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Contribution of Vasoactive Intestinal Peptide to Immune Homeostasis in Trophoblast-Maternal Leukocyte Interaction under LPS Stimulation

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Key Words
Vasoactive intestinal peptide · Early pregnancy · Tolerance and pregnancy · Human implantation

Abstract
Background/Aims: The maternal-fetal interface is a unique immunological site that generates an adequate microenvironment during pregnancy, recognizing and eliminating infections and tolerating the trophoblast/placenta unit. For that purpose, trophoblast cells display several tolerogenic mechanisms to allow fetal survival, such as production of the neuropeptide vasoactive intestinal peptide (VIP). Here we investigated the contribution of VIP to maintaining homeostasis at the maternal-placental interface under lipopolysaccharide (LPS) stimulation. ***Methods:*** We performed cocultures between trophoblast cells (Swan-71 cell line) and maternal leukocytes obtained from fertile women as an in vitro model of maternal-placental interaction, and we focused on the effects of LPS on the modulation of VIP and their receptors ($VPAC_1$ and $VPAC_2$). ***Results:*** VIP could prevent the upregulation of IL-6, MCP-1, and nitrite production and maintain the production of IL-10 and TGF-β under LPS (10 μg/ml) stimulation after 48 h of coculture. To gain deeper insight into the mechanisms of how VIP could contribute to a tolerogenic microenvironment even in the presence of LPS, we investigated VIP production by maternal leukocytes and observed a significant increase in the frequency of CD4+VIP+ cells after interaction with Swan-71 cells in the presence of LPS. LPS increased VIP and inducible receptor $VPAC_2$ expression directly on trophoblast cells in a dose- and time-dependent manner. ***Conclusions:*** The present results suggest that VIP might act as an additional homeostatic mechanism during early stages at the maternal-placental interface to control exacerbated inflammatory responses such as the ones observed in intrauterine infections.

Introduction

The maternal-fetal interface is a unique immunological site that promotes tolerance to the allogeneic fetus while maintaining the host defense against pathogens.

In this sense, the innate immune system, as the first line of immunological defense, provides an immediate response against invading pathogens through its ability to distinguish specific sequences on microorganisms like

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pathogen-associated molecular patterns (PAMPs) by specific pattern recognition receptors (PRR), thus constituting a critical step for the later specific adaptive response against antigens [1, 2].

Recent evidence shows that the innate immunity at the maternal-fetal interface has a fundamental role in establishing an adequate microenvironment during pregnancy, recognizing and eliminating infections and tolerating the trophoblast/placenta unit [2].

In line with this, trophoblast cells express proteins of the PRR families, such as the Toll-like receptors (TLR), whose expression is regulated temporally and spatially. Hence, through interaction with specific PAMPs, these receptors could trigger the activation of the NFkB pathway, which would result in an inflammatory and Th1 microenvironment.

Generation of a sterile and controlled inflammatory response by first-trimester trophoblast cells induces a slow inflammatory response characterized by a modest upregulation of cytokines that might contribute to the trophoblast invasion, tissue remodeling, and embryo implantation [3, 4, 5]. However, an exacerbated inflammatory response during the peri-implantation period, such as that observed in intrauterine infections, is associated with pregnancy complications, such as preterm labor and delivery, intrauterine growth restriction, and preeclampsia [6–8].

In fact, using in vitro and in vivo mouse models, Cardenas et al. [9] demostrated that stimulation of TLR-3 by poly(I:C), a synthetic dsRNA representing viral infection, induces a potent proinflammatory response as well as the production of antimicrobial products that could be responsible for early miscarriage and preterm labor [10]. Moreover, treatment of first-trimester trophoblast cells with the TLR-2 ligand peptidoglycan (PDG) could directly induce trophoblast cell apoptosis, while ligation of TLR-4 by the lipopolysaccharide (LPS) representing a Gram-negative bacterial infection promotes a classical inflammatory response characterized by the induction of cytokine production [11]. Thus, a successful pregnancy depends on a tight homeostatic and temporal control which is provided by redundant circuits of cell-to-cell interaction as well as local mediators targeting multiple cells to sustain the suppressor/tolerant microenvironment [12–14].

The neuropeptide vasoactive intestinal peptide (VIP) has been proposed to have anti-inflammatory and tolerogenic effects based on several in vitro models in human and murine cells and on studies in animal models of viral disease and chronic inflammation [15–21]. Moreover, this neuropeptide promotes secretion in the glandular epithelium and contributes to smooth muscle relaxation and vasodilatation in the uterus [22–24]. At the maternal-placental interface, an increasing amount of evidence on human and murine pregnancy supports an immunomodulatory role of VIP at early stages, i.e. an increase in CD4+CD25+Foxp3+ Treg cells and LIF expression at the implantation site explants of normal mice at day 9 [25]. Consistent with a trophic and suppressor effect of the peptide at these early stages, lower levels of decidual VIP expression were found in viable implantation sites of prediabetic nonobese diabetic (NOD) mice (a high-resorption-rate mouse strain due to an inflammatory background) [25].

As an autocrine regulatory peptide, VIP might contribute to fetal survival growth through two relevant strategies: on one side a direct trophic effect on trophoblast cells and on the other side an immunomodulatory effect that favors tolerance towards trophoblast antigens. VIP regulatory properties were evidenced using coculture systems with an immortalized trophoblast cell line (Swan-71) and peripheral blood mononuclear cells (PBMCs) obtained from fertile women as a representative model of maternal-placental interaction. In this model, VIP increased the frequency of maternal CD4+CD25+Foxp3+ cells, TGF-β expression, and IL-10 secretion. Accordingly, it reduced proinflammatory mediators such as MCP-1, IL-6, and nitric oxide production [26].

Based on the observation that trophoblast cells produce VIP and that it can mediate tolerogenic responses contributing to tissue homeostasis maintenance, in the present work we investigated the effect of VIP at the maternal-placental interface under LPS stimulation. For that purpose, we performed cocultures between trophoblast cells and maternal leukocytes obtained from fertile women as an in vitro model of maternal-placental interaction focusing on modulation of the VIP/VIP receptor ($VPAC_1$ and $VPAC_2$) system under LPS stimulation.

Material and Methods

Reagents and Antibodies

Endotoxin-free reagents and plastic materials were used in all experiments. RPMI-1640 and PBS were purchased from HyClone Laboratories (Logan, Utah, USA). DMEM-F12 and penicillin/streptomycin were purchased from Invitrogen Life Technologies (Grand Island, N.Y., USA). Fetal bovine serum (FBS) was from Natocor (Cordoba, Argentina). Twenty-four-well flat bottom polystyrene plates and ELISA cytokines were from BD Pharmingen (San Diego, Calif., USA). LPS from *Escherichia coli* and Ponceau S staining were from Sigma-Aldrich (St. Louis, Mo., USA).

Fluorescein isothiocyanate (FITC) or phycoerythrin (PE)-conjugated anti-CD4, CD14, CD19, and CD56 and control isotype-matched antibodies (Ab) were from BD Pharmingen.

Peripheral Blood Mononuclear Cells

PBMCs from fertile women, defined as nonpregnant women who had had two or more previous normal pregnancies without any miscarriage, were isolated from heparinized peripheral blood via density gradient centrifugation on Ficoll-Hypaque (Amersham Pharmacia Biotech, Uppsala, Sweden). Cells were extensively washed and resuspended in DMEM-F12 supplemented with 10% FBS, 2 mM glutamine, and penicillin/streptomycin.

The Investigation and Ethics Committee of the Argentinean Society of Gynecological and Reproductive Endocrinology (SAEGRE) approved this study, and all women provided their written consent to participate in it.

Cocultures

Trophoblast cells (Swan-71 cell line, derived from the telomerase-mediated transformation of a 7-week cytotrophoblast isolate described by Straszewski-Chavez et al. [27]) were cultured in 24-well flat bottom polystyrene plates in DMEM-F12 supplemented with 10% FBS, 2 mM glutamine, and penicillin/streptomycin. The cell line was mycoplasma free tested via DAPI staining. For cocultures, trophoblast cells (70% confluent) were cultured in the absence/presence of PBMCs from fertile women (5×10^5 cells/well) with or without VIP (Polypeptide Group, France) (10^{-7} M) or LPS (L4005; Sigma, St. Louis, Mo., USA) (0.1 or 10 μg/ml). After 24 or 48 h of culture, supernatants were collected for ELISA determinations and maternal PBMCs were recovered and then used for flow cytometry, RT-PCR, or Western blot analysis.

Cytokine Quantification

IL-6, MCP-1, and IL-10 were assayed by ELISA in supernatants collected from the cocultures performed in the presence of maternal PBMCs during 48 h. The ELISA test was performed according to the manufacturer's instructions (Becton Dickinson, Franklin Lakes, N.J., USA). Results were expressed as picograms per milliliter.

Nitrite Determination

The nitrite concentration was determined in supernatants obtained as described above for cytokine measurements using the Griess method with N-(1-naphthyl) 9 ethylenediamine dihydrochloride (NEDA) and sulfanilamide [28]. Results were expressed as micromoles per liter of nitrites synthesized for 48 h in the cocultures performed with maternal PBMCs in the absence or presence of VIP and LPS.

Western Blot Assays

Cells recovered from the cocultures were analyzed for TGF-β expression via Western blot. Cells were extensively washed with PBS, and then the cell pellet was mixed gently with 1 ml ice-cold lysis buffer (PBS containing 5 mM EDTA, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 142.5 mM KCl, 5 mM $MgCl_2$, and 10 mM HEPES, pH 7.2) with freshly added protease inhibitor cocktail (0.2 mM PMSF, 0.1% aprotinin, 0.7 μg/ml pepstatin, and 1 μg/ml leupeptin) and incubated for 1 h on ice. Samples were finally centrifuged at 12,000 *g* for 20 min at 4°C and the supernatant fluids, representing the whole cell protein lysates, were stored at –70°C until use.

The protein concentration was estimated using a micro-BCA TM Protein Assay Reagent Kit (Pierce, Rockford, Ill., USA). Equal amounts of proteins were diluted in sample buffer and resolved on 15% SDS-polyacrylamide gel. After electrophoresis, the separated proteins were transferred onto nitrocellulose membranes and probed with a 1:500 anti-TGF-β Ab (R&D Systems, Minneapolis, Minn, USA). Blots were then incubated with a 1:3,000 dilution of an HRP-conjugated anti-rabbit Ab and developed using an enhanced chemoluminescence detection kit (Amersham, Uppsala, Sweden). Equal loading and absence of protein degradation were checked via Ponceau S staining. The immunoreactive protein bands were analyzed with a Fotodyne Image Analyzer® (Fotodyne, Inc., Hartland, Wisc., USA). Results were expressed as relative densitometric values by means of Image J software (NIH, Bethesda, Md., USA) normalized to β-actin expression.

Flow Cytometry Analysis for Intracellular VIP Detection

To assess VIP production, Swan-71 cells and maternal PBMCs, cocultured with Swan-71 cells, were stimulated for 12 or 24 h in the absence or presence of LPS 0.1 or 10 μg/ml, and incubated with Stop Golgi in the last 4 h of culture following the manufacturer's instructions (Becton Dickinson), to promote intracellular accumulation of proteins. After washing with PBS, cells were fixed and permeabilized with a Fix/Perm kit at the manufacturer's recommended concentrations (Becton Dickinson), and incubated for 30 min with rabbit anti-VIP polyclonal Ab (Peninsula-Bachem Inc., San Carlos, Calif., USA) and then washed and incubated with FITC-conjugated anti-rabbit Ab (Santa Cruz, Palo Alto, Calif., USA). Cells were then washed with 2% FBS PBS to allow membrane closure and finally the surface was stained with anti-CD4, CD14, CD19, and CD56 mAb conjugated with PE or PeCy5 fluorochromes.

Ten thousand events were acquired in a FACSAria II® cytometer and the results were analyzed using WinMDI® software. Negative control samples were incubated at the same time with an irrelevant, isotype-matched Ab. Results for positive cells were expressed as a percentage of the respective population.

RT-PCR for VIP, $VPAC_1$, and $VPAC_2$ Expression

Determination of VIP, $VPAC_1$, and $VPAC_2$ expression levels was performed in Swan-71 cells cultured with or without LPS (0.1 and 10 μg/ml) for 0, 6, and 12 h by RT-PCR. Briefly, RNA was obtained using Trizol reagent (Life Technologies, Grand Island, N.Y., USA) and cDNAs were generated using a commercial kit (Clontech; Palo Alto, Calif., USA) and stored at –20°C for batched analyses. For amplification of the resulting cDNA, 2 μl of the RT mixture were used. The sample volume was increased to 25 μl with the solution containing 50 mM KCl, 10 mM Tris (pH 8.3), 2 mM $MgCl_2$, 0.1 μM up- and downstream primers, and 1 U Taq polymerase in a DNA thermocycler (PerkinElmer/Cetus, Boston, Mass., USA). The primers and thermal profile were selected with the software Primer3 as previous reported [26]. In the thermal cycler, after a predenaturation step at 94°C for 5 min, we used a denaturation step at 95°C for 30 s, an annealing step at 60°C for 30 s, and an elongation step at 72°C for 30 s for a total of 35 cycles. An additional extension step at 72°C for 10 min was carried out. PCR products were electrophoresed through a 2% ethidium bromide-stained agarose gel, visualized by transilumination, and scanned. Densitometry was performed and the results were expressed as arbitrary units (AU) normalized to GAPDH expres-

sion. The nomenclature for $VPAC_1$ and $VPAC_2$ also conforms with the Guide to Receptors and Channels (GRAC), 3rd edition [29].

Statistical Analysis

The significance of the results was analyzed by Student's t test and the Mann-Whitney test for nonparametric samples using GraphPad Prism4 software (GraphPad, San Diego, Calif., USA). $p < 0.05$ was considered statistically significant.

Results

VIP Prevents the Upregulation of Inflammatory Mediators Induced by LPS in Trophoblast-Maternal Leukocyte Cross Talk under LPS Stimulation

Since the local response to various inflammatory stimuli at the maternal-placental interface requires strict modulation at the early stages of pregnancy to avoid tissue damage and embryo resorption, we first investigated the effects of VIP on the production of inflammatory mediators during the interaction between trophoblast cells and maternal leukocytes under LPS stimulation. For that purpose, Swan-71 cells (a human cytotrophoblast cell line) were cocultured with maternal PBMCs in the absence or presence of VIP (10^{-7} M) and LPS (10 μg/ml), as this is the reported concentration that induces a proinflammatory response via the upregulation of cytokine production [11]. After 48 h of coculture, the inflammatory mediators involved in the maternal-placental cross talk were evaluated.

We observed that in the cocultures performed in the presence of VIP and LPS, MCP-1 and IL-6 secretion was not increased (fig. 1a, b); the same was observed for nitrite production, which did not increase compared to the condition with LPS alone (fig. 1c). Moreover, no significant differences were found when the cocultures were performed in the presence of VIP alone or VIP plus LPS for the three mediators under study. On the other hand, VIP maintained the levels of IL-10 secretion and TGF-β expression in maternal PBMCs after 48 h of interaction with trophoblast cells in the presence of LPS (fig. 1d, e). As an additional control, we also investigated the mentioned mediators in Swan-71 cells in the absence or presence of LPS.

Taken together, these data suggest that VIP contributes to controlling the inflammatory response in trophoblast-maternal leukocyte cross talk under LPS stimulation, preventing the increase of inflammatory mediator production and maintaining the levels of IL-10 production and TGF-β expression.

LPS Increases VIP Production in Maternal CD4 T Lymphocytes after the Dialogue with Trophoblast Cells

Taking into account that VIP could act as a homeostatic factor limiting the inflammatory response at the early stages of the immune-trophoblast cell interaction in the presence of LPS, we investigated the effect of LPS on the maternal leukocyte response during the interaction with trophoblast cells. Therefore, we analyzed whether LPS could modulate VIP expression in maternal PBMCs after the dialogue with Swan-71 trophoblast cells in the absence or presence of LPS (10 μg/ml). After 24 h, suspension cells were recovered and VIP production was quantified in the CD4+ T lymphocytes, CD19+ B lymphocytes, CD14+ monocytes, and CD56+ NK cells via FACS analysis. As shown figure 2, the main source of VIP production in the maternal leukocytes after trophoblast interaction was the CD4+ cell population. Figure 2b displays representative dot plots showing the percentage of CD4+VIP+ cells in the absence or presence of LPS.

We did not detect CD19+VIP+, CD56+VIP+, or CD14+VIP+ cells after cocultures of maternal PBMCs with trophoblast cells.

LPS Modulates VIP and $VPAC_2$ Expression in Trophoblast Cells

To provide more insight into the mechanisms by which VIP could contribute as a homeostatic mediator at the placental-maternal interface, we focused on the effects of LPS on VIP/VPACs expression directly on trophoblast cells. Moreover, we investigated whether the VIP/VPAC system is being modulated after 6 and 12 h of stimulation with two concentrations of LPS (0.1 and 10 μg/ml). We used these reported concentrations because they allow the production of pro- and anti-inflammatory cytokines by trophoblast cells, maintaining their survival [11, 30].

We could observe that VIP expression in trophoblast cells increased in a time-dependent manner after stimulation with LPS at both concentrations tested (fig. 3a–d). The present results were also confirmed by FACS analysis after 12 and 24 h of stimulation with LPS in both concentrations. Figure 3f shows the percentage of VIP+ Swan-71 cells, and figure 3e shows representative histogram profiles of VIP intracellular production after LPS stimulation.

Next, we investigated the effect of LPS on VIP receptors $VPAC_1$ and $VPAC_2$. As shown in figure 4a–h, LPS significantly increased the expression of $VPAC_2$ but did

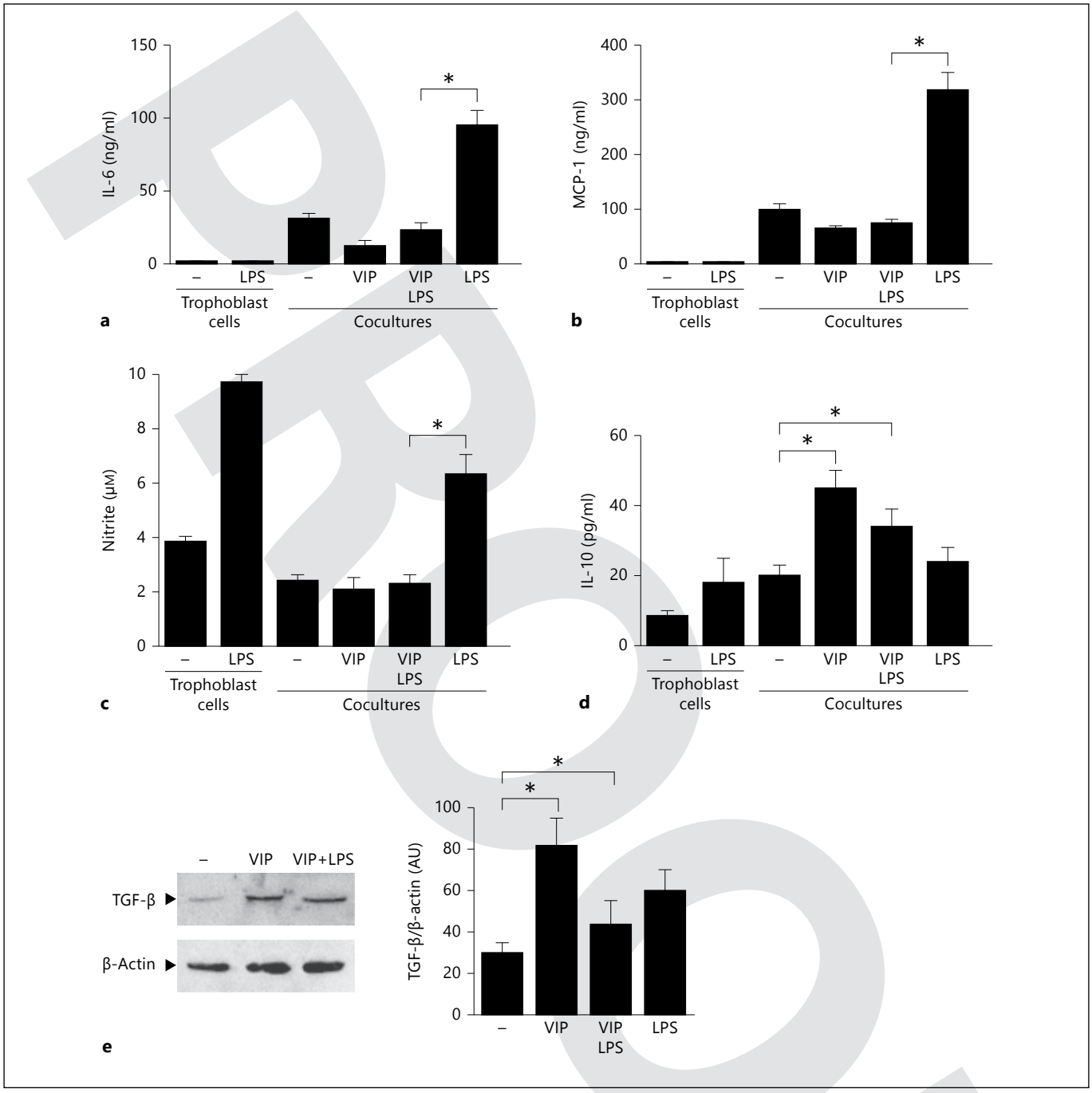

Fig. 1. VIP prevents the upregulation of inflammatory mediators induced by LPS into trophoblast-maternal leukocyte cross talk under LPS stimulation. The Swan-71 cell line was cultured with or without maternal PBMCs in the absence or presence of VIP (10^{-7} M) and LPS (10 μg/ml). After 48 h of culture, supernatants were collected and IL-6 (**a**), MCP-1 (**b**), and IL-10 (**d**) were quantified by ELISA. Nitrite production was quantified via the Griess method (**c**) and TGF-β expression was quantified via Western blot (**e**). Representative immunoreactive bands are shown and semi-quantification expressed relative to β-actin in AU is shown by the bars. Results are representative of 3 independent experiments using different fertile PBMCs (* $p < 0.05$, Mann-Whitney test).

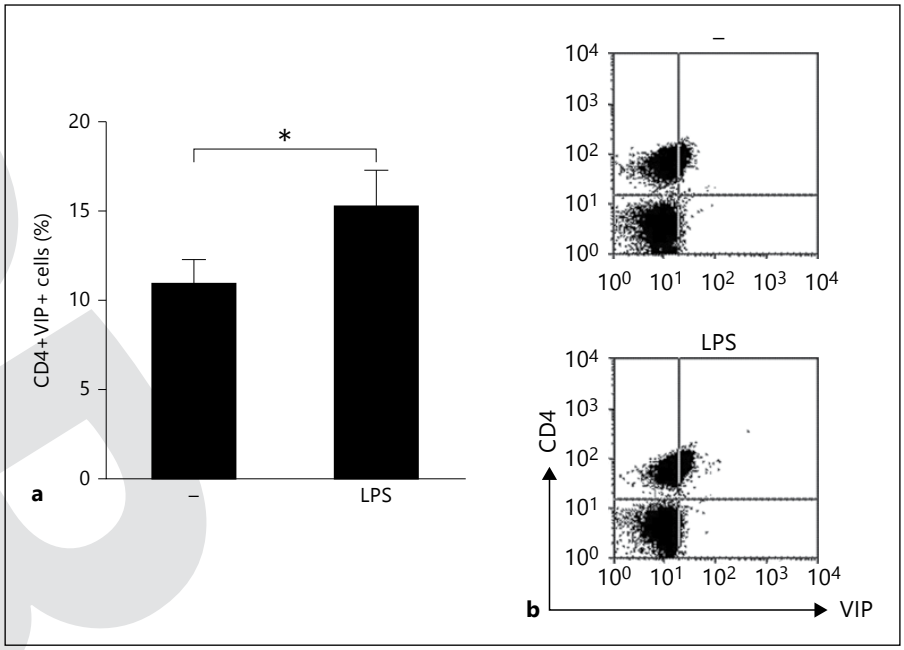

Fig. 2. LPS increases VIP production in maternal CD4+ T lymphocytes after interaction with trophoblast cells. Swan-71 cells at 70% confluence in a 24-well flat bottom plate were cultured in the presence of PBMCs from fertile women in the absence or presence of LPS (10 μg/ml). After 24 h of culture, cells in suspension were recovered and double stained for surface CD4, CD19, CD56, and CD14 and intracellular VIP production and analyzed by FACS. **a** Percentage of CD4+VIP+ cells in the absence or presence of LPS after interaction with trophoblast cells. **b** Representative dot plots of PBMCs from fertile women. Results are expressed as means ± SEM of 5 different fertile women (* $p < 0.05$, Mann-Whitney test).

not modulate $VPAC_1$ expression. In addition, the effect of LPS on $VPAC_2$ expression was time and concentration dependent within the first 6 h (fig. 4e–h).

The present data suggest that, in the presence of LPS, trophoblast cells may be more sensitive to the stimulation of VPAC receptors through the activation of autocrine mechanisms.

Discussion

The results presented herein provide evidence of a potential homeostatic autocrine mechanism mediated by VIP at the maternal-placental interface. Our results indicate that LPS modulates VIP/VPAC system expression in immune and trophoblast cells, as an approach to both sides of the maternal-placental cross talk.

The first stages of a normal pregnancy are characterized by a proinflammatory response that is finely regulated by various immune circuits [1–5]. As expected, when an exacerbated proinflammatory response or an infection precedes implantation or is established early on in pregnancy and homeostatic mechanisms are exceeded, pregnancy complications such as abortion, preeclampsia, intrauterine growth restriction, and preterm labor may appear [6–8, 31]. The results shown here support that VIP may contribute to the control of the deleterious and exacerbated inflammatory response in the interaction between maternal leukocytes and trophoblast cells under LPS stimulation, as an additional mechanism to prevent tissue damage.

The addition of LPS at 10 μg/ml in the in vitro model of maternal-placental interaction targeted not only the maternal CD4+ cells but also the trophoblast cells. In this sense, a direct effect was observed in Swan-71 cells via a significant increase in VIP and $VPAC_2$ expression. On the other hand, LPS also increased VIP production by maternal CD4+ T lymphocytes after interacting with trophoblast cells, which helped to decrease inflammatory mediators such as MCP-1, IL-6, and nitrite production. In this sense, Martinez et al. [32] showed that VIP is produced and secreted by lymphocytes after their stimulation with LPS, TNF-α, or IL-1β and could influence the differentiation and/or downregulation of the ongoing response.

The initial inflammatory response, during the early stages of implantation, with migration of immune cells to the maternal-fetal interface is concomitant with trophoblast cells breaking the epithelial lining of the uterus in order to adhere to and invade the endometrial tissue, as well as the replacement of vascular smooth muscle of the uterine spiral arteries [33]. However, trophoblast cells, just like innate immune cells, could also be able to recognize PAMPs from a viral or bacterial infection and may generate very distinct patterns of responses depending on the type of stimuli and, therefore, the specific TLR

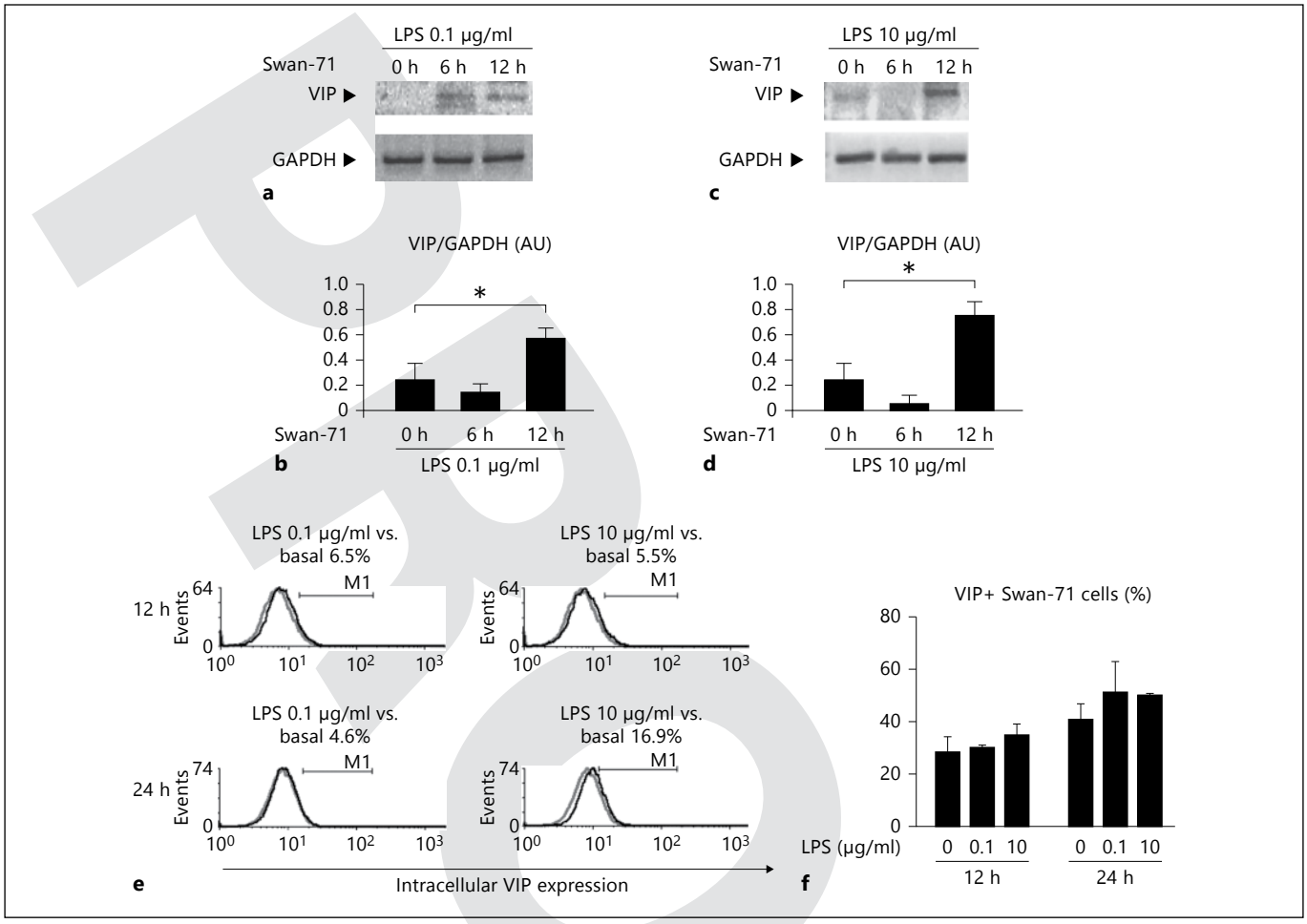

Fig. 3. LPS induces VIP expression on trophoblast cells. The Swan-71 cell line was cultured in the absence or presence LPS 0.1 µg/ml (**a**, **b**) and 10 µg/ml (**c**, **d**), and after 0, 6, and 12 h cells were recovered and VIP expression was quantified by RT-PCR. Representative amplification bands are shown in **a** and **c** and the semiquantification of bands from at least 3 assays expressed as AU relative to GAPDH (mean ± SEM) is shown in **b** and **d** (* $p < 0.05$, Mann-Whitney test). VIP production by trophoblast cells was performed via intracellular staining and FACS analysis. **f** Frequency of VIP+ Swan-71 cells after 12 or 24 h of stimulation with LPS 0.1 and 10 µg/ml. **e** Representative histogram profiles of VIP production after LPS stimulation.

that is activated [10]. In a murine pregnancy model, LPS administration was shown to change the cytokine profile via an increasing maternal serum concentration of TNF-α and IL-6, as well as placental expression of TNF-α, IL-6, and IL-1a, implying that both systemic and local inflammatory responses followed by LPS administration may cause preterm labor and septic abortion [31, 34, 35]. In addition, LPS increased IL-8 and IL-6 production and decreased extra villous trophoblast invasion through the activation of mitogen-activated protein kinase (MAPK) signaling and the MEK1/2 (MAPK extracellular signal-regulated kinase) activation contributing to placental dysfunction [36].

In the present work, we tested two concentrations of LPS (0.1 and 10 µg/ml) on trophoblast cells, both acting on the induction of $VPAC_2$ receptors and also inducing VIP expression with a peak of expression of the peptide at 12 h. Even though the differences in VIP protein production were not significant, we observed the same effect using FACS analysis. These differences could be explained by different regulation mechanisms at the gene and protein level. The fact that LPS increased VIP production and its receptor $VPAC_2$ through a direct effect on trophoblast cells strongly suggests that it could sensitize the VIP/VPAC autocrine circuit at the immune-trophoblast interaction contributing to embryotrophic and local immuno-

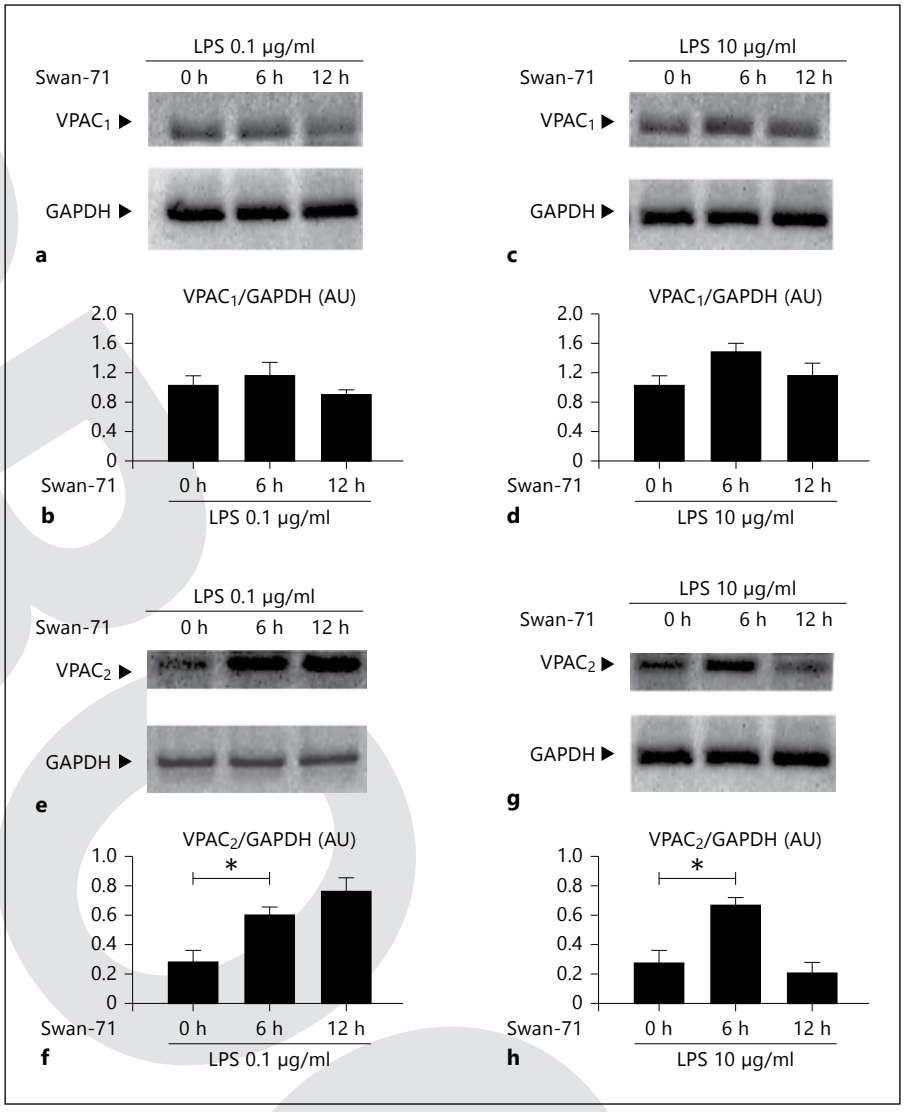

Fig. 4. LPS induces $VPAC_2$ expression on trophoblast cells. The Swan-71 cell line was cultured in the absence or presence 0.1 and 10 µg/ml LPS, and after 0, 6, and 12 h cells were recovered and $VPAC_1$ (**a–d**) and $VPAC_2$ expression (**e–h**) was quantified via RT-PCR. Representative amplification bands are shown in **a**, **c**, **e**, and **g** and the semiquantification of bands from at least 3 assays expressed as AU relative to GAPDH (mean ± SEM) is shown in the **b**, **d**, **f**, and **h** (* $p < 0.05$, Mann-Whitney test).

modulatory effects. In this sense, Uh et al. [37] reported that LPS induces corticotrophin-releasing hormone (CRH) expression in trophoblast cells through the MyD88 intracellular pathway. Interestingly, in this in vitro model of choriocarcinoma cell line JEG-3, LPS, chlamydial heat shock protein 60, and IL-1 induced the expression of CRH, a factor that is produced by the placenta to determine the timing of the onset of parturition in humans [38].

Moreover, our results are consistent with previous data obtained in other first trimester trophoblast cell lines, such as 3A and HTR8, in which ligation of TLR-4 by LPS stimulation increased pro- and anti-inflammatory cytokine production and maintained trophoblast cell survival [11]. The proposed homeostatic effects of VIP in the maternal-fetal interface shown here agree with previous results in several acute and chronic inflammatory processes [39–42]. In this sense, the ability of an inflammatory stimulus to trigger an anti-inflammatory response is a well-known strategy to maintain the homeostasis of the immune system. As an example, macrophages can be activated through TLR by different inflammatory stimuli, responding with the production of several inflammatory mediators. However, a switch to a suppressor phenotype of the macrophage during gestation is needed and the impairment of these regulatory loops is associated with immunotolerance loss during gestation. In line with this, a role of VIP in macrophage phenotype switch has been

proposed in normal pregnant mice and in NOD [39, 43, 44]. Accordingly, in the NOD strain of mice, at the prediabetic stage, failure of homeostatic control involves lower levels of VIP in the fetal-maternal interface and a local reduction of anti-inflammatory/suppressant markers [44, 45].

Inflammatory disorders account for a significant percentage of reproductive failures and pregnancy complications as they affect the trophoblast and trophoblast-endometrial interactions [46, 47]. The results presented herein provide experimental evidence of the effects of LPS on the VIP/VPAC system in immune-trophoblast cell interactions as a potential homeostatic autocrine mechanism during the early stages of maternal-placental cross talk that would be central for the switch among certain phenotypic profiles of surrounding cells.

Acknowledgments

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