

Lipopolysaccharide induces the expression of interleukin-1 α distinctly in different compartments of term and preterm human placentae

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ABSTRACT. The aim of the study was to investigate the stimulatory effect of lipopolysaccharide (LPS) on IL-1 α production in different compartments of term and preterm placental tissues. Homogenates from amnion, chorion, and from fetal (subchorionic placental tissues, maternal decidua, and mid-placental tissue before and after perfusion of isolated placental cotyledons of 5 term placentas and 4 placentas obtained after preterm birth (28-34 W of gestation) were examined. Isolated placental cotyledons were dually perfused LPS (100 ng/kg perfused placental tissue) was perfused into the maternal side during 10 hours. Homogenates of the samples were examined by ELISA for IL-1 α levels, and paraffin sections of the samples were stained by immunohistochemical staining, to characterize the cellular origin of placental IL-1 α . Paired t test and ANOVA determined statistical significance. In the homogenates, there was a tendency towards higher IL-1 α levels in all preterm placental compartments as compared to the term compartments before perfusion. A significant increase was observed only in the chorion compartment ($p = 0.035$). LPS had significantly increased IL-1 α levels only in the decidua compartment of term placentas as compared to other placental compartments ($p = 0.0004$), and had decreased IL-1 α levels in the mid-placenta ($p = 0.034$). In preterm placentas, addition of LPS did not affect the expression levels of IL-1 α in either fetal or maternal compartments as determined by ELISA and immunohistochemical staining. IL-1 α levels in the chorion compartment of preterm placenta were significantly higher as compared to term placenta. LPS affects placental tissues of term and preterm placentas differently. Also, in the term placentas, LPS affected the different compartments differently. Thus, IL-1 α may have a key role (as a autocrine/paracrine factor) in the regulation of normal and pathological pregnancy and parturition.

Keywords: chorion/amnion/trophoblasts/IL-1/lipopolysaccharide/term and preterm delivery

Cytokines are paracrine/autocrine immune-regulatory growth factors produced mainly by immune cells following immune challenges (antigen/pathogen or inflammation). However, non-immune cells also produce these factors [1]. Cytokines produced by placental tissues are involved in the regulation of implantation, fetal growth and development. They control placental development through the regulation of trophoblast proliferation, differentiation and function [2-6]. Immunoregulatory molecules in the placental compartments have been shown to be involved in immunological tolerance [2].

The interleukin-1 family exerts growth-inducing, growth-inhibitory and differentiation-inducing activities and members are autocrine/paracrine factors with pleiotropic activity [1, 7].

Molecular studies have demonstrated three types of IL-1, namely; IL-1 α , IL-1 β and IL-1 receptor antagonist (IL-

1ra) [1, 7]. Both IL-1 α and IL-1 β bind to the same receptor, and share a spectrum of activities involved in physiological and pathophysiological functions [1, 7]. IL-1 β is the major secreted form, while IL-1 α is active in the cytoplasm or is membrane-associated, in a variety of cell types [1].

A unique feature of the IL-1 system is the naturally occurring IL-1ra. Its genetic structure is homologous with the IL-1 α and IL-1 β genes to certain degree [8, 9]. It binds to the same receptors without transmitting any signal; thus it has an important role in the regulation of the action of IL-1 [1, 7].

Premature birth is the major contributory factor in both perinatal morbidity and mortality worldwide [10], and 20-30% of preterm labor occurs in the setting of infection [11]. It is suggested that microbial invasion of the chorionic and amniotic membranes (chorioamnionitis) leads to the release of endotoxins [such as lipopolysaccharide

(LPS)], which increase the production of cytokines by maternal immune cells and placental tissues [6]. Indeed, increased levels of pro-inflammatory cytokines such as TNF- α , IL-1 β , IL-6 and IL-8 have been detected in the amniotic fluid of patients with preterm labor and infection [12]. LPS induces inflammatory responses in human gestational tissues, resulting in increased release of proinflammatory cytokines such as IL-1 and phospholipid metabolites such as prostanoids [13-15]. In addition, injection of LPS into pregnant mice and rats, increased the levels of pro-inflammatory cytokines and prostaglandins, and induced preterm delivery [16-18].

IL-1 β has been demonstrated in decidual tissue cells [19], and in cultures of first trimester trophoblast cells and explants of chorionic villous [20]. *In vivo* and *in vitro* studies demonstrated increase levels of IL-1 α , IL-1 β and IL-1ra in amniotic fluid or gestational tissues from patients with intra-amniotic infection or after exposure to LPS [21-28].

Furthermore, administration of IL-1 α or IL-1 β induces preterm delivery in mice, rabbits, and monkeys [29-31]; blocking of IL-1 activity using IL-1ra reduces stress-triggered or IL-1 α -induced abortion in mice [32, 33].

IL-1 receptor (IL-1R) type I was detected on extravillous trophoblast, villous syncytiotrophoblast and mesenchymal cells, but not villous cytotrophoblasts. High levels of expression were also detected on glandular epithelium [34], macrophages and NK of the deciduas [19].

In the present study, we examined the stimulatory effect of lipopolysaccharide (LPS) on IL-1 α production in different compartments of term and preterm placental tissues.

MATERIALS AND METHODS

Study design

Placentas from 10, uncomplicated term and preterm (34-36 W of gestation) pregnancies were collected immediately after either vaginal or cesarean delivery. Five of these placentas were perfused with medium alone [M199 media (Beit HaEmek, Israel), enriched with bovine serum albumin (0.1 mg/ml), glucose (1.0 g/L) (Sigma Chemicals Co., St. Louis, USA), heparin (20 IU/ml) (Beit Kama, Israel) and Gentamycin (48 μ g/ml)] (Teva, Petah Tekva, Israel) and another 5 placentas were perfused with medium containing LPS [(1 μ g/kg perfused placental tissue (Sigma)], for 10 hours. The perfusion experiments were performed using the method of Schneider and Huch [35]. Placentas were taken to the laboratory where a fetal artery and a fetal vein from a single cotyledon were cannulated, within 20 min of delivery. Following successful establishment of the fetal circulation, the placenta was mounted in a perfusion chamber, and the maternal circulation was cannulated by placing four catheters into the intervillous space of the lobe corresponding with the perfused isolated cotyledon. Maternal perfusate that returned from the intervillous space was continuously drained by a maternal venous catheter placed at the lowest level on the maternal decidual surface to avoid significant pooling of perfusate. Perfusion medium with or without LPS was adjusted to 7.4 with bicarbonate and gassed with 95% oxygen and 5% carbon dioxide at 37 °C, PO₂ > 110 mmHg.

Perfusion rates were 4-6 ml/min and 10-12 ml/min in the fetal and maternal circulation, respectively. Lateral pres-

sure was measured in the fetal inflow line adjusted to the point of cannulation. A Hewlett Packard 1290c universal quartz pressure transducer recorded the pressure. Placental tissues before and after perfusion were separated into compartments; Placental tissues, chorion, amnion, subchorionic placental tissue, mid-placental tissue, maternal surface with decidua, were collected at the end of the perfusion process (10 hours) and were formalin-fixed for IL-1 α cellular origin characterization by immunohistochemical staining, or stored at -70 °C to be homogenized for IL-1 α level evaluation.

Immunohistochemical staining [36]

Four micron thick sections from formalin-fixed, paraffin-embedded tissue blocks were mounted on saline-coated slides, dried at 37 °C for 48 hrs and stored at room temperature. Before the primary antibodies (polyclonal rabbit anti-human IL-1 α) (Endogen, MA, USA) were applied, blocking of the nonspecific background was achieved with PBS (Beit HaEmek) containing 0.05% casein (Sigma). This solution was also used to dilute the primary antibodies. In order to choose an optimal procedure for antigen unmasking, we used sections from two of the cases. These were exposed to trypsin, proteinase K, PBS, urea (Sigma) and boiled or to citrate (Sigma) and boiled. The best results were obtained after boiling in 6-M urea for 15 min., using polyclonal rabbit anti-human IL-1 α antibodies (100 μ g/ml) diluted in PBS/casein pH 7.5. In order to dilute the remaining antibodies and for the purpose of washing the sections, we added 0.05% Tween 20 (Sigma) to the PBS/casein solution. The biotinylated antibody (polyclonal goat anti-rabbit) (Zymed, San Francisco, CA, USA) and the streptavidin-peroxidase conjugates (Zymed) were applied according to the supplier's directions. Blocking of the endogenous peroxidase was achieved with 3% H₂O₂ in 80% methanol for 15 min. before the streptavidin-peroxidase conjugate (Frutarom, Ltd, Israel) was applied. Development was performed with 0.06% DAB, and Mayer's hematoxylin (Sigma) was used for counterstaining. The sections were mounted in Eukitt. Negative controls were performed for each specimen using PBS/casein instead of the primary antibodies.

Preparation of placental homogenates: placental homogenates were prepared from different compartments of 10 placentas. Placental tissues were homogenized in 1 ml cold PBS. At the end of the homogenization process, the mixture was centrifuged at 13000 RPM for 15 min. and the supernatant was collected and stored at -70°C. Total protein was determined using Biorad reagent.

The levels of IL-1 α in the homogenates of the placental fetal and maternal sites were examined using ELISA kits specific for human IL-1 α (Endogen).

The results are presented as pg/ μ g protein \pm SD.

Statistical significance was determined by analysis of variance (ANOVA) and the paired t-test.

RESULTS

Expression of IL-1 α in fetal and maternal compartments of term and preterm placentas

There was a tendency towards higher IL-1 α levels in all preterm placental compartments as presented in *Figure 1*.

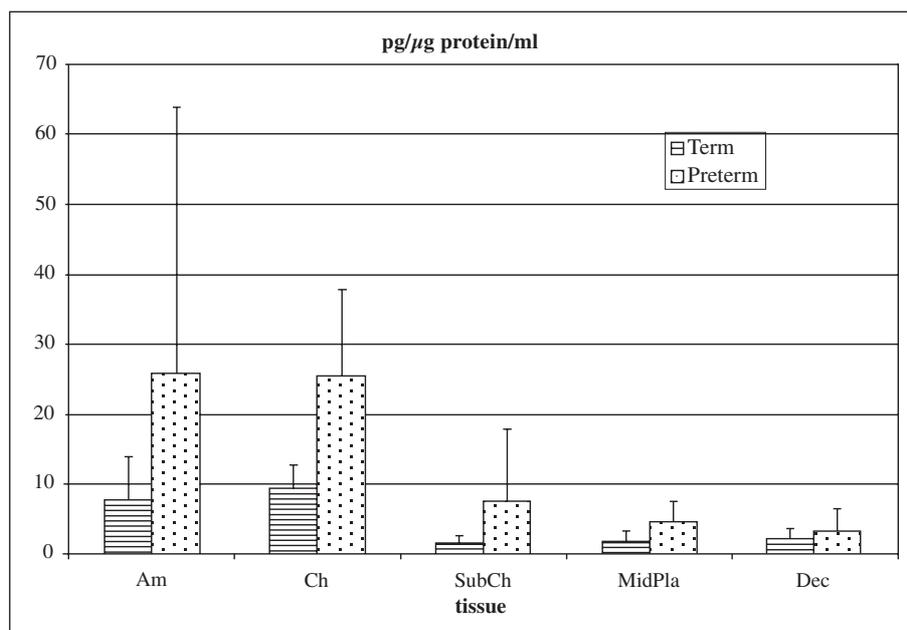


Figure 1

Expression of IL-1 α in fetal and maternal compartments of term and preterm placentas. IL-1 α levels were detected in the homogenates of amnion (Am) and chorion (Ch) tissues, and in the homogenates of subchorionic (SubCh), mid-placenta (MidPla) and decidua (Dec) of term and preterm placentas before perfusion. Results are expressed as mean \pm SD. ELISA kit sensitivity was 20 pg/ml.

The levels of IL-1 α in the following preterm and term placental compartments were not statistically significant: amnion; 26 ± 38 and 8 ± 6 pg/mg protein respectively, subchorionic placental tissue; 8 ± 10 and 2 ± 1 respectively, mid-placenta; 5 ± 3 and 2 ± 1.5 respectively, and decidua; 3 ± 3 and 2 ± 1.4 respectively. However, a significant difference was observed only in the chorion compartment of preterm and term placentas (25 ± 12 and 9 ± 3 respectively; $p = 0.035$).

The levels of IL-1 α in the amnion of term placentas were significantly higher than in subchorionic placental tissue ($p = 0.038$), and mid-placenta ($p = 0.044$) but not in chorion or decidua tissues. However, the levels of IL-1 α in chorion tissue were significantly higher than in subchorionic placental tissue ($p = 0.00015$), mid-placenta ($p = 0.00014$) and decidua ($p = 0.00021$), but not than levels in the amnion.

The levels of IL-1 α in the chorion of preterm placentas were significantly higher than in subchorionic placental tissue ($p = 0.03$), mid-placenta ($p = 0.018$) and decidua ($p = 0.014$), but not than levels in the amnion. However, the levels of IL-1 α in the amnion of preterm placental tissues were not significantly different than any those found in other placental tissue.

Stimulation of term placentas with LPS significantly increased IL-1 α levels in decidual tissue only, and decreased it in mid-placental tissue

Perfusion of term placentas with medium containing LPS, or medium alone (Figure 2) did not affect the levels of IL-1 α in the homogenates of amnion (10 ± 4 , 15 ± 10 respectively), chorion (7 ± 5 , 14 ± 5) or subchorionic placental tissues (2 ± 0.3 , 2.5 ± 1.5). However, it significantly increased IL-1 α levels in decidual tissue (76 ± 4 , 2.7 ± 1.6 , 2 ± 1.4 respectively; $p = 0.0004$). On the other hand, it significantly decreased IL-1 α levels in mid-placental tissues (0.8 ± 0.8 , 2 ± 0.6 respectively; $p = 0.034$).

Stimulation of preterm placentas with LPS did not affect the capacity of the maternal and fetal compartments to produce IL-1 α

Perfusion of preterm placentas with medium containing LPS, or medium alone (Figure 3) did not significantly affect the levels of IL-1 α in the homogenates of amnion (62 ± 13 , 178 ± 113 respectively), chorion (14 ± 9 , 65 ± 79), subchorionic placental tissues (25 ± 0.3 , 32 ± 19), mid-placental tissues (4 ± 3 , 20 ± 17) or decidua (31 ± 11 , 24 ± 27).

Localization of IL-1 α in the fetal and maternal compartments of term and preterm placentas

Immunohistochemical studies showed that IL-1 α is localized in the epithelial cells of the amnion tissue, and in the fibroblasts and macrophages of the chorion tissue (Figure 4A). In the subchorionic and mid-placental tissues (Figure 4B), IL-1 α was expressed mainly in cytotrophoblasts and syncytiotrophoblasts. In addition, IL-1 α was expressed in decidual tissue cells (Figure 4C). No significant difference was shown in the IL-1 α levels between the placental tissues (both maternal and fetal tissues) perfused in the absence or in the presence of LPS (data not presented). Negative controls showed no staining for IL-1 α in the examined tissues of placentas perfused in the presence or absence of LPS (Figure 4D).

DISCUSSION

This is the first work showing an expression of IL-1 α in fetal and maternal compartments of term and preterm placentas under stimulation with LPS, in a system that seems to be close to *in vivo* conditions. Our results show that IL-1 α levels are significantly higher, only in the chorionic tissue of preterm placentas as compared to term placentas. Stimulation with LPS affected term and preterm

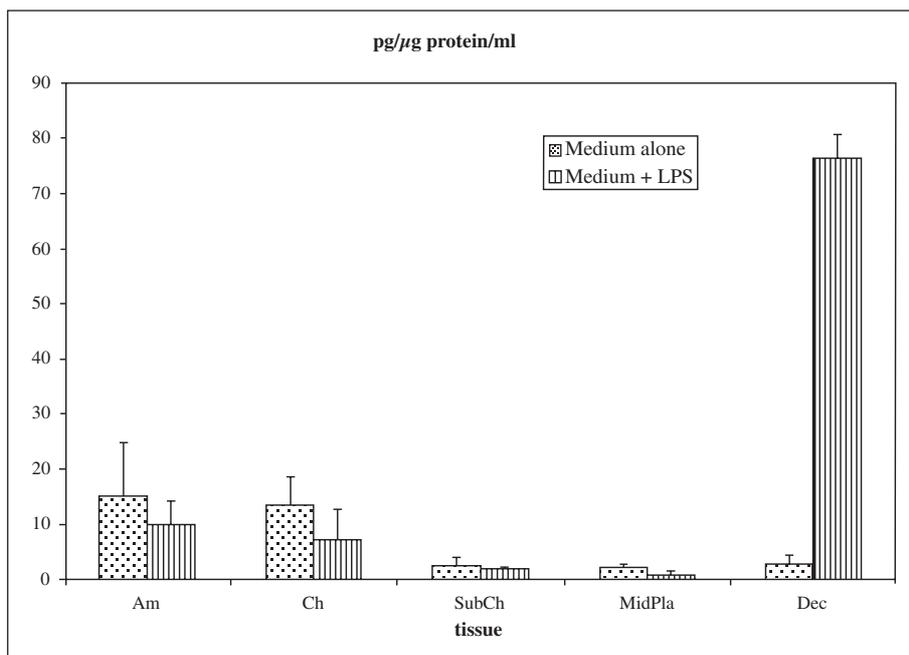


Figure 2

Effect of LPS on the expression levels of IL-1 α in fetal and maternal compartments of term placentas. IL-1 α levels were detected in the homogenates of amnion (Am) and chorion (Ch) tissues, and in the homogenates of subchorionic (SubCh), mid-placenta (MidPla) and decidua (Dec), following 10 hours of perfusion with medium alone or medium containing LPS (1 μ g/kg). Results are expressed as mean \pm SD.

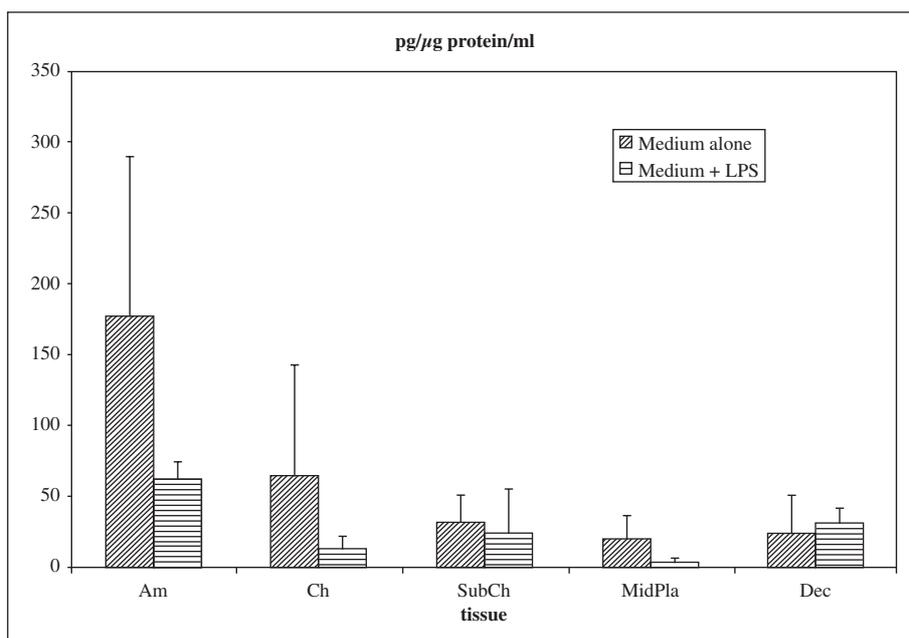


Figure 3

Effect of LPS on the expression levels of IL-1 α in fetal and maternal compartments of preterm placentas. IL-1 α levels were detected in the homogenates of amnion (Am) and chorion (Ch) tissues, and in the homogenates of subchorionic (SubCh), mid-placenta (MidPla) and decidua (Dec), following 10 hours of perfusion with medium alone or medium containing LPS (1 μ g/kg). Results are expressed as mean \pm SD.

placental tissues differently. In addition, LPS distinctly affects IL-1 α production by the different compartments of term placentas. The levels of IL-1 α were higher in the chorion and amnion of both term and preterm placentas, as compared to other placental compartments. These results suggest the possible involvement of IL-1 α , as a paracrine factor (intracellular or membrane-associated) of the pla-

cental tissues, in the process of initiation of labor. The increased levels in the preterm placental tissues could be a result of the preterm process or as a signal inducing this process. The significantly high levels of IL-1 α , mainly in the chorionic tissues, may indicate a key role of this compartment in the process of parturition, and specifically in preterm labor.

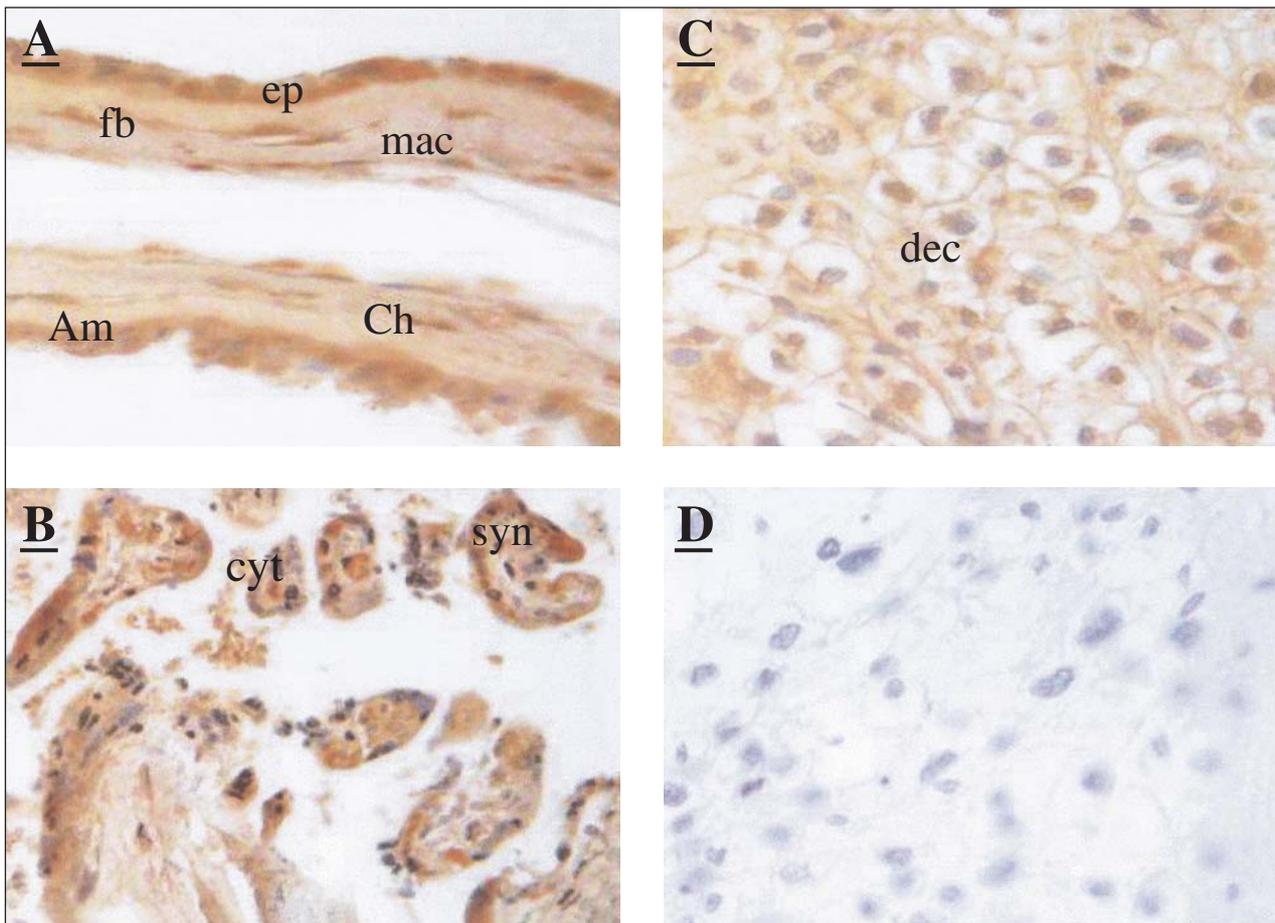


Figure 4

Localization of IL-1 α in the fetal and maternal compartments of term and preterm placentas. Immunohistochemical staining of IL-1 α in the epithelial cells (ep) of the amnion tissue (Am), and in the fibroblasts (fb) and macrophages (mac) of the chorion tissue (Ch) (Figure A). In the other placental sites: subchorionic and mid-placenta (B), and decidua (C), IL-1 α was expressed mainly in cytotrophoblasts (cyt), syncytiotrophoblasts (syn) and decidual cells (dec) respectively. Negative controls (D). Magnification of the slides was $\times 200$.

On the other hand, LPS did not significantly induce any compartment of preterm placental tissues to produce IL-1 α , although IL-1 α was significantly increased in decidual tissue attached to the placenta and decreased the mid-placental tissues of term placentas. We suggest that IL-1 α , in preterm placental tissues, might have been presenting under maximal (optimal) expression, and therefore stimulation with LPS did not affect this expression. However, in term placentas, the levels of IL-1 α are basal (low). On the other hand, the only placental compartments which were affected by LPS were decidua and mid-placental tissues. Decidual tissue contains macrophages and therefore, it responded to LPS by increasing IL-1 α production. These results are in harmony with other studies showing strong IL-1 production by decidual macrophages [37]. As this compartment has low IL-1 α levels, it may be speculated that placenta needs to be more mature in order to be involved in cytokine production. Since IL-1 α could be expressed as a membrane-associated molecule, it is possible that this form of IL-1 may be secreted into the placental microenvironment after LPS induction (in our case into the perfusate, which is now under examination). Therefore, after the shedding of this membrane-associated IL-1, we could not find any induced levels in all placental compartment tissues. Indeed, *in vitro* studies have demonstrated an increased secretion of various pro-inflammatory

cytokines such as IL-1, IL-6 and TNF by LPS-stimulated decidual explants and cells [38]. It has been previously reported and suggested that microorganisms, or their products, may enter the fetomaternal compartment and recruit the decidual macrophages that produce inflammatory cytokines [6]. In the present study, we have shown that not only was decidual tissue (maternal compartment) induced by LPS to increase IL-1 α production, but the mid-placental tissue (fetal compartment) was too. The capacity of placental tissues to produce IL-1 α in selected parts of the placenta, under infection conditions, may indicate the possible involvement of IL-1 α in the preterm labor process related to infection. IL-1 α could affect placental tissue activity directly or indirectly through the induction of various pro-inflammatory cytokines such as growth factors, adhesion molecules, cytokine receptors, acute phase protein and prostaglandins. Recently, IL-1 was demonstrated to induce COX-2 gene expression and prostaglandin synthesis in various cultured cells and placental tissue explants [39, 40]. In addition, stimulation of amnion cells with IL-1 α increased their capacity to produce PGE₂, which promotes uterine contractions [41-43]. Also, IL-1 α was shown to reduce IL-1-induced prostaglandin biosynthesis by human amnion and chorion and prevent IL-1-induced preterm parturition in mice [33, 44].

Our results may also indicate that the source of IL-1 α and other cytokines in the amniotic fluid (which is in close contact with chorion, amnion and decidual tissues), could be these placental compartments themselves.

Our immunohistochemical studies support our suggestion that the cellular origins of IL-1 in the placental tissues are the trophoblasts, syncytiotrophoblasts, endothelial cells and stromal cells, in addition to decidual cells.

Both IL-1 α and IL-1 β have been demonstrated in endometrial macrophages and endothelial cells [45-47]. In addition, high levels of these cytokines in conditioned media of cultured embryos correlated with successful implantation [46, 47].

Thus, the results demonstrate IL-1RI expression in the maternal-embryonic interface [34]. This may suggest a possible key role of the IL-1 system (produced by both fetal and maternal compartments) in the regulation of normal and pathological pregnancy, and parturition.

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REFERENCES

- Dinarelo CA. 1996. Biological basis for Interleukin-1 in disease. *Blood* 87: 2095.
- Entrican G. 2002. Immune regulation during pregnancy and host-pathogen interactions in infectious abortion. *J. Comp. Pathol.* 126: 79.
- Yang ZM, Chen DB, Le SP, Harper MJ. 1995. Interleukin-1 α in the rabbit uterus during early pregnancy. *Early Pregnancy* 1: 201.
- Robertson SA, Seamark RF, Guilbert LJ, Wegmann TG. 1994. The role of cytokines in gestation. *Crit. Rev. Immunol.* 14: 239.
- Frank GR, Brar AK, Jikihara H, Cedars MI, Handwerger MS. 1995. Interleukin-1 β and the endometrium: an inhibitor of stromal cell differentiation and possible autoregulator of decidualization in humans. *Biol. Reprod.* 52: 184.
- Dudley DJ. 1997. Preterm labor: intrauterine inflammatory response syndrom. *J Reprod Immunol* 36: 93.
- Roux-Lombard P. 1998. The interleukins-1 family. *Eur. Cytokine Netw.* 9: 565.
- Eisenberg SP, Evans RJ, Arend WP, Verderber E, Brewer MT, Hannum CH, Thompson RC. 1990. Primary structure and functional expression from complementary DNA of a human interleukin-1 receptor antagonist. *Nature* 343: 341.
- Carter DB, Deibel MR Jr, Dunn CJ, Tomich CS, Laborde AL, Slightom JL, Berger AE, Bienkowski MJ, Sun FF, McEwan RN, et al. 1990. Purification, cloning, expression and biological characterization of interleukins-1 receptor antagonist protein. *Nature* 344: 633.
- Cooper RL, Goldenberg RL, Greasy RK, DuBard MB, Davis RO, Entman SS, et al. 1993. A multicenter study of preterm birth weight and gestational age-specific mortality. *Am. J. Obstet. Gynecol.* 168:78.
- Romero R, Sirtori M, Oyarzum E, et al. 1989. Infection and labor V: prevalence, microbiology and clinical significance of intra-amniotic infection in women with preterm labor and intact membranes. *Am. J. Obstet. Gynecol.* 161: 817.
- Saji F, Samejima Y, Kamimura S, Sawai K, Shimoya K, Kimura T. 2000. Cytokine production in chorioamnionitis. *J. Reprod. Immunol.* 47: 185.
- Kauma SW, Walsh SW, Nestler JE, Turner TT. 1992. Interleukin-1 is induced in the human placenta by endotoxin and isolation procedures for trophoblasts. *J. Clin. Endocrinol. Metabol.* 75: 951.
- Taniguchi T, Matsuzaki N, Kameda T, Shimoya K, Jo T, Saji F, Tanizawa O. 1991. The enhanced production of placental interleukin-1 during labor and intrauterine infection. *Am. J. Obstet. Gynecol.* 165: 131.
- Lundin-Schiller S, Mitchell MD. 1991. Prostaglandin production by human chorion leave cells in response to inflammatory mediators. *Placenta* 12: 353.
- Bennett WA, Terrone DA, Rinehart BK, Kassab S, Martin JN, Granger JP. 2000. Intrauterine endotoxin infusion in rat pregnancy induces preterm delivery and increases prostaglandin F2 α metabolite levels. *Am. J. Obstet. Gynecol.* 182: 1496S.
- Kaga N, Katshuki Y, Obata M, Shibutani Y. 1996. Repeated administration of low-dose lipopolysaccharide induces preterm delivery in mice: a model for human preterm parturition and for assessment of the therapeutic ability of drugs against preterm delivery. *Am. J. Obstet. Gynecol.* 174: 754.
- Mussalli GM, Blanchard R, Brunnert SR, Hirsch E. 1999. Inflammatory cytokines in a murine model of infection-induced preterm labor: cause or effect ?. *J. Soc. Gynecol. Invest.* 6: 188.
- Jokhi PP, King A, Loke YW. 1997. Cytokine production and cytokine receptor expression by cells of the human first trimester placental-uterine interface. *Cytokine* 9: 126.
- Main EK, Strizki J, Sochet P. 1987. Placental production of immunoregulatory factors: trophoblast is a source of interleukin-1. In Miller RK, Thiede HA (Eds) *Trophoblast research*, vol 2, Plenum Press, New York, pp.149.
- Romero R, Mazor M, Brandt F, Sepulveda W, Avila C, Cotton DB, Dinarelo CA. 1992. Interleukin-1 α and interleukin-1 β in preterm and term human parturition. *Am. J. Reprod. Immunol.* 27: 117.
- McDuffi RSJ, Sherman MP, Gibbs RS. 1992. Amniotic fluid tumor necrosis factor- α and interleukin-1 in a rabbit model of bacterially induced preterm pregnancy loss. *Am. J. Obstet. Gynecol.* 167: 1583.
- Fidel PLJ, Romero R, Wolf N, Cutright J, Ramirez M, Aranedo H, Cotton DB. 1994. Systemic and local cytokine profiles in endotoxin-induced preterm parturition in mice. *Am. J. Obstet. Gynecol.* 170: 1467.
- Osghiro B, Silver RM, Dudley DJ, Mitchell MD, Branch DW. 1994. The mouse as an investigational model for pre-term labor. *Semin. Reprod. Endocrinol.* 12: 240.
- Gavett MG, Witkin SS, Haluska GJ, Edwards JL. 1994. An experimental model for intraamniotic infection and preterm labor in rhesus monkeys. *Am. J. Obstet. Gynecol.* 171: 1660.
- Liang L, Kover K, Dey SK, Andrews GK. 1996. Regulation of interleukin-6 and interleukin-1 β gene expression in the mouse deciduum. *J. Reprod. Immunol.* 30: 29.
- Silver RM, Edwin SS, Umar F, Dudley DJ, Branch DW, Mitchell MD. 1997. Bacterial lipopolysaccharide-mediated murine fetal death: the role of interleukin-1. *Am. J. Obstet. Gynecol.* 176: 544.
- Fidel PLJ, Romero R, Cutright J, Wolf N, Gomez R, Aranedo H, Ramirez M, Yoon BH. 1997. Treatment with the interleukin-1 receptor antagonist and soluble tumor necrosis factor Fc fusion protein does not prevent endotoxin-induced preterm parturition in mice. *J. Soc. Gynecol. Incest.* 4: 22.
- Romero R, Mazor M, Tartakovsky B. 1991. Systemic administration of interleukin-1 induces preterm parturition in mice. *Am J Obstet Gynecol* 165: 969S.
- Bry K, Hallman M. 1993. Transforming growth factor- β 2 prevents preterm delivery induced by interleukin-1 α and tumor necrosis factor- α in the rabbit. *Am. J. Obstet. Gynecol.* 168: 1318.

31. Baggia S, Gravett MG, Witkin SS, Haluska GJ, Novy MJ. 1996. Interleukin-1 β intra-amniotic infusion induces tumor necrosis factor- α , prostaglandin production, and preterm contractions in pregnant rhesus monkeys. *J. Soc. Gynecol. Invest.* 3: 121.
32. Arck PC, Troutt AB, Clark DA. 1997. Soluble receptors neutralizing TNF- α and IL-1 block stress-triggered murine abortion. *Am. J. Reprod. Immunol.* 37: 262.
33. Romero R, Tartakovsky B. 1992. The natural interleukin-1 receptor antagonist prevents interleukin-1-induced preterm delivery in mice. *Am. J. Obstet. Gynecol.* 167: 1041.
34. Simon C, Frances A, Piquette G, Hendrickson M, Milki A, Polan ML. 1994. Interleukin-1 system in the materno-trophoblast unit in human implantation: immunohistochemical evidence for autocrine/paracrine function. *J. Clin. Endocrin. Metab.* 78: 847.
35. Schneider H, Huch A. 1985. Dual in vitro perfusion of an isolated lobe of human placenta: method and instrumentation. In: Schneider H, Dancis J (Eds). In: *in vitro* perfusion of human placental tissue. Basel: Karger. pp. 40.
36. Huleihel M. 2001. Detection of cytokines by immunohistochemistry. In "Interleukin protocols; Methods in Molecular Medicine". (O'Neill LAJ and Bowie A, eds.). The Humana Press. Inc., Totowa, N.J. 60: 157.
37. Stallmach T, Hebisch G, Joller-Jemelka HI, Orban P, Schwaller J, Engelmann M. 1995. Cytokine production and visualized effects in the feto-maternal unit. Quantitative and topographic data on cytokines during intrauterine disease. *Lab. Invest.* 73: 384-3
38. Kleen JA, Coleman M, Mitchell MD. 1997. The molecular mechanisms of term and preterm labor: Recent progress and clinical implications. *Clin. J. Obstet. Gynecol.* 40: 460.
39. Arslan A, Zingg HH. 1996. Regulation of COX-2 gene expression in rat uterus in vivo and in vitro. *Prostaglandins* 52: 463.
40. Ishihara O, Numari H, Saitoh M, Arai Y, Takanashi H, Kitagawa H, Kinoshita K. 1996. Prostaglandin E2 production by endogenous secretion of interleukin-1 in decidual cells obtained before and after the labor. *Prostaglandins* 52: 199.
41. Furuta I, Yamada H, Sagawa T, Fujimoto S. 2000. Effects of inflammatory cytokines on prostaglandin E2 production from human amnion cells cultured in serum-free condition. *Gynecol. Obstet. Invest.* 49: 93.
42. Romero R, Durum S, Dinarello CA, Oyarzun E, Hobbins JC, Mitchell MD. 1989. Interleukin-1 stimulates prostaglandin biosynthesis by human amnion. *Prostaglandins* 37: 13.
43. Kent ASH, Sun M-Y, Sullivan MHF, Elder MG. 1993. The effects of interleukins 1 α and 1 β on prostaglandin production by cultured human fetal membranes. *Prostaglandins* 46: 51.
44. Romero R, Sepulveda W, Mazor M, *et al.* 1992. The natural interleukin-1 receptor antagonist in term and preterm parturition. *Am. J. Obstet. Gynecol.* 167: 863.
45. Zolti M, Ben-Rafael Z, Meirom R, *et al.* 1991. Cytokines involvement in oocytes and early embryos. *Fertil. Steril.* 56: 265.
46. Sheth KV, Roca GL, Al-Sedairy ST, Parhar RS, Hamilton CJCM, Al-Abdul Jabbar F. 1991. Prediction of successful embryo implantation by measuring interleukin-1 α and immunosuppressive factor(s) in preimplantation embryo culture fluid. *Fertil. Steril.* 55: 952.
47. Baranano RI, Pizza A, Rumi L, Polak de Fried E. 1997. Interleukin-1 beta levels in human embryo culture supernatants and their predictive value for pregnancy. *Early Hum. Dev.* 48: 71.