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) (Arecchavaleta-Velasco et al., 2002). Prostaglandins can exert further utoerotic 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 Perinatología approved this project (Approval Number 212250-02061). Consecutive patients with uncomplicated pregnancies were included. Women with twin pregnancies, cervical incompetence, polyhydramnios 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 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*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*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*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.

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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.

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 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).

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.

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.
Caffeese, R.G., De La Rosa, R.M., De La Rosa, G.M. and Weltman, R. (2002) Adverse pregnancy outcomes and clinical risk factors are needed to identify women at risk of preterm labour and/or PROM may have immediate application to the field of obstetrics as additional tools beyond existing biochemical tests.

References


