Perfusion of human term placentas with lipopolysaccharide did not affect the capacity of the fetal and maternal tissues to produce interleukin-10

Mahmoud Huleihel[1, 4], Amash Alaa[1, 3], Sapir Olga[2, 3], Maor E[5], Sharon Levy[1, 3], Miriam Katz[2, 3], Lesly Myatt[6], Holcberg Gershon[2, 3]

[1] Department of Microbiology and Immunology and [2] Department of Obstetrics and Gynecology, [5] Institute of Pathology, Soroka University Medical Center and [3] Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel. [6] University of Cincinnati, College of Medicine, OH, USA.

Correspondence: Mahmoud Huleihel, Ph.D, Department of Microbiology and Immunology, Faculty of Health Sciences, Ben Gurion University of the Negev, Beer-Sheva, Israel. Tel/fax: 972-8-6400932 E-mail: huleihel@bgumail.bgu.ac.il

ABSTRACT. IL-10 is anti-inflammatory cytokine that is involved in the regulation of the pregnancy process. We examined the capacity of fetal and maternal placental tissues from human term placentas, to produce IL-10, in the presence and absence of LPS. The levels of IL-10 were examined (by ELISA and immunohistochemical staining) in the fetal and maternal tissues of human placentas after 10 hours of perfusion, in the presence or absence of lipopolysaccharide (LPS; 1 µg/k"g perfused tissue). We could detect IL-10 in amnion (A; 13.91 ± 11.35 pg/ml) and chorion (CH; 7.85 ± 6.38 pg/ml) tissue homogenates, and in the homogenates of three different sites of the placental tissue compartment (subchorionic placenta (SubCH); 7.39 ± 4.39 pg/ml, mid-placenta (MidPL); 8.9 ± 4.73 pg/ml and decidua (Decid); 16.48 + 11.86 pg/ml). Immunohistochemical studies showed that IL-10 was localized in the epithelial cells of the amnion, and in the fibroblasts and macrophages of the chorion. In the placenta and mid-placental sites, IL-10 is localized mainly in cytotrophoblasts and syncytotrophoblasts. The presence of LPS in the perfusion media of the placentas for 10 hours, did not significantly affect the capacity of the fetal and maternal tissues to produce IL-10. Thus, our results may indicate the involvement of the fetal compartment in the down-regulation of the cell-mediated response of the maternal compartment against the fetus, by producing IL-10 under physiological conditions. Infection/inflammation agents such as LPS did not affect the expression levels of IL-10 in the placenta

Keywords: chorion, amnion, trophoblasts, IL-10, lipopolysaccharide, term delivery

INTRODUCTION

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]. Immunoregulatory molecules in the placental compartments have been shown to be involved in immunological tolerance [2].

Interleukin-10 is considered to be one of the major inhibitors of inflammatory reactions. It down-regulates the production of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α. Also, it interferes with antigen presentation, T cells and natural killer (NK) cell functions [3]. The production of IL-10 is induced by cytokines such as TNF-α and IL-12, prostaglandins and progesterone, factors which are locally produced by placental cells [3-6]. It has been demonstrated that IL-10 prevents naturally occurring fetal loss in a mouse model [7]. It has been argued that IL-10 is a possible mediator of the immunological, pregnancy protective effect of progesterone [8].

Reproductive tract bacterial infection has been implicated as a significant risk factor for preterm delivery [9, 10]. High levels of induced pro-inflammatory cytokines, which may directly or indirectly promote myometrial responsiveness or activity, have been suggested to be behind this process [9-11]. Lipopolysaccharide (LPS) induced inflammatory responses in human gestational tissues, resulting in increased release of proinflammatory cytokines such as IL-1, and phospholipid metabolites such as prostanoids [12-14].

The disregulation of IL-10 production in some reproductive pathologies, including spontaneous abortion [7,15], intrauterine growth restriction [16,17], and intrauterine infection-associated preterm labor [18] high lights the protective role of IL-10 in human pregnancy.

Purified human trophoblasts were shown to secrete physiological amounts of IL-10, *in vitro* [19]. IL-10 expression in placental tissues was demostrated to be gestational age-dependent, and that cytotrophoblasts express IL-10 receptors [20, 21].

In the present study, we have examined the capacity of fetal and maternal placental tissues from human term placentas, to produce IL-10, in a perfusion system, when LPS was present or absent in the perfusion medium.

MATERIALS AND METHODS

Materials: Bovine serum albumin, glucose, lipopolysaccharide (LPS; from *E Coli*, serotype 055:B5; Cat no. L-2880), bicarbonate, casein, urea, Tween 20 and DAB (Sigma Chemicals Co., St. Louis, USA), heparin (Bet-Kama, Israel), gentamycin (Tva, Petah Tekva, Israel), buffered formaldehyde (BioLab, Jerusalem, Israel), recombinant IL-10 (Genzyme Diagnostic, MA, USA), Monoclonal rabbit anti-human IL-10, biotinylated polyclonal anti-human IL-10 (Endogen, MA, USA), polyclonal goat anti-human IL-10 (R&D system, Cat no. AF-217-NA, USA), PBS, M199 (Beit HaEmek, Israel), biotinylated polyclonal rabbit anti-goat antibodies and streptavidin-peroxidase conjugate (Zymed, San Francisco, CA, USA), H_2O_2, ethanol, methanol, xylene (Frutarom LTD, Israel).

Methods: Placentas from 10 uncomplicated, term pregnancies were collected immediately after either vaginal or cesarean delivery. Five of these placentas were perfused with medium alone [M199 medium containing bovine serum albumin (0.1 mg/ml), glucose (1.0 g/L), heparin (20 IU/ml) and Gentamycin (48 µg/ml)], and another five placentas were perfused with medium containing LPS (1 µg/k"g perfused placental tissue), for 10 hours. The perfusion experiments were performed using the method of Schneider and Huch [22]. 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 to 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, PO_2 > 110 mmHg.

Perfusion rates were 4-6 ml/min and 10-12 ml/min in the fetal and maternal circulation, respectively. Lateral pressure 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 included chorion, amnion, subchorionic placental site, mid-placental maternal surface and decidua were collected at the end of the perfusion process (after 10 hours of perfusion) and formalin fixed for IL-10 cellular origin characterization by immunohistochemical staining, or stored at – 70 °C to be homogenized for IL-10 level evaluation.

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

Immunohistochemical staining [23]: 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 goat anti-human IL-10) were applied, blocking of the nonspecific background was achieved with PBS containing 0.05 % casein and suitable normal serum. 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, which were exposed to trypsin, proteinase K, PBS, urea by boiling, or citrate by boiling. The best results were obtained with both primary antibodies after boiling in 6 M urea for 15 min., using polyclonal goat anti-human IL-10 antibodies (100 µg/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 to the PBS/casein solution. The biotinylated antibody (polyclonal rabbit anti-goat) and the streptavidin-peroxidase conjugates were applied according to the suppliers' directions. Blocking of the endogenous peroxidase was performed with 3 % H_2O_2 in 80 % methanol for 15 min. before the streptavidin-peroxidase conjugate was applied. Development was done with 0.06 % DAB, and Mayer's hematoxylin was used for counter-staining. The sections were mounted in Eukitt. Negative controls were performed for each specimen using the normal goat serum/PBS/casein instead of the primary antibodies, or by pre-absorption of the first antibodies with recombinant IL-10 (Figures 2E and F respectively).

Pre-absorption of the first antibodies: Antibody anti-IL-10 (1 µg/ml) was incubated with various concentrations of hrIL-10 (0.1-100 pg/ml). After overnight incubation at 4 °C, the mixture was used as a first antibody to stain amnion tissue (as an example).

Preparation of placental homogenates: Placental homogenates were prepared from 10 placentas. Placental tissues were homogenized in 1 ml cold PBS on ice. 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 examined by Biorad reagent.

The levels of IL-10 in the homogenates of the placental fetal and maternal sites were examined by using ELISA kits specific for human IL-10. Duplicates for each sample and a standard curve between 4-1000 pg/ml were examined.

Results are presented as mean ± SD. Five placentas were untreated, and the other 5 placentas were stimulated with LPS.

RESULTS

Expression of IL-10 in fetal and maternal compartments of term placentas following LPS stimulation. IL-10 levels were detected in amnion (A –; 13.91 ± 11.35 pg/ml) and chorion (CH –; 7.85 ± 6.38 pg/ml) tissue homogenates and in the homogenates of the other three sites of the placental compartments: subchorionic placenta (SubCH); 7.39 ± 4.39 pg/ml, mid-placenta (MidPL); 8.9 ± 4.73 pg/ml and decidua (Decid); 16.48 ± 11.86 pg/ml (Figure 1). The presence of LPS in the perfusion medium of the placentas for 10 hours did not significantly affect the capacity of the amnion; 16.13 ± 15.74 pg/ml, chorion; 21.6 ± 38.67 pg/ml, and the other placental tissues examined: subchorionic placenta; 13.18 ± 10.04 pg/ml, mid placenta; 15.5 ± 13.7 pg/ml, and decidua; 10.9 ± 6.5 pg/ml to

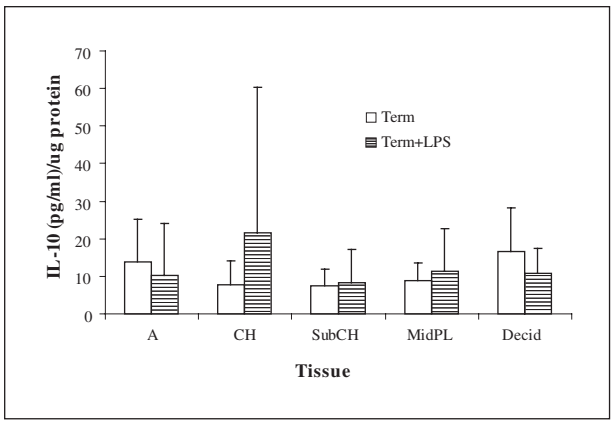

Figure 1
Expression of IL-10 in fetal and maternal compartments of term placentas following LPS stimulation. IL-10 levels were detected in the homogenates of amnion (A) and chorion (CH) tissues and in the homogenates of subchorionic (SubCH) and mid-placenta (MidPL) and decidua (Decid) in the absence (□) or in the presence of LPS (1 mg/kg) (▤) in the perfusion media of the placentas for 10 hours. Results are expressed as mean ± SD. ELISA kit sensitivity was 20 pg/ml.

produce IL-10 (Figure 1). The levels of IL-10 were not significantly different between the various placental tissues in the presence or absence of LPS.

Localization of IL-10 in the fetal and maternal compartments of term placentas. Immunohistochemical studies showed that IL-10 is localized in the epithelial cells of the amnion tissue, and in the fibroblasts and macrophages of the chorion tissue (Figure 2A). In the subchorionic (Figure 2B) and mid-placental tissues (Figure 2C), IL-10 was expressed mainly in cytotrophoblasts and syncytotrophoblasts. In addition, IL-10 was expressed in decidual tissue cells (Figure 2D). No significant difference was shown in the IL-10 levels between the placental tissues (maternal and fetal tissues) perfused in the presence or in the absence of LPS (Figure 2 and data not shown, respectively). Negative controls, using normal serum or PBS showed no staining (Figure 2E), and preabsorption of the primary antibodies with rhIL-10 (50 pg/ml) showed dramatic decrease (amnion tissue is presented; Figure 2D) in IL-10 staining in the examined tissues.

DISCUSSION

This is the first work showing the expression levels of IL-10 in fetal and maternal compartments of term placentas under LPS stimulation, in a system that seems to be closed to the *in vivo* conditions of live placentas,. Our results show that stimulation of placental tissues with LPS

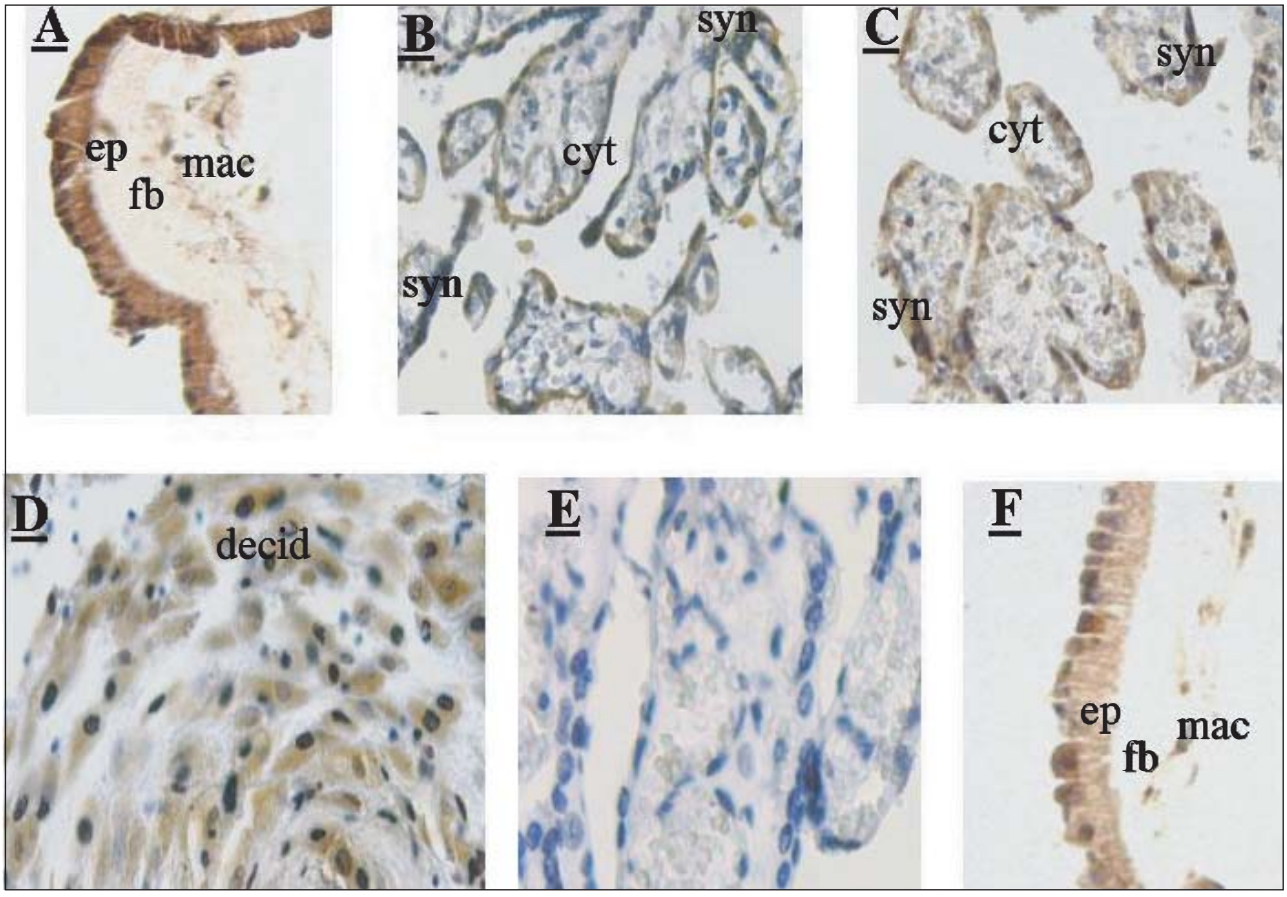

Figure 2
Localization of IL-10 in the fetal and maternal compartments of term placentas perfused with medium alone or in the presence of LPS for 10 hours. Immunohistochemical studies show the localization of IL-10 in the epithelial cells (ep) of the amnion (A), and in the fibroblasts (fb) and macrophages (mac) of the chorion (CH) (A). In the other placental sites: subchorionic (B) and mid-placenta (C), and decidua (D), IL-10 was expressed mainly in cytotrophoblasts (cyt) syncytotrophoblasts (syn) and decidual cells (decid). Negative controls using normal serum and the second antibodies, or preabsorption of the first antibodies with examined tissues (E and F respectively). Magnification of the slides was × 200.

did not affect the capacity of the different placental compartments to produce IL-10.

IL-10 is considered to be an inhibitory factor of the immune response. It decreases immune functions including pro-inflammatory cytokine production [3]. Placental tissues also produce proinflammatory cytokines, in addition to IL-10 during all stages of gestation [18, 24, 25]. In the placenta, IL-10 is regarded as one of the major factors involved in reducing the capacity for maternal-fetal rejection [3, 26, 27]. It has been suggested that IL-10 induces type 2 helper T cell cytokines, through the suppression of IFN-γ production by T and NK cells [3, 25, 26], and of IL-12 by dendretic cells and macrophages [3, 26]. Also, it was shown that IL-10, of cytotrophoblast origin, could suppress IFN-γ secretion in allogenic, mixed lymphocyte reactions [3, 27, 28]. Recently, it was demonstrated that IL-10, in addition to its capacity to decrease the expression levels of co-stimulatory molecules and MHC class I and II antigen in monocytes [24, 29-31], also selectively enhances HLA-G cell-surface expression in human monocytes [31]. In addition, it was demonstrated that IL-10 enhances steady-state levels of HLA-G transcription in cultures of trophoblast cells [31].

Thus, it is possible that the fetal compartments are actively participating in the protection of the fetus against cell-mediated immune responses of the maternal side, by constitutive production of high levels of IL-10. It should be emphasize that IL-10 is not the only suppression factor of this system. Other factors such as IL-4, TGF-β and others, could also participate in this immunosuppression.

More than 20 % of preterm deliveries occur in the presence of infection. There is a growing body of evidence showing that under chorioamnionitis conditions, involving endotoxins of invading bacteria, cytokine production was increased by maternal immune cells and gestational tissues [32]. This hypothesis is supported by various studies, which have demonstrated the secretion of high levels of pro-inflammatory cytokines including TNF-α, IL-1, IL-6 and IL-8 in the amniotic fluid of patients with preterm labor in the setting of infection [33-36]. In addition, injection of LPS into pregnant mice and rats, increased the levels of pro-inflammatory cytokines and prostaglandins, and induced preterm delivery [37-39]. Recently, it was shown that stimulation of decidual cultures and chorion cell cultures, organ cultures of decidua or chorionic villi and choriodecidual explant cultures, with LPS, increased their capacity to produce IL-10 [40-42]. These results are in contrast to our results presented here. This difference could be related to the different systems used in our study (perfused whole placental cotyledon) and their studies (cell monolayers, organ cultures and explants). It should be mentioned that we could not detect IL-10 in the perfusates of our placentas but only in their homogenates, which may indicate that placental tissues behave differently under *in vivo* conditions as compared to *in vitro* conditions. In our present study, the inability of LPS to induce IL-10 production by both the maternal and fetal tissues (as detected by ELISA and IHC) support our suggestion that placental cells of non-leukocyte origin are the source of IL-10, as LPS is well known to be a potent inducer of IL-10 production by leukocytes [4]. In addition, according to our results, it is possible that the levels of IL-10 (or the mechanisms involved in IL-10 production) in the placental tissues examined are present under the maximum expression levels. Thus, additional stimulation (with LPS) is unable to induce more IL-10. On the other hand, the balance between Th1 and Th2 cytokines and the expression of other regulatory molecules (such as HLA-G, integrins and other cytokines), in the placenta, should be considered under physiological and pathological conditions, and not only the expression levels of one cytokine. Also, it could be suggested that using one dose of LPS and examining IL-10 levels after 10 hours of placental perfusion maybe the reason for this maximal effect, even though other cytokines examined such as TNF, IL-1β and IL-6 showed increased levels under these conditions (Huleihel *et al., unpublished data).*

Recently, it was suggested that IL-10 could also regulate placental morphogenesis, acting to delay expansion of the placental labyrinth and to modify the architecture of the maternal blood sinuses [43].

In conclusion, the fetal compartment seems to play an active role in prohibiting fetal rejection. It seems that placental IL-10 is not the main signal involved in the preterm delivery related to maternal genital infection. Other mechanisms should be considered for a better understanding the mechanisms and signals involved in this process.

ACKNOWLEDGEMENTS. * This work was partially supported by a grant (No.97-00368) from the Israel-USA Binational Science Foundation (BSF).

REFERENCES

1. Dinarello CA. 1996. Biological basis for Interleukin-1 in disease. *Blood* 87: 2095.
2. Entrican G. 2002. Immune regulation during pregnancy and host-pathogen interactions in infectious abortion. *J. Comp. Pathol.* 126: 79.
3. Moore KW, O'Garra O, de Waal Malefyt R, Viera P, Mosmann TR. 1993 Interleukin-10. *Ann Rev Immunol* 11: 165-90.
4. P.M. Daftarian, A. Kumar, M. Kryworuchko, F. Diaz-Mitoma. 1996. IL-10 production is enhanced in human T cells by IL-12 and IL-6 in monocytes by tumor necrosis factor-α. *J. Immunol.* 157: 12.
5. Shreedhar V, GieseT, Sung VW, Ullrich SE. 1998. A cytokine cascade including prostaglandin E2, IL-4, and IL-10 is responsible for UV-induced systemic immune suppression. *J. Immunol.* 160: 3783.
6. Woiciechowsky C, Asadullah K, Nestler D, Eberhardt B, Platzer C, Schoning B, Glockner F, Lankesh WR, Volk H-D, Docke W-D. 1998. Sympathetic activation triggers systemic interleukin-10 release in immunodepression induced brain injury. *Nat. Med.* 4: 808.
7. Chaouat G, Meliani AA, Martal G, Raghupathy R, Elliot J, Mosmann T, Wegmann TG. 1995. IL-10 prevents naturally occuring fetal loss in the CBA X DBA/2 mating combination, and local defect in IL-10 production in this abortion-prone combination is corrected by in vivo injection of IFN-tau. *J. Immunol.* 154: 4261.
8. Hashii K, Fujiwara H, Yoshioka S, Kataoka N, Yamada S, Hirano T, Mori T, Fujii S, Maeda M. 1998. Peripheral blood mononuclear cells stimulate progesterone production by luteal cells derived from pregnant and non-pregnant women: possible involvement of interleukin-4 and interleukin-10 in corpus luteum function and differentiation. *Hum. Reprod.* 13: 2738.
9. 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.

10. Dudley DJ. 1997. Preterm labor: an intra-uterine inflammatory response syndrom ? *J. Reprod. Immunol.* 36: 93.

11. Farrugia W, Nichollas L, Rice GE. 1999. Effect of bacterial endotoxin on the in vitro release of type II phospholipase – A2 and prostaglandin E2 from human placenta. *J. Endocrinol.* 160: 291.

12. 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. Endocrinnol. Metabol.* 75: 951.

13. 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.

14. Lundin-Schiller S, Mitchell MD. 1991. Prostaglandin production by human chorion leave cells in response to inflammatory mediators. *Placenta* 12: 353.

15. Downing LG, Strieter RM, Kadell AM, Wilke CA, Austin JC, Hare BD, Burdick MD, Greenfield LJ, Wakefield TW. 1998. IL-10 regulates thrombus-induced vein wall inflammation and thrombosis. *J. Immunol.* 161: 1471.

16. Rivera DL, Olister SM, Liu X, Thompson JH, Zhang ZJ, Pennline K, Aznero R, Clark DA, Miller MJ. 1998. Interleukin-10 attenuates experimental fetal growth restriction and demise. *FASEB. J.* 12: 189.

17. Heyborne KD, McGregor JA, Henry G, *et al.* 1994. Interleukin-10 in amniotic fluid at mid-trimester: immune activation and suppression in relation to fetal growth. *Am. J. Obstet. Gynecol.* 171: 55.

18. Greig PC, Herbert WN, Robinette BL, Teot LA. 1995. Amniotic fluid interleukin-10 concentrations increase through pregnancy and are elevated in patients with preterm labor associated with intrauterine infection. *Am. J. Obstet. Gynecol.* 173: 1223.

19. Bennett WA, Lagoo-Deenadayalan S, Brackin MN, Hale E, Cowan BD. 1996. Cytokine expression by models of human trophoblast as assessed by a semiquantitative reverse transcription polymerase chain reaction technique. *Am. J. Reprod. Immunol.* 36: 285.

20. Szony BJ, Bata-Csorgo Z, Bartfai G, Kemeny L, Dobozy A, Kovacs L. 1999. Interleukin-10 receptors are expressed by basement membrane anchored, alpha(6) integrin (+) cytotrophoblast cells in early human placenta. *Mol. Hum. Reprod.* 11: 1059.

21. Hanna N, Hanna I, Hleb M, Wagner E, Dougherty J, Balkundi D, Padbury JF, Sharma S. 2000. Gestational age-dependent expression of interleukin-10 and its receptor in human placental tissues and isolated cytotrophoblasts. *J. Immunol.* 164: 5721.

22. Schneider H, Huch A. Dual in vitro perfusion of an isolated lobe of human placenta: method and instrumentation. In: Schneider H, Dancis J (Eds.). In vitro perfusion of human placental tissu. Basel, Karger, 1985, pp. 40.

23. Huleihel M. Detection of cytokines by immunohistochemistry, in: O'Neill LAJ, Bowie A (Eds.). Interleukin protocols; Methods in Molecular Medicine. The Humana Press. Inc., Totowa, N.J, 60, 2001, pp. 157.

24. Marzi M, Vigano A, Trabattoni D, Villa ML, Salvaggio A, Clerici E. 1996. Characterization of type 1 and type 2 cytokine production profile in physiologic and pathologic human pregnancy. *Clin. Exp. Immunol.* 106: 127.

25. Bennett WA, Lagoo-Deenadayalan M, Whitworth NS, Stopple JA, Barber WH, Hale E, Brackin MN, Cowan BD. 1999. First-trimester human chorionic villi express both immunoregulatory cytokines: a role for interleukin-10 in regulating the cytokine network of pregancy. *Am. J. Reprod. Immunol.* 41: 70.

26. Wegmann TG, Lin H, Guilbert L, Mosmann TR. 1993. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon ?. *Immunol. Today* 14: 353.

27. Cadet P, Rady PL, Tyring SK, Yandell RB, Hughes TK. 1995. Interleukin-10 messenger RNA in human placenta: Implications for a role for interleukin-10 in fetal allograft protection. *Am. J. Obstet. Gynecol.* 173: 25.

28. Roth L, Corry DB, Locksley RM, Abrams JS, Litton MJ, Fisher SJ. 1996. Human placental cyto-trophoblasts produce the immunosuppressive cytokine interleukin-10. *J. Exp. Med.* 184: 539.

29. Hill JA, Polgar K, Anderson DJ. 1995. T-helper 1-type immunity to trophoblast in women with recurrent spontaneous abortion. *J. Am. Med. Ass.* 273: 1933.

30. Raghupathy R. 1997. T_h1-type immunity is incompatible with successful pregnancy. *Immunol. Today* 18: 478.

31. Moreau P, Adrian-Cabestre F, Menier C, Guiard V, Gourand L, Dausset J, Carosella ED, Paul P. 1999. IL-10 selectively induces HLA-G expression in human trophoblasts and monocytes. *Int. Immunol.* 11: 803.

32. Romero R, Manogue KR, Mitchell MD, Wu YK, Oyarzun E, Hobbins JC, et al. 1998. Infection and labor. IV. Cachectin-tumor necrosis factor in amniotic fluid of women with intraamniotic infection and preterm labor. *Am. J. Obstet. Gynecol.* 161: 336.

33. Romero R, Brody DT, Oyarzun E, Major M, Wu YK, Hobbins JC, *et al.* 1998. Infection and labor. III. Interleukin-1: a signal for the onset of parturition. *Am. J. Obstet. Gynecol.* 160: 1117.

34. Romero R, Avila C, Santhanam U, Sehgal PB. 1990. Amniotic interleukin-6 in preterm labor: association with infection. *J. Clin. Invest.* 85: 1392.

35. Romero R, Ceska M, Avila C, Mazor M, Behnke E, Lindley I. 1990. Neutrophil attractant/activating peptide-1/interleukin-8 in term and preterm parturition. *Am. J. Obstet. Gynecol.* 165: 813.

36. Field PI, Romero R, Maymon E, Hertelendy F. 1998. Bacteria-induced or bacterial product-induced parturition in mice and rabbits is proceded by a significant fall in serum progesterone concentrations. *J. Matern. Fetal. Med.* 7: 222.

37. 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 F2a metabolite levels. *Am. J. Obstet. Gynecol.* 182: 1496.

38. 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 therapautic ability of drugs against preterm delivery. *Am. J. Obstet. Gynecol.* 174: 754.

39. 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.

40. Dudley DJ, Edwin SS, Dangerfield A, Jackson K, Trautman S. 1997. Regulation of decidual cell and chorion cell production of interleukin-10 by purified bacterial products. *Am. J. Reprod. Immunol.* 38: 246.

41. Paradowska E, Blach-Olszewska Z, Gejdel E. 1997. Constitutive and induced cytokine production by human placenta and amniotic membrane at term. *Placenta* 18: 441.

42. Sato TA, Keelan JK, Mitchell MD. 2003. Critical paracrine interactions between TNF-α and IL-10 regulate lipopolysaccharide-stimulated human choriodecidual cytokine and prostaglandin E_2 production. *J. Immunol.* 170: 158.

43. Roberts CT, White CA, Wiemer NG, Ramsay A, Robertson SA. 2003. Altered placental development in interleukin-10 null mutant mice. *Placenta* 24: S94.