS effect on the IFN-γ production of hAECs. We found that the IFN-γ level was below the detection limit in culture supernatants of hAECs, and the level was unchanged after stimulation

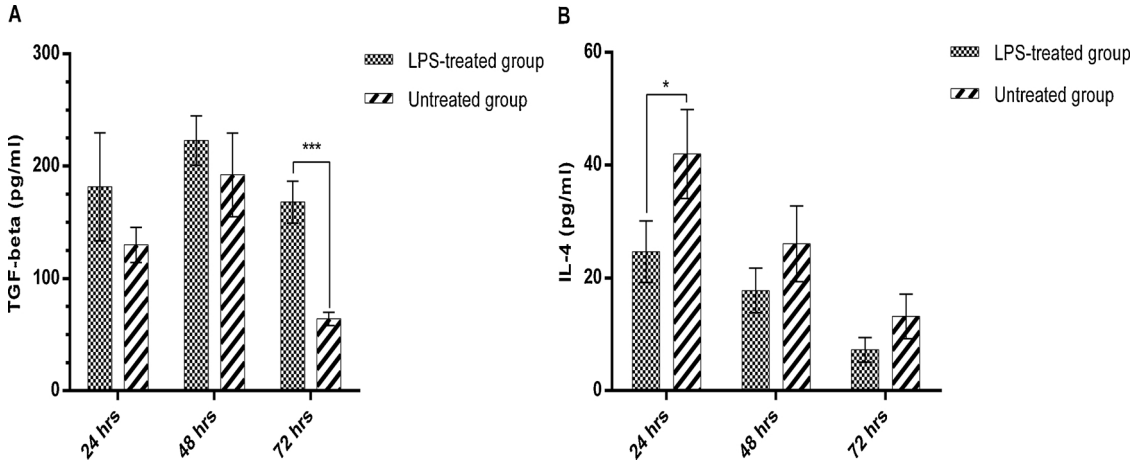

Fig. 3. LPS effects on anti-inflammatory cytokines production of hAECs. The isolated hAECs from the amnion membrane were cultured in the presence or absence of LPS. After 24, 48 and 72 h, the culture supernatants of hAECs were collected and the effects of LPS on the production of TGF-β1 and IL-4 of hAECs were measured by ELISA (A and B). Data are representative of six independent experiments. Values are shown as mean ± SEM. * $p < 0.05$, ***$p < 0.001$.

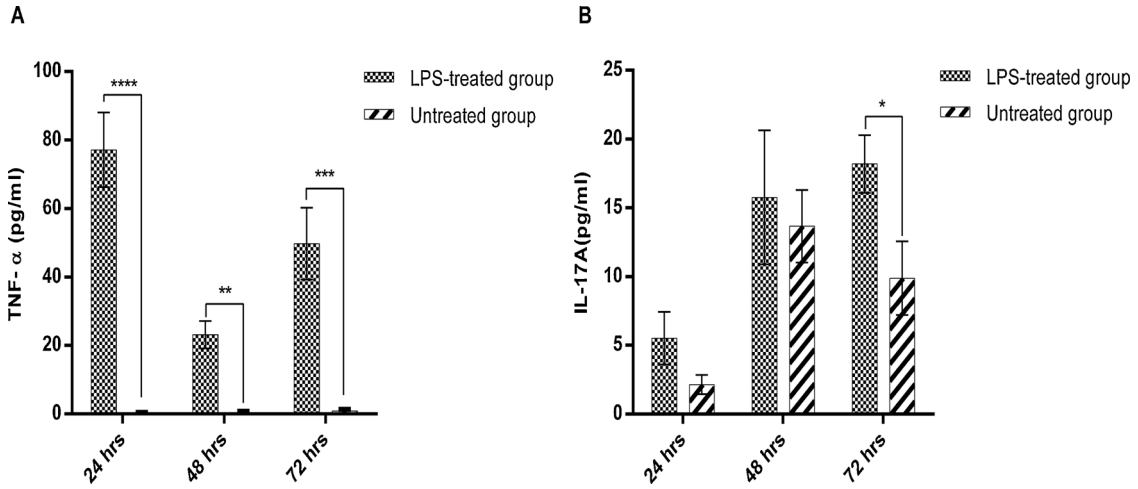

Fig. 4. The Effects of LPS on Pro-inflammatory Cytokines Production of hAECs. hAECs were isolated from the amnion membrane and cultured in the presence or absence of LPS. The culture supernatants of hAECs were collected after 24, 48 and 72 h of incubation and LPS effect on TNF-α and IL-17A production of hAECs was measured using ELISA (A and B). The results are representative of six independent experiments. Data show mean ± SEM. *p < 0.05, **p < 0.001, ***p < 0.001, ****p < 0.0001.

with LPS. The IFN-γ level was only detectable in hAEC samples no. two and three (data not shown).

4. Discussion

Pregnancy is a semi-allograft and immunologically foreign to the mother [3]. Maternal immunological tolerance to the fetus plays a pivotal role in the implantation and maintenance of pregnancy [22]. However, infectious agents is a major cause of the failure of feto-maternal immunologic tolerance [5,23]. Therefore, we investigated whether hAECs activation by LPS may contribute to abnormalities in immunological tolerance at the maternal-fetal interface through changing the production of anti-inflammatory and pro-inflammatory cytokines of hAECs.

Despite numerous studies on pathogenic agents and their role in pregnancy complications, there are a few reports about intra-amniotic bacteria and their effects on hAECs function [5,6,11]. Some studies on hAECs have shown that activation of these cells by infectious agents or their components exerts different effects on activities of hAECs, depending upon which TLR is activated [6]. On the other hand, immunomodulatoy effects of hAECs are dependent on the secretion of immunosuppressive mediators [20]. Thus, the critical question was how LPS treatment influences TGF-β1 production, as an anti-inflammatory cytokines, of hAECs. Our data indicated that stimulation of hAECs by LPS significantly improved TGF-β1 production after 72 h of culture. This finding was consistent with our previous study that showed LPS significantly induced the expression of TGF-β1 in hAECs [10]. Although these findings suggest that production of LPS-mediated TGF-β1 of hAECs can participate in modulating pro-inflammatory responses against intra-amniotic bacteria and regulating the immune system at the feto-maternal interface, there are some reports that showed TGF-β1 is an important cytokine in activation, proliferation, and differentiation of T cells [24–26]. These data suggest that hAECs activation by LPS may influence T cell-mediated cellular immune responses against intra-amniotic infections. However, further studies are required to determine the molecular mechanisms involved in the effects of LPS on the TGF-β1 production of hAECs.

It has been reported that intrauterine infections result in a shift in the maternal immune system away from Th2 immunity towards Th1 responses [2,27,28]. In our knowledge, the possible role of hAECs in shifting Th2 immunity towards Th1 responses is not investigated yet. Therefore, we focused on determining whether hAECs activation may participate in a shift in pregnancy-friendly immune responses toward deleterious responses at the feto-maternal interface. In the current study, we observed that in the presence of LPS, the IL-4 production of hAECs was significantly reduced after 24 h of incubation, which is in agreement with similar studies conducted on TLRs and their functions in the cervicovaginal tract pointing to the activation of TLRs by intrauterine infection results in spontaneous abortion and PTB due to a defect in maternal immunological tolerance [6]. Regarding the fact that Th1 and Th2-type cytokines are mutually inhibitory, it is likely that the reduction in IL-4 secretion of hAECs participate in enhancing immune responses at the maternal-fetal interface, which are harmful to the maintenance of pregnancy [27,29]. In line with this notion, we assessed the production level of some cytokines which are considered harmful to the successful pregnancy. The results of this study revealed that TNF-α and IL-17 A production of hAECs were significantly increased when the cells were cultured in the presence of LPS. Gillaux et al. indicated that hAECs stimulation by TLR5 and TLR6/2 agonists produced pro-inflammatory cytokines such as IL-6 and IL-8, however TLR4 activation on hAECs could not affect the production of these pro-inflammatory cytokines of hAECs, which is disagreed with the results of this study [6]. In contrast with our data, the authors observed that stimulation of hAECs by LPS was only contributed to the decreased viability of hAECs and increased rate of cell apoptosis [6]. Moreover, an *in vitro* study on hAEC responses to intra-amniotic infections has demonstrated that the stimulation of TLR2, TLR6, and TLR9 on hAECs by *Ureaplasma parvum* and *urealyticum serovars* induced pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, and TNF-α of hAECs, while there was no effect on production of IL-10 [19]. These findings propose that hAECs activation by intra-amniotic bacteria or bacterial products may participate in the breakdown of maternal immunological tolerance to the fetus, which is critical to the maintenance of pregnancy. In an effort to discover the effects of LPS on the IFN-γ secretion of hAECs, we observed that IFN-γ was only produced in very low amounts in two culture supernatants of hAECs cultured in the presence or absence of LPS, which its production was independent of stimulation with LPS.

Taken together, the results of this study for the first time provide evidence to show that hAECs stimulation by LPS may contribute to immune imbalance at the maternal-fetal interface through inducing TNF-α, IL-17 A, and TGF-β1 production and reducing IL-4 production of hAECs.

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University of Medical Sciences (IUMS).

Conflicts of interest

The authors declare that there are no conflicts of interest.

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References

[1] Arenas-Hernandez M, Romero R, St Louis D, Hassan SS, Kaye EB, Gomez-Lopez N. An imbalance between innate and adaptive immune cells at the maternal–fetal interface occurs prior to endotoxin-induced preterm birth. Cell Mol Immunol. 2016;13:462.
[2] Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? Immunol Today 1993;14:353–6.
[3] Piccinni M-P. T cell tolerance towards the fetal allograft. J Reprod Immunol 2010;85:71–5.
[4] Sacks G, Sargent I, Redman C. An innate view of human pregnancy. Immunol Today 1999;20:114–8.
[5] Mor G, Cardenas I. The immune system in pregnancy: a unique complexity. Am J Reprod Immunol 2010;63:425–33.
[6] Gillaux C, Méhats C, Vaiman D, Cabrol D, Breuiller-Fouché M. Functional screening of TLRs in human amniotic epithelial cells. J Immunol 2011;187:2766–74.
[7] Fortunato SJ, Menon R, Bryant C, Lombardi SJ. Programmed cell death (apoptosis) as a possible pathway to metalloproteinase activation and fetal membrane degradation in premature rupture of membranes. Am J Obstet Gynecol 2000;182:1468–76.
[8] Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. New Eng J Med 2000;342:1500–7.
[9] Reynolds JM, Dong C. Toll-like receptor regulation of effector T lymphocyte function. Trend Immunol 2013;34:511–9.
[10] Taheri RA, Motedayyen H, Ghotloo S, Masjedi M, Mosaffa N, Mirshafiey A, et al. The effect of lipopolysaccharide on the expression level of immunomodulatory and immunostimulatory factors of human amniotic epithelial cells. BMC Res Notes 2018;11:343.
[11] Nasu K, Narahara H. Pattern recognition via the toll-like receptor system in the human female genital tract. Mediators Inflamm 2010;2010.
[12] Thaxton JE, Nevers TA, Sharma S. TLR-mediated preterm birth in response to pathogenic agents. Infect Dis Obstet Gynecol 2010;2010.
[13] Patni S, Flynn P, Wynen L, Seager A, Morgan G, White J, et al. An introduction to toll-like receptors and their possible role in the initiation of labour. BJOG: Int J Obstet Gynaecol 2007;114:1326–34.
[14] Patni S, Wynen LP, Seager AL, Morgan G, White JO, Thornton CA. Expression and activity of Toll-like receptors 1–9 in the human term placenta and changes associated with labor at term. Biol. Reprod 2009;80:243–8.
[15] Motedayyen H, Esmaeil N, Tajik N, Khadem F, Ghotloo S, Khani B, et al. Method and key points for isolation of human amniotic epithelial cells with high yield, viability and purity. BMC Res Notes 2017;10:552.
[16] Ueta M, Kweon MN, Sano Y, Sotozono C, Yamada J, Koizumi N, et al. Immunosuppressive properties of human amniotic membrane for mixed lymphocyte reaction. Clin Exp Immunol 2002;129:464–70.
[17] Motedayyen H, Rezaei A, Zarnani A-H, Tajik N. Human amniotic epithelial cells inhibit activation and pro-inflammatory cytokines production of naive CD4+ T cells from women with unexplained recurrent spontaneous abortion. Reprod Biol 2018.
[18] Szukiewicz D, Szewczyk G, Pyzlak M, Klimkiewicz J, Maslinska D. Increased production of β-defensin 3 (hBD-3) by human amniotic epithelial cells (HAEC) after activation of toll-like receptor 4 in chorioamnionitis. Inflam Res 2008;57:67–8.
[19] Triantafilou M, De Glanville B, Aboklaish AF, Spiller OB, Kotecha S, Triantafilou K. Synergic activation of toll-like receptor (TLR) 2/6 and 9 in response to ureaplasma parvum & urealyticum in human amniotic epithelial cells. PLoS One 2013;8:e61199.
[20] Insausti CL, Blanquer M, García-Hernández AM, Castellanos G, Moraleda JM. Amniotic membrane-derived stem cells: immunomodulatory properties and potential clinical application. Stem Cells Cloning 2014;7:53–63.
[21] Motedayyen H, Zarnani A-H, Tajik N, Ghotloo S, Rezaei A. Immunomodulatory effects of human amniotic epithelial cells on naive CD4+ T cells from women with unexplained recurrent spontaneous abortion. Placenta 2018.
[22] Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. Nat Immunol 2004;5:266.
[23] Kanellopoulos-Langevin C, Caucheteux SM, Verbeke P, Ojcius DM. Tolerance of the fetus by the maternal immune system: role of inflammatory mediators at the feto-maternal interface. Reprod Biol Endocrinol 2003;1:121.
[24] Ramezan Ali Taheri, Ghotloo Somayeh, Masjedi Mohsen, Mosaffa Nariman, Mirshafiey Abbas, Saffari Mahmood, et al. The effect of lipopolysaccharide on the expression level of immunomodulatory and immunostimulatory factors of human amniotic epithelial cells. BMC Res Notes 2018:343.
[25] Letterio JJ, Roberts AB. Regulation of immune responses by TGF-β. Ann Rev Immunol 1998;16:137–61.
[26] Gonzalez CR, Matzkin ME, Frungieri MB, Terradas C, Ponzio R, Puigdomenech E, et al. Expression of the TGF-beta1 system in human testicular pathologies. Reprod Biol Endocrinol 2010;8:148.
[27] Kehrl JH, Wakefield LM, Roberts AB, Jakowlew S, Alvarez-Mon M, Derynck R, et al. Production of transforming growth factor beta by human T lymphocytes and its potential role in the regulation of T cell growth. J Exp Med 1986;163:1037–50.
[28] Raghupathy R, Ma Makhseed, Azizieh F, Hassan N, Al-Azemi M, Al-Shamali E, et al. Maternal Th1-and Th2-type reactivity to placental antigens in normal human pregnancy and unexplained recurrent spontaneous abortions. Cell Immunol 1999;196:122–30.
[29] Zen M, Ghirardello A, Iaccarino L, Tonon M, Campana C, Arienti S, et al. Hormones, immune response, and pregnancy in healthy women and SLE patients. Swiss Med Wkly 2010;140:187.