


Effect of pregnancy hormone mixtures on cytokine production and surface marker expression in naïve and LPS-activated THP-1 differentiated monocytes/macrophages

Innate Immunity
2020, Vol. 26(2) 84–96
© The Author(s) 2019
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/1753425919864658
journals.sagepub.com/home/ini


María Isabel Mendoza-Cabrera¹,
Rosa-Elena Navarro-Hernández², Anne Santerre³,
Pablo Cesar Ortiz-Lazareno⁴, Ana Laura Pereira-Suárez¹  and
Ciro Estrada-Chávez⁵

Abstract

In pregnancy, maternal monocytes and macrophages acquire a specific phenotype that enables them to maintain immune tolerance and facilitate hormone-immune cell interactions, which are necessary for gestational progression. The aim of this study was to determine the effect of pregnancy hormone mixtures of the first and third trimesters on both resting and activated monocytes and macrophages. Pregnancy hormone levels (cortisol, estradiol, progesterone, and prolactin) were quantified at the first and third trimesters. The average of the levels obtained was used to prepare two mixtures of synthetic hormones: low and high. These mixtures were then used to stimulate THP-1 monocytes and macrophages, resting or activated with LPS. Cytokine production in the culture supernatants and surface marker expression (CD14, CD86, and CD163) were evaluated by ELISA and flow cytometry, respectively. We found that the hormones modulated the pro-inflammatory response of THP-1 cells, LPS-activated monocytes, and macrophages, inducing high levels of IL-10 and low levels of IL-8, IL-1- β , and IL-6. All hormone stimulation increased the CD163 receptor in both resting and LPS-activated monocytes and macrophages in a dose-independent manner, unlike CD14 and CD86. Pregnancy hormones promote the expression of the markers associated with the M2-like phenotype, modulating their pro-inflammatory response. This phenotype regulation by hormones could be a determinant in pregnancy.

Keywords

Surface marker CD163, monocyte phenotype, macrophage phenotype, pregnancy hormones

Date Received: 21 November 2018; revised: 27 March 2018; accepted: 27 June 2019

Introduction

Human pregnancy is characterized by the production of placental, maternal, and fetal hormones that allow the development of a new individual by regulating implantation and uterine quiescence, culminating in the onset of labor. During gestational progression, hormone interactions with immune cells are necessary to avoid the rejection of paternal fetal Ags, achieve proper trophoblast invasion, decrease myometrial contractility, and permit the action of inflammatory mediators at delivery.¹

As the pregnancy develops, monocytes acquire a specific phenotype that enables them to maintain

¹Departamento de Fisiología, Universidad de Guadalajara, Centro Universitario de Ciencias de la Salud, Mexico

²Universidad de Guadalajara, Centro Universitario de Ciencias de la Salud, Instituto de Investigación en Reumatología y Sistema Músculo-Esquelético, Mexico

³Departamento de Biología Celular y Molecular, Universidad de Guadalajara, Centro Universitario de Ciencias Biológicas y Agropecuarias, Mexico

⁴División de Inmunología, Instituto Mexicano del Seguro Social, Centro de Investigación Biomédica de Occidente, Mexico

⁵Unidad de Biotecnología Médica y Farmacéutica, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, Mexico

Corresponding author:

Ciro Estrada-Chávez, iTRANS, Av. José Parres Arias # 5, Rinconada de la Azalea, Industrial Belenes, 45150 Zapopan, Jalisco, México.
Emails: proyectos.itrans@cucei.udg.mx; estradaciro@hotmail.com



immune maternal tolerance. In the first trimester of normal pregnancy, monocytes possess higher membrane expression of CD200, enabling the transmission of inhibitory signals to hematopoietic cells.² The phagocytic function also decreases, suggesting an important immunomodulatory role in the maternal–fetal interface.³

Hence, monocyte and macrophage plasticity in response to this microenvironment provides these cells with the ability to display inflammatory functions for defense against pathogens, as well as immunomodulatory and angiogenic activities during the resolution of inflammation and tissue-damage repair.⁴ Their function and phenotype regulation may be modulated by changes in the endocrine system during pregnancy, since they possess receptors for prolactin and steroid hormones.^{5–8}

In pregnancy, cortisol, estradiol, progesterone, and prolactin serum levels progressively increase,¹ while human chorionic gonadotropin (hGC) gradually decreases.⁹ Since the effects of hormones on immune response are concentration-dependent, increased estrogen levels suppress inflammation, while low levels have been associated with pro-inflammatory effects post menopause.¹⁰ In addition, cortisol at a diurnal concentration (5–10 µg/dl) exerts inflammatory effects; on the other hand, under stress concentrations (35–45 µg/dl) and pharmacologic concentrations (70–80 µg/dl), it achieves its maximal anti-inflammatory effect.¹¹

Previous reports have shown that during pregnancy, the loss of normal function of monocytes and macrophages is associated with different pathologies. For example, the monocytes of patients with pre-eclampsia polarize toward the M1 phenotype because they possess high expression of TLR4, CD64, and inflammatory cytokines, in addition decreasing the synthesis of IL-10 as well as D206 and CD163 surface markers, compared to normotensive pregnant women.¹²

Similarly, in the basal decidua of pregnant women with pre-eclampsia and preterm delivery, the number of M2 macrophages and the CD163:CD14 ratio decreased compared to normotensive and preterm pregnancies. This suggests that a smaller amount of M2 macrophages may contribute to the development of pre-eclampsia.¹³

Therefore, alterations in the pregnancy hormone mixture, as well as in monocyte and macrophage phenotypes, could give rise to pathologies such as pre-eclampsia and premature labor,^{12,14–16} suggesting that phenotype regulation of monocytes and macrophages by hormones could be a determinant in pregnancy. The aim of this study was to determine the effect of pregnancy hormone mixture in the first and third trimesters on both resting and activated monocytes and macrophages.

Material and methods

Experimental design

In order to evaluate the effect of hormones associated with pregnancy on the phenotype of monocytes and macrophages either resting or activated by the inflammatory stimulus (LPS), we performed treatments on cultures of monocytes and macrophages derived from THP-1 cells, with hormone mixtures mimicking low and high concentrations present during the first and third trimesters. The hormonal treatment was applied to both resting and activated cells.

First, for hormonal stimulation, we determined the concentration levels of cortisol, estradiol, progesterone, and prolactin taken from the sera of pregnant women during the first and third trimesters of their second pregnancy. According to the confidence interval, we designed low and high doses for the two stages (Table 1). Second, we differentiated THP-1 cells toward a macrophage phenotype and also obtained

Table 1. Serum hormone levels in women in the first and third trimesters.

Hormones	Levels in first trimester			Levels in third trimester		
	Pregnancy	Representative values ^a		Pregnancy	Representative values ^a	
		Low	High		Low	High
Cortisol* (nmol/l)	129 (65–194)	60	200	276 (205–348)	200	350
Estradiol* (pg/ml)	1.3 (1.1–1.6)	1.0	1.5	18.6 (16.8–20.5)	17.0	20.0
Progesterone* (ng/ml)	26.8 (10.6–42.9)	10.0	40.0	107.4 (44.3–170.4)	40.0	170.0
Prolactin* (µIU/ml)	41.7 (28.8–54.6)	30.0	55.0	223.8 (110.5–337.0)	100.0	300.0

Data shown are the mean (95% confidence interval).

*Difference between pregnancy group in the first trimester and the third trimester; Bonferroni test, $P < 0.0001$.

^aRepresentative levels designed to stimulate THP-1 cells *in vitro*. The hormone levels were determined in sera from pregnant women during two stages of their second pregnancy.

activated monocytes and macrophages with LPS stimulation. Third, we analyzed cell membrane receptors, cytokine profile, and nitrite and polyamine production from cells and supernatants in each of the conditioned cell cultures, performed with or without hormonal stimulation.

Serum samples from pregnant women

Thirty healthy pregnant women in the first and third trimesters of their second pregnancy (age range 20–35 yr; body mass index ≤ 27 kg/m²) from the Hospital Materno-Infantil, Secretaría de Salud Jalisco in Guadalajara, Mexico, were included. We screened each participant with routine laboratory tests. All participants abstained from taking medication and signed an informed consent. The study was approved by a local ethics committee (office no. 7282. 18/UG-JAL/2008) and was conducted according to the Declaration of Helsinki guidelines.

Pregnancy hormone assessment and profile design

To establish hormone reference ranges, blood samples were taken in the middle of the trimester between 8:00 am and 9:00 am following a 12 h fasting period. The blood was allowed to clot at room temperature and was centrifuged at 1509 RCF (Rotanta 460R; Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany) for 10 min at 20°C. Serum was collected and stored at –80°C until further analysis.

The serum concentration of cortisol, estradiol, progesterone, and prolactin was performed with Elecsys Enzyme Immunoassay II Roche kits, with a detection limit of 415 nmol/l, 5 pmol/l, 0.03 ng/ml, and 1.00 μ IU/m, respectively, using Roche Modular Analytics 917 (Roche Diagnostics, Indianapolis, IN), following the manufacturer's instructions.

For preparation of the hormone mixtures, the manufacturer's recommendations were followed (Sigma–Aldrich, St. Louis, MO): 200 mg/ml hydrocortisone in 4 ml chloroform:ethanol (1:1) solution, 50 mg/ml β -estradiol in ethanol, 50 mg/ml of progesterone in chloroform, and 100 μ g/ml of PRL in sterile 4 mM HCl. Taking these solutions into account, the corresponding concentrations were prepared for each treatment in accordance with the levels of the hormones determined in the sera of the pregnant women in this study (Table 1).

THP-1 cell line culture and stimulation

As a model for monocytes, the THP-1 cell line (number TIB-202; American Type Culture Collection, Manassas, VA) was used, realizing the cell culture within RPMI 1640 culture media, which was

supplemented with 10% inactivated FBS and 1% antibiotic-antimycotic (Life Technologies, Grand Island, NY).

In order to obtain activated monocytes, THP-1 cells were exposed to 1 μ g/ml LPS from *Escherichia coli* serotype 0111:B4 (Sigma–Aldrich) for 48 h. The THP-1 cells (resting monocytes) and the activated monocytes that were obtained were seeded at a density of 7×10^5 cells/ml on a six-well plate, either without hormonal stimulus (W/S) or with hormonal stimulus (i.e. LLH (low) and HLH (high) hormone concentrations in the first trimester or third trimester). To obtain macrophages, 3×10^6 THP-1 cells were differentiated with 200 nM phorbol myristate acetate for 3 d, followed by resting culture with fresh RPMI for 5 d.¹⁷ Thereafter, the macrophages that were obtained were exposed to the same treatments as previously described for monocytes. All cell cultures were carried out in a humidified atmosphere at 37°C and CO₂ 5%, in triplicate for each condition.

Next, we evaluated the effect of the pregnancy hormone mixture on cytokine production and surface marker expression in resting and activated monocytes and macrophages derived from THP-1 cells with measurements detailed below.

Flow cytometry: surface marker expression of CD14, CD86, and CD163

The following reagents and mAbs were used:

- Fixable Near-IR Dead Cell Staining Kit (Life Technologies) subsequently excluding dead cells from the analysis;
- Fc receptors blocked with 5 μ l human TruStain FcX™ (BioLegend, San Diego, CA) to block Fc receptors and avoid nonspecific binding of Abs to these membrane receptors; and
- anti-human primary Abs (BioLegend; PE anti-human CD86 Ab (305406) clone IT2.2, PerCP/Cyanine5.5 anti-human CD14 Ab (325622) clone HCD14 and PE anti-human CD163 Ab (333606) clone GHI/61) and the respective isotype (PE Mouse IgG2b κ Isotype Ctrl Ab (400314) clone MPC-11, PerCP/Cy5.5 Mouse IgG1, κ Isotype Ctrl Ab (400149) clone MOCP-21 and PE Mouse IgG1, κ Isotype Ctrl Ab (400111) clone MOPC-21) was employed in each test to adjust for background fluorescence, and we performed fluorescence minus one (FMO) and staining controls to establish the parameters.

Three panels were made: the first with the Fixable Near Kit plus CD163-PE, the second with the Fixable Near Kit plus CD86-PE, and the third with

the Fixable Near Kit plus CD14-PerCP-Cy5.5. Isotype control was used in each test to adjust the background fluorescence. A FMO was also performed to define the positive and negative for each marker. The cells of the different experimental groups were harvested, washed twice with PBS, re-suspended in 100 μ l Fixable Near, and incubated at room temperature for 30 min. Subsequently, two washes were performed with 2 ml PBS, the cells were re-suspended in 100 μ l PBS, and 5 μ l human TruStain FcXTM (BioLegend) was added and incubated for 5 min. Then, 10 μ l of each of the Abs were added, as previously mentioned. The cells were incubated at room temperature in the dark for 30 min. Then, they were washed with PBS and centrifuged at 560 *g* for 7 min. The supernatant was decanted, and the cells were re-suspended in 500 μ l PBS and then analyzed in a FACSAria ITM cell sorter (BD Biosciences, San Jose, CA). In a first dot plot (FSC-A vs. FSC-H), doublets were discarded. Later, in a second dot plot (SSC-A vs. Live/Dead), a gate was made in the live cells (negative for the staining of the Live/Dead dye), and finally, in a histogram, the expression of CD14-PerCP-Cy5 was analyzed. In the expression of CD86-PE or CD163-PE or each sample, at least 20,000 viable cell region events were acquired (negative for Live/Dead). Data were processed with FlowJo vX.0.7 (Tree Star, Inc., Ashland, OR), and the results are reported as geometric mean fluorescence intensity and represent the mean \pm SD of three independent experiments carried out in triplicate.

Gating controls were utilized to adjust for background fluorescence, and the results are reported as % of expression and geometric mean fluorescence intensity. For each sample, at least 10,000 events were acquired in a FACSAria ITM cell sorter (BD Biosciences). Data were processed with FlowJo vX.0.7 (Tree Star, Inc.).

Quantitation of cytokine production

Cytokine assays were also conducted on supernatants of the cell cultures, which were harvested and stored at -80°C until analysis. Levels of cytokines were measured according to the manufacturer's instructions using a quantitative ELISA kit. Sensitivity and range (pg/ml) of IL-8 (0.4, 1.0–64) and IL-10 (0.17, 0.8–50; R&D Systems, Minneapolis, MN) and high sensitivity TNF- α (7.8–500), IL-1 β (2.0–125), IL-6 (7.8–500), and IL-12/IL-23 (p40; 62.5–4000; BioLegend) were determined.

Measurement of NO concentration

Analysis of nitrite production by the Griess reaction was performed on a 96-well plate, to which 100 μ l culture supernatants was added and mixed with 100 μ l Griess reagent, and a series of solutions with known

concentrations of sodium nitrite (Sigma–Aldrich, Inc., Merck KGaA, Darmstadt, Germany) prepared in the same media were employed to construct a standard curve. Then, these were incubated for 15 min in the dark and measured for optical density at 540 nm using an xMarkTM Microplate Spectrophotometer. Data were analyzed with Microplate Manager v6.1 (Bio-Rad Laboratories, Inc., Hercules, CA).

Measurement of polyamine synthesis

The analysis of the polyamine concentration of the cultured cells was performed by HPLC analysis, the ion-exchange principle was applied, and post-column derivatization employing ortho-phthaldialdehyde (Sigma–Aldrich, Inc.) and fluorescence detection (Shimadzu RF-530 Fluorescence Detector; Shimadzu, Kyoto, Japan) was utilized. In order to deproteinize the sample, the cells were treated with a solution containing 5% trichloroacetic acid (Merck) and 0.05 N hydrochloric acid (Mallinckrodt Baker, Edo. de México, México) at a ratio of 1:2 v/v, which was then vortexed and allowed to stand at room temperature for 20 min. The mix was centrifuged at 9200 *g* for 20 min (Spectrofuge 24D; Labnet International, Edison, NJ), and the supernatant was filtered using syringe-driven 0.20 μ m filters (Millex-LG; Millipore Corp., Bedford, MA). As an internal standard, 25 nmol/100 μ l 1,7-diaminoheptane (Sigma–Aldrich, Inc.) was employed. An autosampler (model AS3000; Spectra-Physics Analytical, Darmstadt, Germany) equipped with a 20 μ l injection loop was utilized to inject the samples into the HPLC system, which consisted of a Waters pump (515; Waters, Milford, MA) and a reverse phase C18 column (150 mm \times 4.6 mm; Inertsil[®] ODS-2, with a 5 μ m particle diameter; Varian GL Sciences, Inc., Walnut Creek, CA). The mobile phase consisted of a gradient of four aqueous buffers containing 1-octane sulfonate sodium salt (Caledon Laboratories Ltd., Georgetown, Canada), sodium acetate (Merck), and acetonitrile (Mallinckrodt Baker), as described by Farkas and Hajós,¹⁸ with a few modifications in the gradient elution program. The flow rate was set at 0.5 ml/min. The compounds were identified according to their retention times and quantified by comparing them to the peak areas of two known commercially available standards – spermidine and spermine (Sigma–Aldrich) – using the Varian Galaxy Chromatography Data Acquisition System v1.9 SP2b (Varian GL Sciences, Inc.). The retention times of spermidine and spermine were 43 and 45 min, respectively. Each sample was analyzed in triplicate.

Statistical analysis

Data were analyzed with GraphPad Prism v6.01 (GraphPad Software, Inc., La Jolla, CA). Two-way ANOVA followed by the Bonferroni post hoc test was performed. All data were plotted in graphs as the mean \pm SEM. Significance was defined as $P \leq 0.05$.

Results

The immune phenotype of monocytes and macrophages derived from THP-1 cells stimulated with low and high levels of pregnancy hormones possessed increased amounts of membrane-expressed markers, namely:

1. The levels of CD14, CD86, and CD163 membrane expression on cells with stimulation of pregnancy hormones were higher than those without (Figure 1).
2. We detected higher CD86 and lower CD163 membrane expression on resting THP-1 monocytes without pregnancy hormone stimulation than membrane expression of resting THP-1 monocytes with pregnancy hormone stimulation (Figure 1a). On the other hand, activated THP-1 monocytes showed a decrease in CD14 and CD86 and an increase in CD163 membrane expression on cells with pregnancy hormone stimulation than those without (Figure 1b).
3. Increased CD163 membrane expression was identified in resting and activated macrophages with pregnancy hormone stimulation compared to those without (Figure 1c and d).
4. Higher CD163 membrane expression was found in resting and activated monocytes with a high level of pregnancy hormone stimulation in the third trimester compared to low-level pregnancy hormone stimulation in the first trimester (Figure 1a and b). In addition, higher CD163 and CD14 expression was determined in resting monocytes with low-level pregnancy hormone stimulation in the third trimester than in the first trimester with low-level pregnancy hormone stimulation, as shown in Figure 1a.

The production of TNF- α , IL-1 β , IL-6, IL-12, IL-8, and IL-10 released by LPS-activated monocytes and macrophages derived from THP-1 cells and from activated monocytes and macrophages were evaluated.

The following differences were observed. With pregnancy hormone stimulation, both activated monocytes and macrophages showed opposing levels of pro-inflammatory and anti-inflammatory cytokines. The amounts of pro-inflammatory cytokines were reduced when the cells were cultured with pregnancy hormones (Figure 2a–e), with the exception TNF- α in the activated monocytes, which was increased (Figure 2a). Meanwhile, the anti-inflammatory cytokine IL-10 was

increased in activated macrophages and decreased in activated monocytes (Figure 2f).

In activated macrophages, with or without pregnancy hormone stimulation, the levels of TNF- α were around 100 times higher, and the levels of IL-1 β and IL-10 were almost 10 times higher than in activated monocytes (Figure 2a, b, and f, respectively). In IL-6, IL-12, and IL-8 levels, an inverse range was observed that was nearly 10 times lower in activated monocytes than in activated macrophages (Figure 2c, d, and e, respectively).

In addition to other important differences shown in Figure 2, interestingly we noted two scenarios: in both activated THP-1 monocytes stimulated with low levels of pregnancy hormones in the third trimester and high levels of pregnancy hormones in the first and third trimesters and activated THP-1 macrophages stimulated with a high level of pregnancy hormones in the third trimester, decreased levels of TNF- α , IL-6, and IL-12 were identified (Figure 2a, c, and d, respectively). However, IL-1 β levels remained constant in activated THP-1 monocytes and increased in activated THP-1 macrophages (Figure 2b).

IL-8 and IL-10 production of resting monocytes and macrophages showed an opposite profile after pregnancy hormone stimulation.

When cells were exposed to pregnancy hormones, only stimulation of IL-8 and IL-10 was detected in the resting monocytes and macrophages (Figure 3). As shown in Figure 2e, activated monocytes and macrophages produced just about 100 times more IL-8 than resting monocytes, with or without pregnancy hormone stimulation.

Simultaneously, the production of IL-8 after stimulation with high amounts of pregnancy hormones in the third trimester was different in activated monocytes (lower) and resting macrophages (higher; Figures 2e and 3a, respectively).

Compared to cells without pregnancy hormone stimulation, the production of IL-8 in resting monocytes and macrophages only decreased when these were cultured with low and high levels of pregnancy hormones in the first trimester (Figure 3a).

In particular, the production of IL-10 by resting and activated monocytes gave the same profile, while in activated macrophages, it was 10 times more than in resting macrophages. On the other hand, IL-10 decreased when resting monocytes were stimulated with the pregnancy hormone mixtures, while it increased in the resting macrophages (Figure 3b).

The production of IL-8 in resting macrophages (Figure 3a) and of IL-10 in activated macrophages (Figure 2f) after pregnancy hormone stimulation in the third trimester contrasted with the first trimester. Moreover, with low levels in the third trimester,

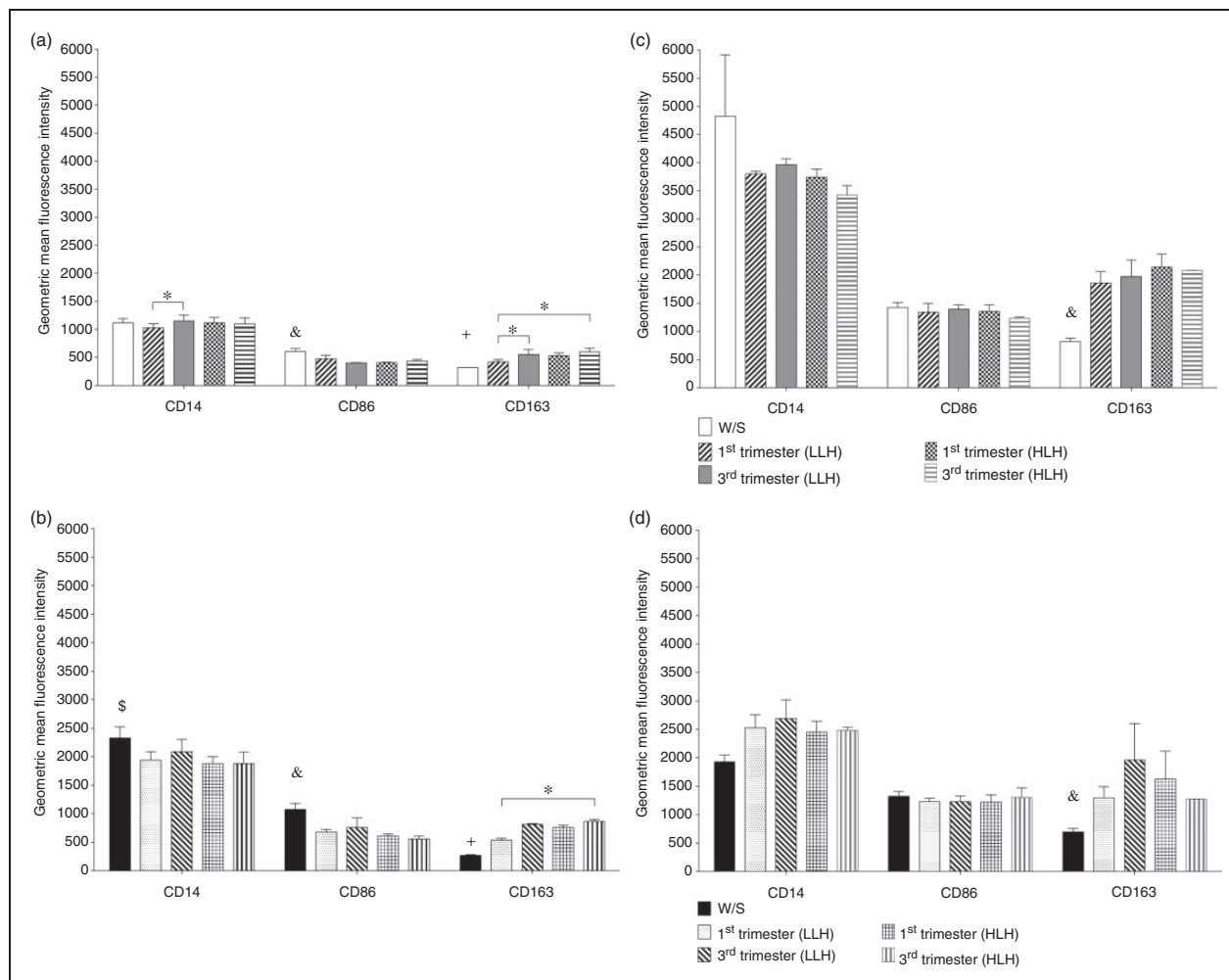


Figure 1. CD14, CD86, and CD163 surface expression on resting and activated monocytes and macrophages derived from THP-1 cells after pregnancy hormone stimulation. (a) Resting THP-1 monocytes, (b) activated THP-1 monocytes, (c) resting THP-1 macrophages, and (d) activated THP-1 macrophages. CD14, CD86, and CD163 flow cytometry analysis of cells stimulated with low and high levels of pregnancy hormones in the first and third trimesters. Bars represent the mean \pm SEM fluorescence intensity. * $P < 0.05$, W/S versus all pregnancy hormone mixture stimulation; ⁺HLH in the first and third trimesters and LLH in the third trimester, and HLH in the first and third trimesters, and LLH in the first trimester. All results were confirmed in triplicate in three independent experiments under the same experimental conditions. W/S: without pregnancy hormone stimulation; LLH: low levels of pregnancy hormone stimulation; HLH: high levels of pregnancy hormone stimulation.

stimulation was higher than the high levels in the first and third trimesters.

Effect of low and high levels of pregnancy hormones on nitrite and polyamine production in resting and activated monocytes and macrophages

Figure 4 summarizes the results of two different experiments. First, we found that nitrite production was increased when resting monocytes were stimulated with high levels of pregnancy hormones in the first and third trimesters. In addition, activated monocytes show reduced nitrite production after stimulation with high levels of pregnancy hormones in the first versus the

third trimesters and after stimulation with a low level of pregnancy hormones in the first trimester (Figure 4a).

Second, the determination of cellular polyamine concentrations by HPLC revealed that in activated monocytes, the production of spermine tended to increase with low and high levels of pregnancy hormones in the third trimester, but tended to decrease with low and high levels of pregnancy hormone stimulation in the first trimester (Figure 4b).

Discussion

Identifying the factors that modify the phenotype of monocytes and macrophages in pregnancy is important

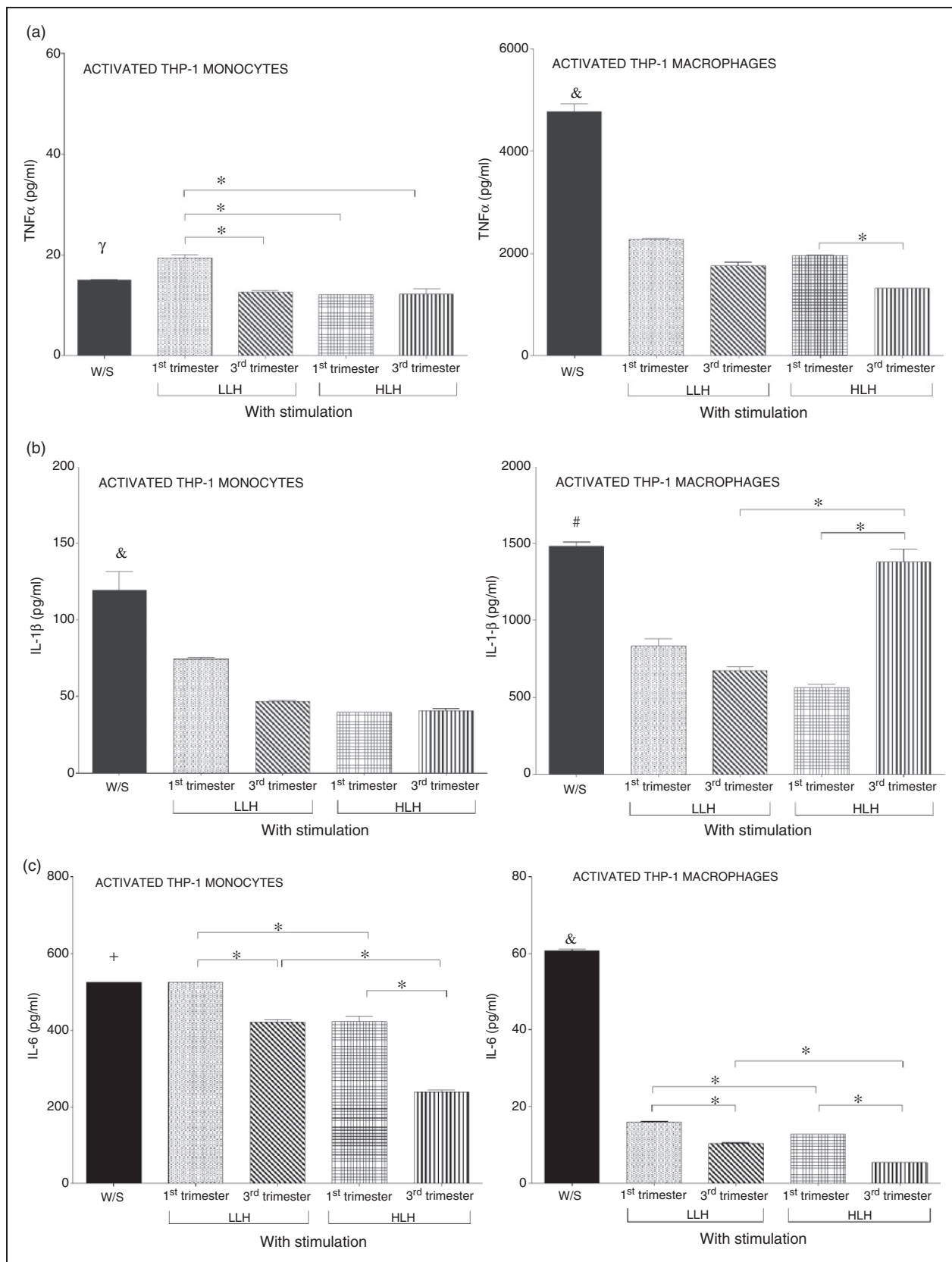


Figure 2. Profile cytokine production of activated monocytes and macrophages derived from THP-1 cells after pregnancy hormone stimulation. (a) TNF- α , (b) IL-1 β , (c) IL-6, (d) IL-12, (e) IL-8, and (f) IL-10 concentrations by ELISA in cell culture supernatants. Bars represent the mean \pm SEM. * $P < 0.05$ W/S versus all pregnancy hormone mixture stimulation, LLH in the first trimester; #HLH and LLH in the first trimester; +HLH in the first and third trimesters and LLH in the third trimester. All results were confirmed in triplicate in three independent experiments under the same experimental conditions.

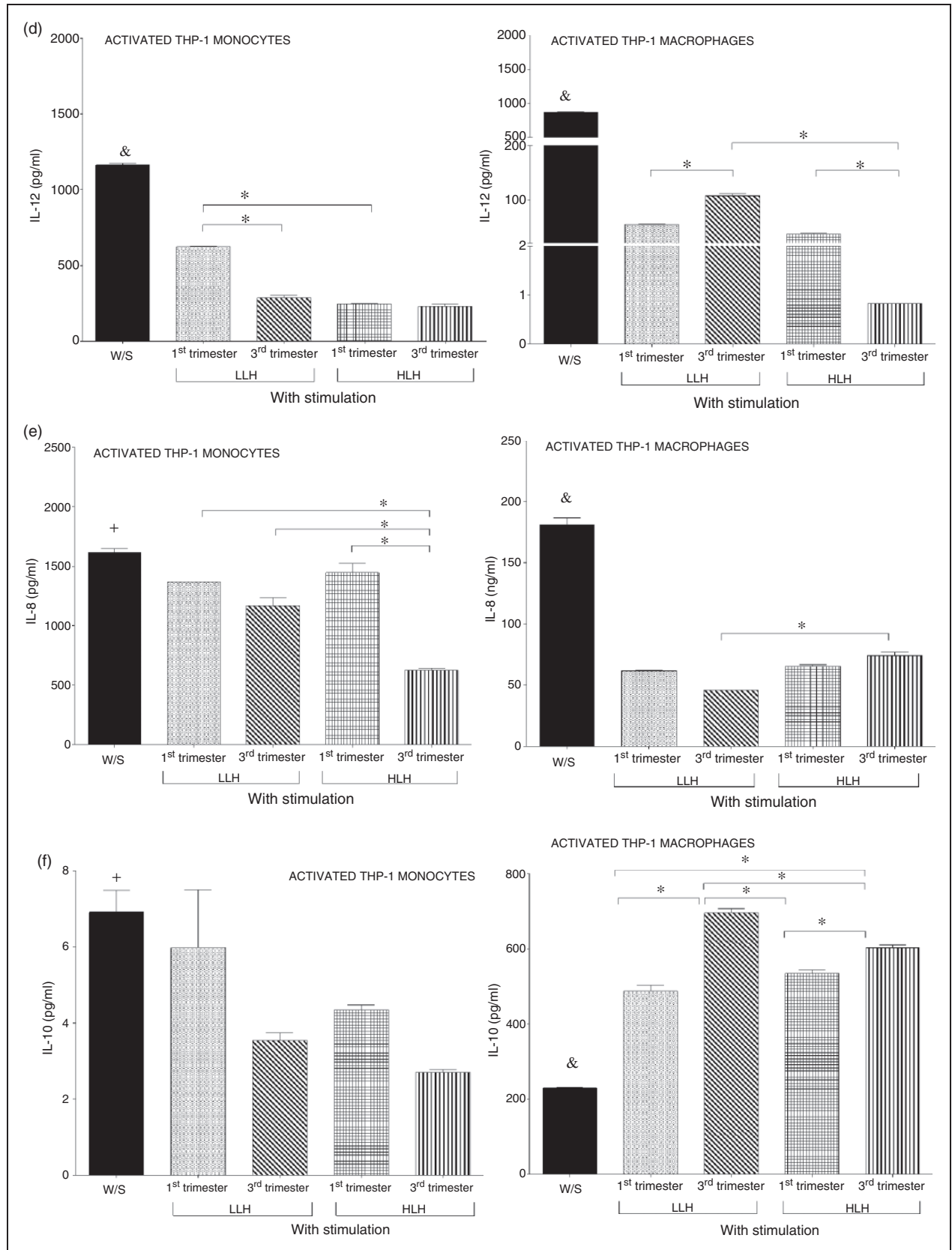


Figure 2. Continued

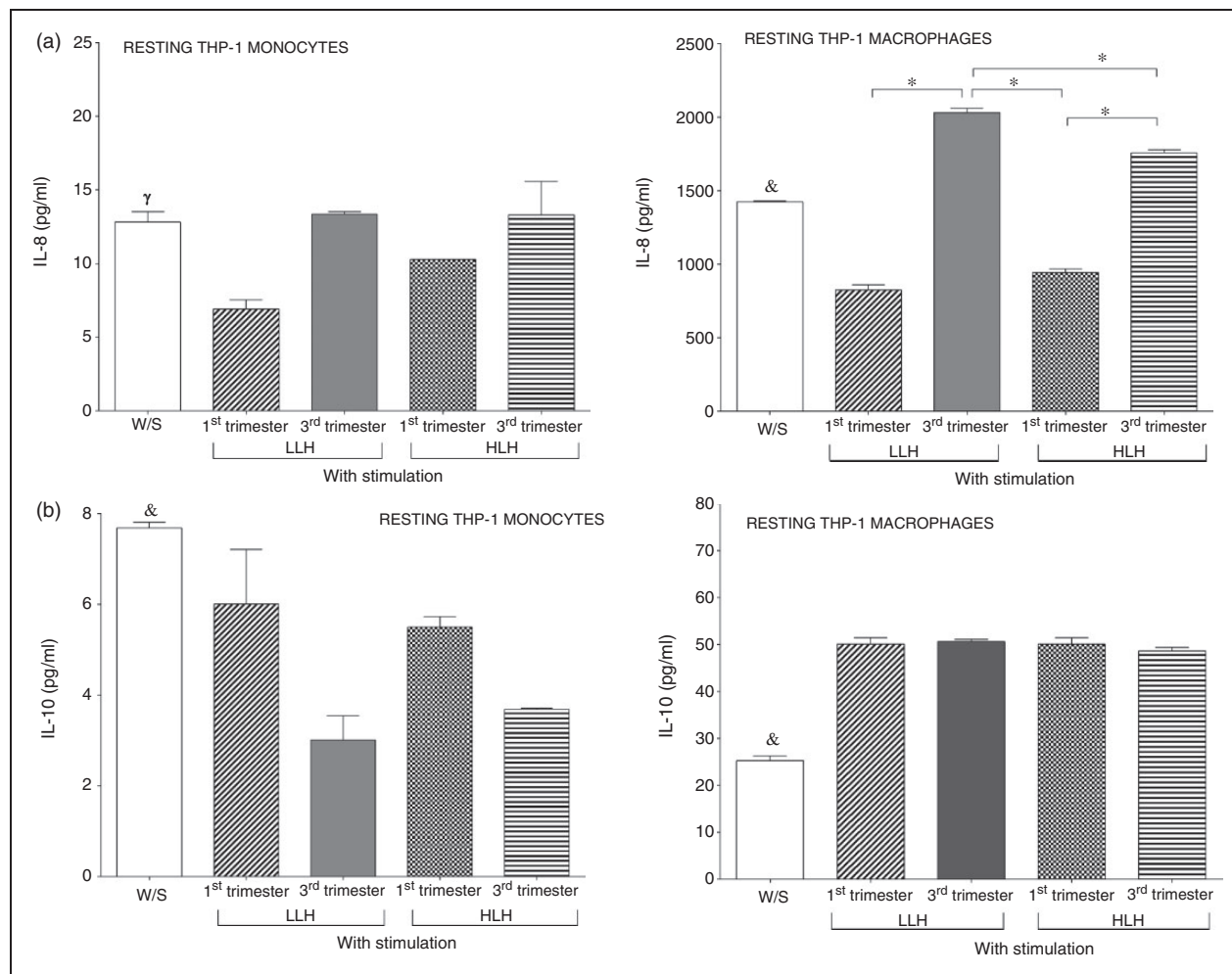


Figure 3. Unsteadiness between IL-8 and IL10 production in resting monocytes and macrophages derived from THP-1 cells after pregnancy hormone stimulation. (a) IL-8 levels and (b) IL-10 levels. Cytokine concentrations by ELISA in cell culture supernatants. Bars represent the mean \pm SEM. * $P < 0.05$ W/S versus all pregnancy hormone mixture stimulation; γ LLH in the first trimester. All results were confirmed in triplicate in three independent experiments under the same experimental conditions.

because macrophages can be hyper-responsive and can induce a dys-regulated release of inflammatory mediators. This could exacerbate tissue damage or could promote the development of chronic diseases, such as pulmonary fibrosis, by releasing cytotoxic, pro- and anti-inflammatory, angiogenic, mitogenic, and fibrogenic factors.¹⁹

In this study, we showed the modulatory effect of pregnancy hormones on the regulation of CD163, as well as on pro-inflammatory cytokine synthesis in both resting and activated monocytes and macrophages.

Previously, modulation of the function of monocytes and macrophages was reported, mainly due to a single effect of steroid hormones, but little is known about the effect obtained by the combination of these hormones in different concentrations. The hormone combination in pregnancy must regulate the

macrophage phenotype differently from that of individual hormones.²⁰

In this study, all the hormonal profiles reduced the pro-inflammatory cytokines in activated monocytes and macrophages. However, in the absence of LPS, the synthesis of IL-8 and IL-10 was increased in resting macrophages, including a higher production of IL-8 with doses in the third trimester. On the contrary, IL-10 synthesis decreased in monocytes.

IL-8 is implicated in the recruitment of leukocytes in pregnancy. It is significantly synthesized by placental cell-type macrophages and trophoblast cells from the first trimester, and its synthesis increases during the second and third trimesters,²¹ while IL-10 plays an anti-inflammatory role in pregnancy.²²

In this regard, it has been found that in THP-1 monocytes stimulated with LPS from *E. coli* or

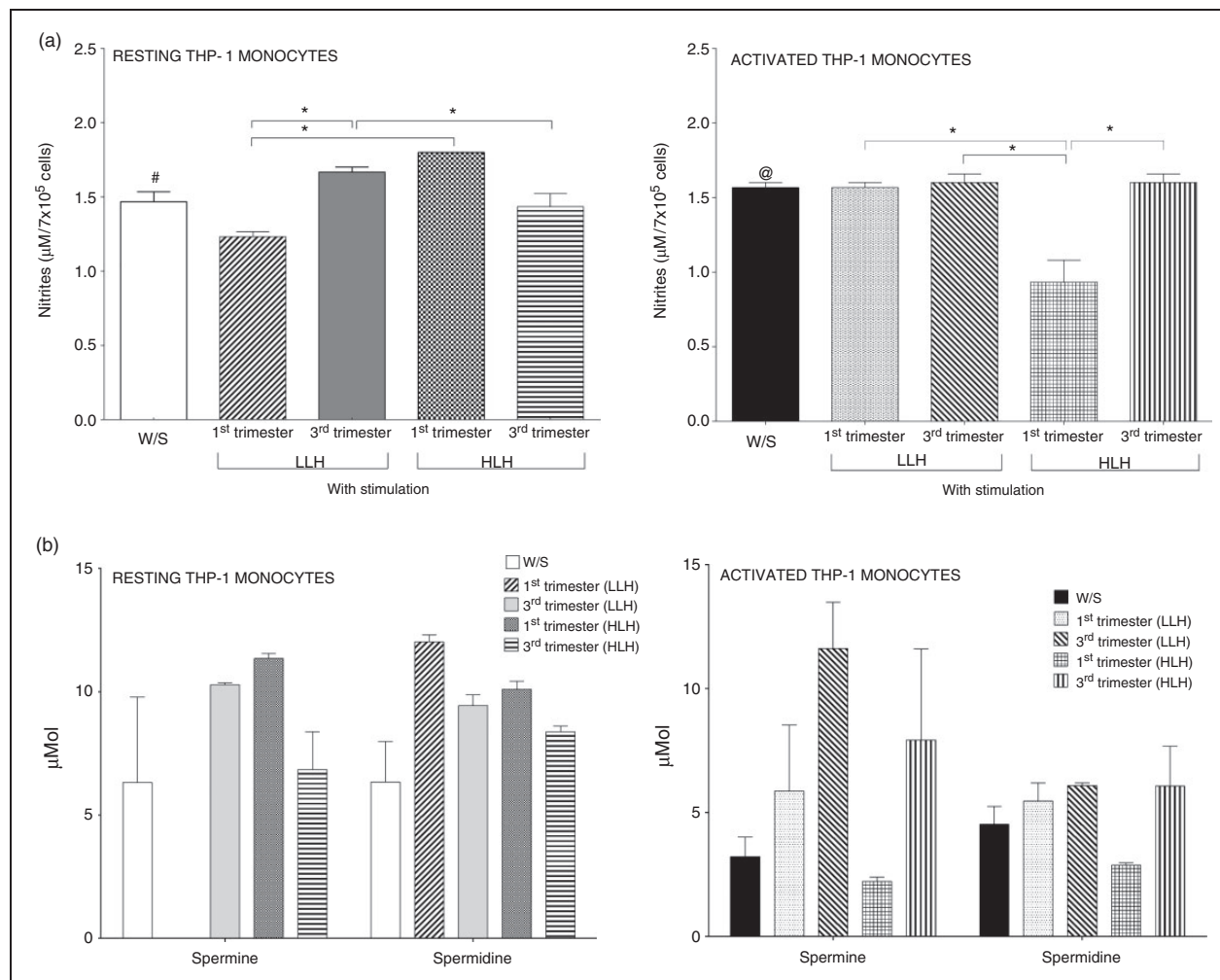


Figure 4. Effect of low and high pregnancy hormone mixture on nitrite and polyamine production in resting and activated monocytes and macrophages derived from THP-1 cells. (a) Nitrite levels and (b) polyamine levels. Bars represent the mean \pm SEM. $P < 0.05$ W/S versus HLH and LLH in the first trimester, and LLH in the third trimester, and high levels in the first trimester. All results were confirmed in triplicate in three independent experiments under the same experimental conditions.

Ureaplasma urealyticum and to which was added high concentrations of progesterone (10,000 ng/ml), an increased production of IL-1 β and IL-8 occurred, while TNF- α synthesis was inhibited. Furthermore, at baseline, progesterone stimulated the synthesis of IL-8,²³ which might indicate a specific regulation of cytokine production due to the effect of this hormone in resting and activated monocytes.

Similarly, prolactin, pretreated or in combination with LPS, induced IL-8 secretion in THP-1 monocytes through cumulative activation of the MEK1-MEK2/MAPK, p38, and JAK/STAT pathways.²⁴

On the other hand, other studies reported that estradiol treatment did not affect the constitutive production of IL-8, but in LPS-activated monocytes, it decreased IL-8 synthesis in a dose-dependent manner. Estradiol regulated IL-8 through its receptor at the

transcriptional level, probably by interfering with activation of transcription factors C/EBP and/or NF- κ B.²⁵ Glucocorticoids inhibit the expression of the IL-8 gene, which contains glucocorticoid response elements in the 5'-flanking region.^{26,27} In addition, glucocorticoids may suppress the transcription of the IL-8 gene by interfering with the activation of NF- κ B.²⁸ However, estradiol treatment did not affect the constitutive expression of IL-1 β but did enhance IL-1 β production in LPS-activated THP-1 monocytes.²⁹

In this study, estradiol, progesterone, and prolactin were able to regulate the synthesis of cytokines differentially, and they could be responsible for the increased synthesis of IL-8 and IL-1 β in monocytes and macrophages, whether resting or activated.

Regarding the expression of surface receptors, up-regulation of CD14 and CD86 by LPS has been

reported,^{30,31} while CD163 was induced by IL-4, IL-13, IL-10, and glucocorticoids³² as a phenotypical characteristic of the M2 macrophage.³³

Based on the results observed in this study, we suggest that the induction of CD163 in monocytes and macrophages could be regulated by the stimulation of cortisol levels, which is in agreement with two previous reports where it was shown that monocytes exposed to glucocorticoids (dexamethasone 2.5×10^{-7}) were differentiated in the M2 phenotype, showing higher expression of CD163 and an improved iron recycling capacity, because the expression of the transferrin receptor is suppressed, while the ferroportin expression of the iron exporter is improved.³⁴ Similarly, in an *ex vivo* culture of fetal macrophages (Hofbauer cells), CD163 and mRNA levels increased in response to cortisol or dexamethasone and to increased uptake of hemoglobin.³²

This can be explained because decidua macrophages have an M2 phenotype that expresses low levels of CD80 and CD86 co-stimulatory molecules, and high levels of CD163, CD206, CD209, NRP-1, and ICAM-3, and synthesizes indoleamine 2,3-di-oxygenase.^{35–37} This immunosuppressive phenotype enables the release of pro-angiogenic factors and remodeling of the uterine wall. However, in late pregnancy and during delivery, macrophages acquire the inflammatory phenotype M1 to drive cervical ripening.³⁸

Thus, pregnancy hormones might promote an anti-inflammatory M2 phenotype, negatively affecting the monocyte and macrophage pro-inflammatory phenotype induced by LPS *in vitro*, without being completely inhibited. As well, this modulation could allow activation of monocytes and macrophages that are useful in parturition and for maternal protection against infections, avoiding the excessive inflammatory response that may affect the viability of the fetus.

It is known that through their nuclear receptors, steroidal hormones interact with NF- κ B, thus preventing the transcription of inflammatory factors.^{39–42} However, it is also known that neither estradiol nor progesterone change the phosphorylation of p38 in LPS-stimulated monocytes,⁴³ while prolactin produces a greater MEK1–MEK2/MAPKs activation response in THP-1 cells stimulated with LPS, resulting in an increase in IL-8 release. This effect is reduced in cells treated with inhibitors for the MEK1 and the p38 or JAK/STAT pathway.²⁴ Further, cortisol through its glucocorticoid receptor could be able to activate different signal pathways. It has been reported that the glucocorticoid receptor attenuates pro-inflammatory responses in mouse macrophages activated with LPS by impairing selectively p38 MAPK, but not PI3K/Akt, ERK, or JNK.⁴⁴ Thus, the response of monocytes

with hormonal treatment to LPS could be independent of NF- κ B induction.

Conclusions

We found that stimulation with sera hormone mixtures containing cortisol, estradiol, progesterone, and prolactin, mimicking concentrations in the first and third trimesters, modulate the pro-inflammatory phenotype and function of monocyte THP-1 cells and macrophages differentiated from THP-1 monocytes *in vitro* because they were able to modify pro-inflammatory cytokine secretion, and CD163 expression in resting and LPS-activated THP-1 cells. Our results suggest that the hormonal environment in pregnancy may be able to modulate both the monocyte and macrophage phenotype toward the M2-like phenotype under resting or inflammatory conditions.

Authors' note

Ciro Estrada-Chávez is now affiliated to Instituto Transdisciplinar de Investigación y Servicios de la Universidad de Guadalajara (iTRANS), CUCEI, Mexico.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: supported by FUNPROJAL grant 0194/11-B (CE-C) and Sectoral Fund for Education Research, SEP-CONACYT (A1-S-51207). CONACyt-México awarded a scholarship (200597) to the first author (MIM-C).

ORCID iD

Ana Laura Pereira-Suárez  <https://orcid.org/0000-0001-9137-6027>

References

1. Mesiano S. *The endocrinology of human pregnancy and fetoplacental neuroendocrine development*. Philadelphia: W.B. Saunders, 2009, pp.249–281.
2. Koldehoff M, Cierna B, Steckel NK, et al. Maternal molecular features and gene profiling of monocytes during first trimester pregnancy. *J Reprod Immunol* 2013;99:62–68.
3. Lampe R, Kover A, Szucs S, et al. Phagocytic index of neutrophil granulocytes and monocytes in healthy and pre-eclamptic pregnancy. *J Reprod Immunol* 2014;107:26–30.
4. Naccasha N, Gervasi MT, Chaiworapongsa T, et al. Phenotypic and metabolic characteristics of monocytes

- and granulocytes in normal pregnancy and maternal infection. *Am J Obstet Gynecol* 2001;185:1118–1123.
5. Cutolo M, Villaggio B, Bisso A, et al. Presence of estrogen receptors in human myeloid monocytic cells (THP-1 cell line). *Eur Cytokine Netw* 2001;12:368–372.
 6. Khan KN, Masuzaki H, Fujishita A, et al. Estrogen and progesterone receptor expression in macrophages and regulation of hepatocyte growth factor by ovarian steroids in women with endometriosis. *Hum Reprod* 2005;20:2004–2013.
 7. Werb Z, Foley R and Munck A. Interaction of glucocorticoids with macrophages. Identification of glucocorticoid receptors in monocytes and macrophages. *J Exp Med* 1978;147:1684–1694.
 8. López-Rincón G, Pereira-Suárez AL, Del Toro-Arreola S, et al. Lipopolysaccharide induces the expression of an autocrine prolactin loop enhancing inflammatory response in monocytes. *J Inflamm* 2013;10:24.
 9. Edelstam G, Karlsson C, Westgren M, et al. Human chorionic gonadotropin (hCG) during third trimester pregnancy. *Scand J Clin Lab Invest* 2007;67:519–525.
 10. Straub RH. The complex role of estrogens in inflammation. *Endocr Rev* 2007;28:521–574.
 11. Yeager MP, Pioli PA and Guyre PM. Cortisol exerts bi-phasic regulation of inflammation in humans. *Dose Response* 2011;9:332–347.
 12. Medeiros LT, Peraçoli JC, Bannwart-Castro CF, et al. Monocytes from pregnant women with pre-eclampsia are polarized to a M1 phenotype. *Am J Reprod Immunol* 2014;72:5–13.
 13. Schonkeren D, van der Hoorn ML, Khedoe P, et al. Differential distribution and phenotype of decidual macrophages in preeclamptic versus control pregnancies. *Am J Pathol* 2011;178:709–717.
 14. Reister F, Frank HG, Kingdom JC, et al. Macrophage-induced apoptosis limits endovascular trophoblast invasion in the uterine wall of preeclamptic women. *Lab Invest* 2001;81:1143–1152.
 15. Kim J, Ko Y, Kwon K, et al. Analysis of monocyte subsets and toll-like receptor 4 expression in peripheral blood monocytes of women in preterm labor. *J Reprod Immunol* 2012;94:190–195.
 16. Faas MM, Spaans F and De Vos P. Monocytes and macrophages in pregnancy and pre-eclampsia. *Front Immunol* 2014;5:298.
 17. Daigneault M, Preston JA, Marriott HM, et al. The identification of markers of macrophage differentiation in PMA-stimulated THP-1 cells and monocyte-derived macrophages. *PLoS One* 2010;5:e8668.
 18. Farkas S and Hajós G. Monitoring of biologically active amines in cereals and cereal based food products by HPLC. *Chromatographia* 1998;48:37–42.
 19. Laskin DL, Sunil VR, Gardner CR, et al. Macrophages and tissue injury: agents of defense or destruction? *Ann Rev Pharmacol Toxicol* 2011;51:267–288.
 20. Rami D, La Bianca M, Agostinis C, et al. The first trimester gravid serum regulates procalcitonin expression in human macrophages skewing their phenotype *in vitro*. *Mediators Inflamm* 2014;2014:248963.
 21. Shimoya K, Matsuzaki N, Taniguchi T, et al. Human placenta constitutively produces interleukin-8 during pregnancy and enhances its production in intrauterine infection. *Biol Reprod* 1992;47:220–226.
 22. Thaxton JE and Sharma S. Interleukin-10: a multifaceted agent of pregnancy. *Am J Reprod Immunol* 2010;63:482–491.
 23. Peltier MR, Tee SC and Smulian JC. Effect of progesterone on proinflammatory cytokine production by monocytes stimulated with pathogens associated with preterm birth. *Am J Reprod Immunol* 2008;60:346–353.
 24. D'Isanto M, Vitiello M, Raieta K, et al. Prolactin modulates IL-8 production induced by porins or LPS through different signaling mechanisms. *Immunobiology* 2004;209:523–533.
 25. Pioli PA, Jensen AL, Weaver LK, et al. Estradiol attenuates lipopolysaccharide-induced CXC chemokine ligand 8 production by human peripheral blood monocytes. *J Immunol* 2007;179:6284–6290.
 26. Mukaida N, Shiroo M and Matsushima K. Genomic structure of the human monocyte-derived neutrophil chemotactic factor IL-8. *J Immunol* 1989;143:1366–1371.
 27. Mukaida N, Gussella GL, Kasahara T, et al. Molecular analysis of the inhibition of interleukin-8 production by dexamethasone in a human fibrosarcoma cell line. *Immunology* 1992;75:674–679.
 28. Mukaida N, Morita M, Ishikawa Y, et al. Novel mechanism of glucocorticoid-mediated gene repression. Nuclear factor-kappa B is target for glucocorticoid-mediated interleukin 8 gene repression. *J Biol Chem* 1994;269:13289–13295.
 29. Pioli PA, Weaver LK, Schaefer TM, et al. Lipopolysaccharide-induced IL-1 β production by human uterine macrophages up-regulates uterine epithelial cell expression of human beta-defensin 2. *J Immunol* 2006;176:6647–6655.
 30. Fleischer J, Soeth E, Reiling N, et al. Differential expression and function of CD80 (B7-1) and CD86 (B7-2) on human peripheral blood monocytes. *Immunology* 1996;89:592–598.
 31. Landmann R, Knopf HP, Link S, et al. Human monocyte CD14 is upregulated by lipopolysaccharide. *Infect Immun* 1996;64:1762–1769.
 32. Tang Z, Niven-Fairchild T, Tadesse S, et al. Glucocorticoids enhance CD163 expression in placental Hofbauer cells. *Endocrinology* 2013;154:471–482.
 33. Mantovani A, Sica A, Sozzani S, et al. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol* 2004;25 677–686.
 34. Vallelan F, Schaer CA, Kaempfer T, et al. Glucocorticoid treatment skews human monocyte differentiation into a hemoglobin-clearance phenotype with enhanced heme-iron recycling and antioxidant capacity. *Blood* 2010;116:5347–5356.
 35. Gustafsson C, Mjösberg J, Matussek A, et al. Gene expression profiling of human decidual macrophages: evidence for immunosuppressive phenotype. *PLoS One* 2008;3:e2078.

36. Svensson J, Jenmalm MC, Matussek A, et al. Macrophages at the fetal–maternal interface express markers of alternative activation and are induced by M-CSF and IL-10. *J Immunol* 2011;187:3671–3682.
37. Heikkinen J, Mottonen M, Komi J, et al. Phenotypic characterization of human decidual macrophages. *Clin Exp Immunol* 2003;131:498–505.
38. Nagamatsu T and Schust DJ. The contribution of macrophages to normal and pathological pregnancies. *Am J Reprod Immunol* 2010;63:460–471.
39. van der Burg B and van der Saag PT. Nuclear factor-kappa-B/steroid hormone receptor interactions as a functional basis of anti-inflammatory action of steroids in reproductive organs. *Mol Hum Reprod* 1996;2:433–438.
40. McKay LI and Cidlowski JA. Cross-talk between nuclear factor-kappa B and the steroid hormone receptors: mechanisms of mutual antagonism. *Mol Endocrinol* 1998;12:45–56.
41. Nelson G, Wilde GJ, Spiller DG, et al. NF-kappaB signalling is inhibited by glucocorticoid receptor and STAT6 via distinct mechanisms. *J Cell Sci* 2003;116:2495–2503.
42. Scheinman RI, Gualberto A, Jewell CM, et al. Characterization of mechanisms involved in transrepression of NF-kappa B by activated glucocorticoid receptors. *Mol Cell Biol* 1995;15:943–953.
43. Jitprasertwong P, Charadram N, Kumphune S, et al. Female sex hormones modulate Porphyromonas gingivalis lipopolysaccharide-induced Toll-like receptor signaling in primary human monocytes. *J Periodontol Res* 2016;51:395–406.
44. Bhattacharyya S, Brown DE, Brewer JA, et al. Macrophage glucocorticoid receptors regulate Toll-like receptor 4-mediated inflammatory responses by selective inhibition of p38 MAP kinase. *Blood* 2007;109:4313–4319.