Molecular Human Reproduction Vol.9, No.10 pp. 625–629, 2003 DOI: 10.1093/molehr/gag076

In-vitro secretion of proinflammatory cytokines by human amniochorion carrying hyper-responsive gene polymorphisms of tumour necrosis factor-α and interleukin-1β

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The identification of polymorphisms in genes encoding proinflammatory cytokines that affect transcription or the secretion rate has opened new ways to understand the variation in responses to infection during pregnancy. In this study, human amniochorion carrying hyper-responsive alleles of tumour necrosis factor-α (TNF-α: TNF*2 at –308) and interleukin-1β (IL-1β: IL-1*2 at +3953) were stimulated *in vitro* with bacterial lipopolysaccharide (LPS) and compared with tissues carrying the common alleles (TNF*1 and IL-1*1). Fetal membranes carrying the TNF*1 allele displayed an identical dose–response pattern to tissues carrying a TNF*2 allele, except at the highest dose of LPS tested (50 ng/ml) there was a significantly greater production of TNF-α in the presence of a TNF*2 allele. Membranes carrying the IL-1*2 polymorphism secreted IL-1β in a dose–response curve that was different from IL-1* tissues when challenged with 5, 10 and 50 ng/ml LPS. These observations support the hypothesis that reproductive tissues carrying hyper-responsive proinflammatory cytokine genes may over-respond to intrauterine infection secreting higher amounts of cytokines, which in turn, may lead to adverse pregnancy outcomes.

Key words: chorioamnion/IL-1 gene polymorphism/infection during pregnancy/preterm labour/TNF-α gene polymorphism

Introduction

The aetiology of preterm labour is still a matter of debate (Alexander *et al*., 1998). Several clinical trials (Romero *et al*., 1997; Yost and Cox, 2000) link a significant number of preterm labour cases to intrauterine infection. Recent information from research on different experimental models (Gravett *et al*., 1994; Reisenberger *et al*.,1998) has increased the understanding of the molecular and cellular mechanisms triggered by infection. Even though it is clear that genital tract or intrauterine infection is not an obligate condition for development of preterm labour, we are starting to understand how infectious agents interact with the reproductive tissues of the pregnant host and result in the expression of an array of clinical manifestations ranging from vaginal discharge to intra-amniotic infection and maternal and neonatal sepsis (Goldenberg *et al*., 2000). The clinical phenotype of the effect of infection on pregnancy depends on a complex balance between the virulence/pathogenicity of the different microorganisms and the host defence mechanisms (Gomez *et al*., 1997). Variation in individual responses to infection has been recognized for a long time, but the discovery of proinflammatory cytokine gene polymorphisms that affect the transcription of these genes or the secretion rate of the cytokines, has opened new avenues for understanding the pathophysiology underlying the variation in clinical manifestations of infection during pregnancy. The human tumour necrosis factor alpha (TNF-α) gene promoter has a single nucleotide polymorphism named TNF*2, that is present in ~10–25% (McGuire *et al*., 1994; Shu *et al*., 2000) of the population, a single G→A transition at position –308 (Wilson *et al*., 1992). This polymorphism may increase up to 10 times the transcriptional rate of this gene as measured in transfected cells (Wilson *et al*., 1997). The biological and clinical significance of this in-vitro finding is reflected in the association of increased risk to developing preterm labour in patients carrying TNF*2 allele (Aidoo *et al*., 2001) and a higher risk for preterm premature rupture of the membranes(PROM) (Roberts *et al*., 1999). A polymorphism in the interleukin-1β (IL-1β) gene at position +3953 results in an increase in the secretion of this cytokine (Pociot *et al*., 1992). This IL-1β polymorphism has been associated with different disease processes (Caffesse *et al*., 2002; Cvetkovic *et al*., 2002; Rogers *et al*., 2002). These observations support the hypothesis that carriage of polymorphisms conferring 'hyper-responsive' on pro-inflammatory cytokine genes may explain individual variation of the inflammatory response to infection.

TNF-α and IL-1β have been postulated to be key mediators in the genesis of preterm labour through deleterious effects on pregnancy homeostasis. Intra-amniotic infusion of IL-1β or TNF-α in different animal models is followed by labour and these effects are well correlated with the documented effect of IL-1β on uterine activity (Baggia *et al*., 1996). Alternatively, TNF-α and IL-1β can induce a second amplified wave of mediators including prostaglandins (Rauk *et al*., 2000) and matrix metalloproteinases (MMPs) (Arechavaleta-Velasco *et al*., 2002). Prostaglandins can exert further uterotonic effects and augment production of MMPs that are involved in connective tissue degradation in the amniochorion and cervix, leading to membrane rupture and cervical ripening.

No direct evaluation of the capacity of reproductive tissues carrying hyper-responsive alleles to respond to an inflammatory challenge has been conducted. In this study, human amniochorion carrying hyper-responsive alleles of TNF-α and IL-1β were stimulated *in vitro* with bacterial lipopolysaccharide (LPS) and compared with respect to cytokine production with tissues carrying the more common alleles.

Materials and methods

Patients and biological samples

The Internal Review Board of the Instituto Nacional de Perinatologia approved this project (Approval Number 212250-02061). Consecutive patients with uncomplicated pregnancies were included. Women with twin pregnancies, cervical incompetence, polyhidramnios and PROM were not included. General microbiological analyses, including analysis for *Ureaplasma urealyticum*, were performed on the placenta and membranes after extraction. Patients with evidence of infection were not included.

Chorioamnion was obtained from each included patient and a fragment was processed for DNA extraction, after blood clots and maternal decidua were eliminated. The IL-1β and TNF-α genotypes were determined. Membranes were maintained in culture for in-vitro stimulation as described in the next section. Once the gene polymorphisms were defined, membranes were allocated according to the genotypes of both cytokine genes. The code of the polymorphism for each sample was not revealed to the laboratory personnel who carried out the biochemical assays. In order to control for the potential effect of IL-1β genotype on TNF-α secretion by membranes carrying either TNF*1 or TNF*2 polymorphisms, only homozygous IL-1β*1 membranes were used in these experiments. The same design was followed when the IL-1β response was assayed, with homozygous TNF*1 membranes being used.

Sample size calculation was based on previous results already reported for TNF-α and IL-1β secretion by LPS-stimulated mononuclear cells (Pociot *et al*., 1992; Bouma *et al*., 1996) considering differences in mean and standard deviation. We accepted a power of 0.8 and an alpha value of 0.05; calculations revealed that four membranes per group were sufficient for TNF-α analysis and three membranes per group were needed for IL-1 comparison.

TNF-α and IL-1β polymorphism gene identification

Genomic DNA was extracted from 2.0 g of membranes using DNAzol according to the manufacturer's instructions (Gibco BRL, Gaithersburg, MD, USA). Identification of TNF-α alleles was carried out using a PCR according to (Wilson *et al*., 1992) by amplification of a 108 bp segment that includes position –308 of the TNF-α gene (forward primer: AGGCAATAGGTTTT-GAGGGCCAT; reverse primer: TCCTCCCTGCTCCGATTCCG). The protocol included 35 cycles of 94°C denaturation, 60°C annealing and 72°C elongation for 30 s each. At the end of the procedure, 10 µl of each sample were digested with 4 IU of *Nco*I restriction enzyme (Roche Molecular Biochem, Mannheim, Germany) for 24 h at 37°C, followed by agarose gel electrophoresis. This resulted in the identification of two bands of 82 and 20 bp corresponding to the TNF*1 allele or a single 108 bp amplification band that revealed the point mutation in the –308 position corresponding to the TNF*2 allele. The IL-1β alleles were characterized using a similar PCR procedure (Kornman *et al*., 1997) that amplifies a 182 bp fragment including position +3953 of the IL-1β gene (forward primer: CTCAGGTGTCCTCGAAG-AAATCAAA; reverse primer: GCTTTTTTGCTGTGAGTCCCG). At the end of the procedure 10 µl of each sample were digested with 7 IU of *Taq*I restriction enzyme (Roche Molecular Biochem) for 24 h at 65°C, followed by 2.5% agarose gel electrophoresis. This resulted in the identification of two bands of 97 and 85 bp corresponding to the IL-1β*1 allele or a single 182 bp amplification band corresponding to the IL-1β*2 allele.

Fetal membrane explants culture and stimulation

Chorioamnion ex-vivo culture was performed using fresh fetal membranes free of placenta. Membranes were immediately placed in ice-cold phosphate buffered saline and transported to the laboratory within 10 min after extraction. Membranes were washed with phosphate buffered saline and blood clots and adherent maternal decidua were eliminated with sterile cotton gauze. Entire membranes were cut into 10 mm diameter pieces using a punch cutter. Experimental conditions for the amount of tissue, incubation time, tissue viability and functional response were previously established (Arechavaleta-Velasco *et al*., 2002). Briefly, two pieces of tissue were placed into a well of a 24 well tissue culture dish (Costar, New York, NY, USA) in 2 ml of Dulbecco's modified Eagle medium (DMEM; Gibco BRL, Bethesda, MD, USA) supplemented with 10% fetal bovine serum (Gibco BRL, Bethesda, MD, USA), 1 mmol/l sodium pyruvate (Gibco BRL, Bethesda, MD, USA) and antibiotic-antimycotic solution (100 IU/ml penicillin, 100 µg/ml streptomycin and 0.25 µg/ml amphotericin B; Gibco, Bethesda, MD, USA). Tissues were incubated in 5% CO_2/95% air at 37°C. The viability of ex-vivo tissues was followed by the XTT reduction method using the Cytotoxicity Assay (Roche Molecular Biochem). The functionality of tissues was followed by measurement of IL-1β and TNF-α secretion into the media culture.

Membranes were independently stimulated with 0.1, 1.0, 5.0, 10.0 and 50.0 ng/ml of bacterial LPS from *Escherichia coli* serotype 055:B5 (Sigma, St Louis, MO, USA) for 24 h. At the end of stimulation, media samples and tissues were collected and stored at –70°C until assayed. All experiments were repeated at least three times for evidence of reproducibility in each membrane.

Enzyme-linked immunosorbent assay (ELISA)

TNF-α and IL-1β levels in culture media were quantified using a multiple-site, two-step sandwich ELISA using monoclonal antibodies according to the manufacturer's suggestions (Pharmingen, San Diego, CA, USA). Both ELISAs were standardized and validated in our laboratory using internal and external standards. Intra-assay variation was <5% and inter-assay variation was <7% for both systems. The TNF-α limit of detection was 17 pg/ml and the IL-1β limit of detection was 10 pg/ml. TNF-α and IL-1β ELISA results were expressed as pg/mg of protein concentration in media culture.

Statistical analysis

ELISA results are presented as mean ± SD of cytokine concentration. Multiple comparisons between groups were performed by Kruskal–Wallis one-way analysis analysis of variance (ANOVA) on ranks followed by Dunnett's test. A P-value <0.05 was considered the limit for statistical significance.

Results

Ex-vivo cultured explants of choriamnion were fully viable for the first 4 days of incubation. In-vitro stimulation was started within this window and after a stabilization period of 48 h when secretion of IL-1β and TNF-α reached a plateau, since initial manipulation of tissues apparently induced secretion of both cytokines (Figure 1).

Five membranes carrying the TNF*2 polymorphism and 13 membranes carrying the TNF*1 allele were assayed. Membranes carrying a TNF*2 allele were all heterozygous; no homozygous tissues for this allele were identified. TNF-α was secreted into the media by LPS-stimulated membranes in a dose-dependent pattern (Figure 2). Membranes with TNF*1 genotype displayed an identical pattern of response to LPS as did membranes carrying a TNF*2 allele, except at the 50.0 ng/ml LPS dose at which membranes carrying TNF*1secreted 0.67 ± 0.31 pg/mg protein and membranes with a TNF*2 allele secreted 1.42 ± 0.56 pg/mg protein ($P < 0.05$, Kruskal–Wallis, one-way analysis on ranks).

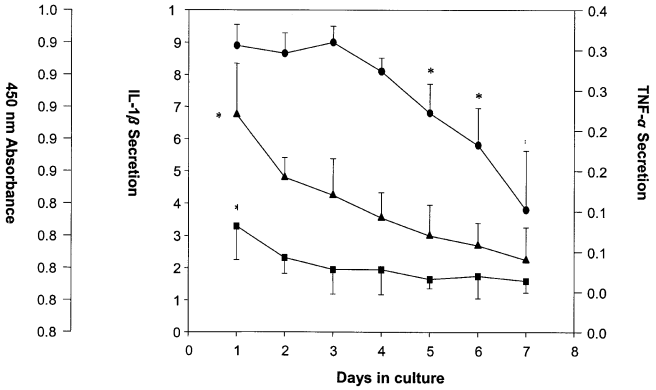

Figure 1. Tissue viability was followed for 7 days in five membranes in triplicate measuring reduction of XTT (450 nm absorbance, closed circles) and functionality was followed by secretion of TNF-α (triangles) and IL-1β (squares). Tissues remained fully viable for 96 h, but after this period viability decreased significantly (*). Secretion of TNF-α and IL-1β was significantly higher during the first day of incubation (*), but after this period a plateau was present for both cytokines. Membranes were used for all experiments on day 2 of culture.

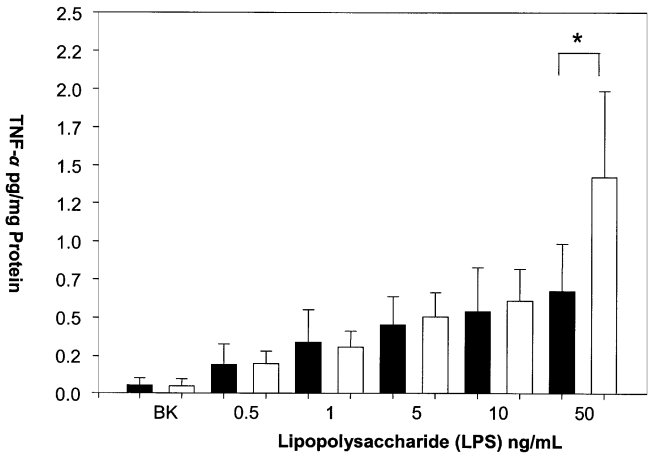

Figure 2. Fetal membranes homozygous for TNF*1 alleles (solid bars, $n = 13$) or heterozygous carrying a TNF*2 allele (open bars, $n = 5$) were stimulated with different concentrations of LPS and secretion of TNF-α into the culture media was measured. A dose–response pattern was documented with significant differences between all doses and the corresponding basal (BK) levels. The response to the dose of 50 ng/ml LPS (*) was significantly different for membranes carrying a TNF*2 allele.

Fourteen membranes carrying the IL-1*1 IL-1β genotype and eight membranes carrying the IL-1*2 polymorphism were stimulated with different concentrations of LPS and a dose–response curve of IL-1β secretion was obtained (Figure 3). Secretion of IL-1β into the media was significantly different at a dose of 5 ng/ml LPS in which allele-1 carrying membranes secreted 1.49 ± 1.76 pg/mg protein and allele-2 membranes secreted 10.50 ± 10.19 pg/mg protein ($P < 0.05$). At a dose of 10.0 ng/ml, LPS induced secretion of 1.38 ± 1.54 pg/mg protein of IL-1β in allele-1 membranes and 15.52 ± 15.99 pg/mg protein in allele-2 membranes ($P < 0.05$). Stimulation with 50.0 ng/ml LPS induced secretion of 2.19 ± 1.87 pg/mg protein in IL-1*1 membranes and 18.78 ±15.68 pg/mg protein in IL-1*2 membranes ($P < 0.05$). Two of the membranes were homozygous for IL-1β*2 and they were included in the same group for analysis purposes.

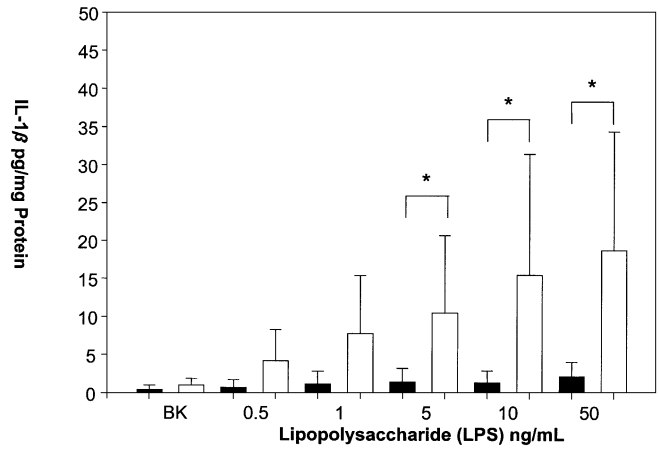

Figure 3. Fetal membranes homozygous for IL-1*1 (solid bars, $n = 14$) or carrying the IL-1*2 allele (open bars, $n = 8$) were stimulated with different concentrations of LPS and secretion of IL-1β into the culture media was measured. A dose–response pattern was documented with significant differences between all doses and the corresponding basal (BK) secretion. Secretion of IL-1β by membranes carrying a IL-1*2 allele was higher than IL-1*1 membranes and significant differences (*) were found at 5.0, 10.0 and 50.0 ng/ml LPS ($P < 0.05$).

DISCUSSION

Although a number of studies clearly link infection to preterm labour, there is scarce information regarding the biological basis of this interaction. Systemic or local responses to bacteria or bacterial products have been explored in some animal models (Witkins *et al*., 1994; Kaga *et al*., 1996; Sakai *et al*., 2001) or in-vitro human tissues (Fortunato *et al*., 1998; Reisenberger *et al*., 1998) and it has been concluded that a network of signals arising from the cellular components of the inflammatory response play a role in the physiopathogenic pathway resulting in preterm labour. A growing body of evidence implicates the mixed effect of cytokines such as TNF-α and IL-1β, that in addition to their role as proinflammatory mediators, also trigger responses in pregnant tissues resulting in secretion of prostaglandins (Kent *et al*., 1993) and direct stimulation of uterine activity (Sadowsky *et al*., 2003) to preterm labour. Furthermore, women carrying specific hyper-responsive polymorphisms in IL-1β and TNF-α genes may have a higher risk for preterm labour or preterm PROM. These findings point to an overactive maternal inflammatory response as being pivotal to the initiation of preterm labour. However, no direct evaluation of the participation of the fetal tissues carrying the 'hyper-responsive' genes has been carried out. Here we provide direct evidence that the presence of the 'hyper-responsive' proinflammatory cytokine gene allele in fetal membranes is correlated with a higher secretion of proinflammatory cytokines upon in-vitro stimulation with bacterial products.

The cultured amniochorion model we are using has been validated previously as a useful tool to study the metabolic response of fetal tissues to several inflammation-related compounds (Fortunato *et al*., 1995; Arechavaleta-Velasco *et al*., 2002). In this study, we measured the amount of TNF-α and IL-1β secreted *in vitro* by fetal membranes stimulated with LPS at a range of concentrations which are compatible with infection (Fortunato *et al*., 1996; Gomez *et al*., 1998) and we compared the amount of secreted cytokines from membranes carrying different alleles for those cytokines. We documented that TNF-α secretion by TNF2-carrying heterozygous membranes was significantly higher only when a higher dose of LPS was used for stimulation. Higher doses of LPS were not tested at levels above 50 ng/ml as they were thought to be excessive.

IL-1 secretion by cultured explants of chorioamnion carrying the IL-1β*2 polymorphism gene was increased dramatically (an average of 10 times) upon stimulation with LPS in comparison with those carrying the more common allele. The presence of one copy of the IL-1*2 allele was sufficient to confer enhanced IL-1 production as there were no significant differences between responses of membranes carrying one or two copies of this allele.

Our observations are consistent with the strong clinical correlation between preterm labour and the presence of the IL-1β*2 reported recently (Genc *et al*., 2002). Collectively, these findings raise the possibility of using IL-1 genotype as a clinical marker to identify women at higher risk of preterm birth. Moreover, our findings using fetal membranes in culture may explain why some but not all pregnancies complicated by infection end in preterm labour, and others are associated with PROM. We postulate that once micro-organisms arrive in the uterine environment, they may trigger a wide inflammatory response in the maternal and fetal compartments involving the secretion of IL-1β among other signals that induce labour. If a woman is a carrier of the IL-1*2 polymorphism-2, the 'hyper-responsive' secretion of the cytokine may potentiate the local inflammatory response in the cervix and uterus. Alternatively, the fetus may be the carrier. Once the fetal membranes are in contact with the microorganisms, an exaggerated inflammatory response affecting mainly chorioamnion may lead to PROM. This hypothesis is reinforced by taking into account that the main source of IL-1β in the chorioamnion is the chorion leave (Menon *et al*., 1995) and this cytokine is a strong inducer of MMP-9 expression in fetal membranes, a key enzyme in connective tissue degradation accompanying PROM (Vadillo-Ortega *et al*., 1995; Parry and Strauss, 1998).

The discovery of genes that contribute to the development of preterm labour and/or PROM may have immediate application to the field of obstetrics as additional tools beyond existing biochemical tests and clinical risk factors are needed to identify women at risk of adverse pregnancy outcomes.

Acknowledgements

This study was supported by CONACyT grants 34743-M and 26177-M, Fellowship 91993 from CONACYT/C.Q.B., Biomedicine Ph.D. Program (to C.H.-G.) and Fellowship COFAA and EDD, IPN (to L.J.-Z.).

References

Aidoo, M., McElroy, P.D., Kolczak, M.S., Terlouw, D.J., ter Kuile, F.O., Nahlen, B., Lal, A.A. and Udhayakumar, V. (2001) Tumor necrosis factor-alpha promoter variant 2 (TNF2) is associated with pre-term delivery, infant mortality and malaria morbidity in western Kenya: Asembo bay Cohort Project IX. *Genet. Epidemiol.*, **21**, 201–211.

Alexander, G.R. (1998) Preterm birth etiology, mechanisms and prevention. *Prenat. Neonat. Med.*, **3**, 3–9.

Arechavaleta-Velasco, F., Ogando, D., Parry, S. and Vadillo-Ortega, F. (2002) Production of matrix metalloproteinase-9 in lipopolysaccharide-stimulated human amnion occurs through an autocrine/paracrine proinflammatory cytokine-dependent system. *Biol. Reprod.*, **67**, 1952–1958.

Baggia, S., Gravett, M.G., Witkin, S.S., Haluska, G.J. and Novy, M.J. (1996) Interleukyn 1-β intra-amniotic infusion induces tumor necrosis factor, prostaglandin production and preterm contractions in pregnant rhesus monkeys. *J. Soc. Gynecol. Invest.*, **3**, 121–126.

Bouma, G., Crusius, J.B., Oudkerk-Pool, M., Kolkman, J.J., Von Blomberg, B.M., Kostense, P.J., Gipharts, M.J., Schreuder, G.M., Meuwissen, S.G. and Pena, A.S. (1996) Secretion of tumor necrosis factor α and lymphotoxin α in relation to polymorphisms in the TNF genes and HLA-DR alleles. Relevance for inflammatory bowel disease. Scand. *J. Immunol.*, **43**, 456–463.

Caffesse, R.G., De La Rosa, R.M., De La Rosa, G.M. and Weltman, R. (2002) Effect of interleukin-1 gene polymorphisms in a periodontal healthy Hispanic population treated with mucogingival surgery. *J. Clin. Periodontol.*, **29**, 177–181.

Cvetkovic, J.T., Wallberg-Jonsson, S., Stegmayr, B., Rantapaa-Dahlqvist, S. and Lefvert, A.K. (2002) Susceptibility for and clinical manifestations of rheumatoid arthritis are associated with polymorphisms of the TNF-alpha, IL-1beta and IL-1Ra genes. *J. Rheumatol.*, **29**, 212–219.

Fortunato, S.J., Menon, R. and Swan, K.F. (1995) Amniochorion: a source of interleukin-8. *Am. J. Reprod. Immunol.*, **34**, 156–162.

Fortunato, S.J., Menon, R.P., Swan, K.F. and Menon, R. (1996) Inflammatory cytokine (interleukins 1, 6, 8 and tumor necrosis factor) release from cultured human fetal membranes in response to endotoxic lipopolysaccharide mirrors amniotic fluid concentrations. *Am. J. Obstet. Gynecol.*, **174**, 1855–1862.

Fortunato, S.J., Lombardi, S.J. and Menon, R. (1998) Immunoreactivity of human fetal membranes to peptidoglycan polysaccharide (PGPS): cytokine response. *J. Perinat. Med.*, **26**, 442–447.

Genc, M.R., Gerber, S., Nesin, M. and Witkin, S.S. (2002) Polymorphism in the interleukin-1 gene complex and spontaneous preterm delivery. *Am. J. Obstet. Gynecol.*, **187**, 157–163.

Goldenberg, R.L., Hauth, J.C. and Andrews, W.W. (2000). Intrauterine infection and preterm delivery. *N. Engl. J. Med.*, **342**, 1500–1507.

Gomez, R., Romero, R., Mazor, M., Ghezzi, F., David, C. and Yoon, B.H. (1997) The role of infection in preterm labor and delivery. In Elder, M.G., Romero, R. and Lamont, R.F. (eds), *Preterm Labor*. Churchill Livingstone, New York, NY, pp. 85–126.

Gomez, R., Romero, R., Ghezzi, F., Yoon, B.H., Mazor, M. and Berry, S.M. (1998) The fetal inflammatory response syndrome. *Am. J. Obstet. Gynecol.*, **179**, 194–202.

Gravett, M.G., Witkin, S.S., Haluska, G.J., Edwars, J.L., Cook, M.J. and Novy, M.J. (1994) An experimental model for intraamniotic infection and preterm labor in rhesus monkey. *Am. J. Obstet. Gynecol.*, **171**, 1660–1667.

Kaga, N., Katsuki, Y., Obata, M. and Shibutani, Y. (1996) Repeated administration of low-dose lipopolisaccharide 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**, 754–759.

Kent, A.S., Sun, M.Y., Sullivan, M.H. and Elder, M.G. (1993) The effects of interleukins 1 alpha and 1 beta on prostaglandin production by cultured human fetal membranes. *Prostaglandins*, **46**, 51–59.

Kornman, K.S., Crane, A., Wang, H.Y., di Giovine, F.S., Newman, M.G., Pirk, F.W., Wilson, T.G., Higginbottom, F.L. and Duff, G.W. (1997) The interleukin-1 genotype as a severity factor in adult periodontal disease. *J. Clin. Periodontol.*, **24**, 72–77.

McGuire, W., Hill, A.V., Allsopp, C.E., Greenwood, B.M. and Kwiatkowski, D. (1994) Variation in the TNF-α promotor region associated with susceptibility to cerebral malaria. *Nature*, **371**, 508–511.

Menon, R., Swan, K.F., Lyden, T.W., Rote, N.S. and Fortunato, S.J. (1995) Expression of inflammatory cytokines (interleukin-1B and interleukin-6) in amniochorion membranes. *Am. J. Obstet. Gynecol.*, **172**, 493–500.

Parry, S. and Strauss, J.F, III (1998) Premature rupture of the fetal membranes. *N. Engl. J. Med.*, **338**, 663–670.

Pociot, F., Molvig, J., Wogensen, L., Worsaae, H. and Nerup, J. (1992) A taq I polymorphism in the human interleukin-1β (IL-1β) gene correlates with IL-1β secretion *in vitro*. *Eur. J. Clin. Invest.*, **22**, 396–402.

Rauk, P.N. and Chiao, J.P. (2000) Interleukin-1 stimulates human uterine prostaglandin production trough induction of cyclooxygenase-2 expression. *Am. J. Reprod. Immunol.*, **43**, 152–159.

Reisenberger, K., Egarter, C., Knofler, M., Schiebel, I., Gregor, H., Hirschl, A.M., Heinze, G. and Husslein, P. (1998) Cytokine and prostaglandin production by amnion cells in response to the addition of different bacteria. *Am. J. Obstet. Gynecol.*, **178**, 50–53.

Roberts, A.K., Monzon-Bordonaba, F., Van Deerlin, P.G., Holder, J., Macones, G.A., Morgan, M.A., Strauss, J.F, III and Parry, S. (1999) Association of polymorphism within the promoter of the tumor necrosis factor alpha gene with increased risk of preterm premature rupture of the fetal membranes. *Am. J. Obstet. Gynecol.*, **180**, 1297–1302.

Rogers, M.A., Figliomeni, L., Baluchova, K., Tan, A.E., Davies, G., Henry, P.J. and Price, P. (2002) Do interleukin-1 polymorphism predict the development of periodontitis or the success of dental implants? *J. Periodontal Res.*, **37**, 37–41.

Romero, R., Gomez, R., Mazor, M., Ghezzi, F. and Yonn, B.H. (1997) The preterm labor syndrome. In Elder, M.G., Romero, R. and Lamont, R.F. (eds), *Preterm Labor*. Churchill Livingstone, New York, NY, pp. 29–49.

Sadowsky, D.W., Novy, M.J., Witkin, S.S. and Gravett, M.G. (2003) Dexamethasone or interleukin-10 blocks interleukin-1β-induced uterine contractions in pregnant rhesus monkeys. *Am. J. Obstet. Gynecol.*, **188**, 252–263

Sakai, M., Tanebe, K., Sasaki, Y., Momma, K., Yoneda, S. and Saito, S. (2001) evaluation of the tocolytic effect of a selective cyclooxygenase 2 inhibitor in a mouse model of lipopolisaccharide-induced preterm delivery. *Mol. Hum. Reprod.*, **7**, 595–602.

Shu, K.H., Lee, S.H., Cheng, C.H., Wu, M.J. and Lian, J.D. (2000) Impact of interleukin-1 receptor antagonist and tumor necrosis factor-alpha gene polymorphism on IgA nephropathy. *Kidney Int.*, **58**, 783–789.

Vadillo-Ortega, F., Gonzalez-Avila, G., Furth, E.E., Lei, H., Muschel, R.J., Stetler-Stevenson, W.G. and Strauss, J.F., III (1995) 92-kd type IV collagenase (matrix metalloproteinase-9) activity in human amniochorion increase with labor. *Am. J. Pathol.*, **146**, 148–156.

Wilson, A.G., di Giovine, F.S., Blakemore, A.I. and Duff, G.W. (1992) Single base polymorphism in the human tumor factor alpha (TNF alpha) gene detectable by NcoI restriction of PCR product. *Hum. Mol. Genet.*, **1**, 353.

Wilson, A.G., Symons, J.A., McDowell, T.L. and McDevitt, H.O. (1997) Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc. Natl Acad. Sci. USA*, **94**, 3195–3199.

Witkins, S.S., Gravett, M.G., Haluska, G.J. and Novy, M.J. (1994) Induction of interleukin-1 receptor antagonist in rhesus monkeys after intraamniotic infection with group B streptococci or interleukin-1 infusion. *Am. J. Obstet. Gynecol.*, **171**, 1668–1672.

Yost, N.P. and Cox, S.M. (2000) Infection and preterm labor. *Clin. Obstet. Gynecol.*, **43**, 759–767.

Submitted on April 7, 2003; accepted on June 11, 2003