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Perfusion with lipopolysaccharide differently affects the secretion of tumor necrosis factor-α and interleukin-6 by term and preterm human placenta

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Abstract

This study has compared the functional capacity of term and preterm placentas in terms of production of pro-inflammatory cytokines in a perfusion system reflecting their ability to react to inflammatory agents, such as lipopolysacharide (LPS), mimicking the situation of chorioamnionitis. We have demonstrated that term placentas secrete higher levels of tumor necrosis factor (TNF)-α compared with preterm placentas. Moreover, TNF-α secretion was significantly higher after exposure to LPS in the maternal and fetal sides of term placentas. In contrast, in preterm placentas, only the fetal side responded with a significant increase in secretion of TNF-α after exposure to LPS. The maternal side of term placentas secreted significantly higher amounts of interleukin (IL)-6 compared with preterm placentas. Exposure to LPS significantly decreased IL-6 secretion from the maternal side in both term and preterm placentas. Moreover, the fetal side of term placentas secreted significantly lower amounts of IL-6 compared with preterm placentas. In summary, this study has indicated that term and preterm placental tissues have a differing responsiveness to LPS stimulation, with term placentas disposed to a higher TNF-α:IL-6 ratio. Release of cytokines into fetal circulation is less than into the maternal side. However, TNF-α is released into fetal circulation after LPS stimulation and this may be relevant to the etiology of chorioamnionitis.

Keywords: Human placenta; Lipopolysaccharide; Tumor necrosis factor-α; Interleukin-6

1. Introduction

Preterm delivery occurs in ∼5–10% of all pregnancies (Romero et al., 1989). Despite advances in understanding mechanisms involved in preterm birth, this rate has remained relatively constant for decades. Between 30% and 70% of all preterm births are associated with an ascending intrauterine infection in which microorganisms, originating in the vagina, migrate through the choriodecidua and subsequently colonize within the chorion, amnion, amniotic fluid and, ultimately the fetus (Hillier et al., 1988). In response to the infective pathogen, the maternal immune system initiates an inflammatory

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response that frequently results in the onset of preterm labor.

Inflammatory cytokines, such as interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)-α, produced by leukocytes of the mononuclear cell lineage and other cell types, are central mediators of the inflammatory response to bacterial pathogens. These cytokines participate in complex signaling networks by which coordinated host responses are mounted against pathogenic stimuli. In the case of intrauterine bacterial infection in pregnancy, these cytokines may induce changes leading to labor, thus providing a functional link between the pathogenic agent and the means of evacuating an infected body cavity that otherwise might lead to death or serious disability in both mother and fetus.

Human gestational membranes produce a number of pro-inflammatory cytokines including IL-1β, TNF-α, IL-6, and IL-8, both constitutively and in response to inflammatory stimuli as well as bacterial cell wall products such as lipopolysacharide (LPS) (Dudley et al., 1993; Mitchell et al., 1991, 1995; Petraglia et al., 1996). At both term and preterm parturition, there is an increase in production of these pro-inflammatory mediators within the uterus but, in cases of intrauterine infection, this response is significantly increased (Mitchell et al., 1995; Dudley et al., 1996; Romero et al., 1996). Previous studies have shown that LPS treatment of pregnant mice leads to an increase in IL-1β and PGE_2 production by decidual caps in vitro (Silver et al., 1997; Sato et al., 2001) and in preterm delivery (Dudley et al., 1993). Mice treated with LPS also have elevated circulating levels of both TNF-α and IL-10 (Barsig et al., 1995). These findings indicate that cytokines, produced by gestational tissues as a result of LPS, treatment might play an important role in the initiation of preterm labor leading to delivery.

The present study was conducted to investigate the interactions between the pro-inflammatory cytokines, IL-6 and TNF-α, in the fetal and maternal compartments of term and preterm placental tissue upon stimulation with LPS in a perfusion system.

2. Materials and methods

2.1. Placental perfusion

Placentas from 10 uncomplicated term and 8 preterm (32–36 weeks gestation) pregnancies were collected immediately after either vaginal or cesarean delivery. The cord was properly clamped to maintain the dilatation of the fetal vascular system. The perfusion experiments were performed using the method of Schneider and Huch (1985). After careful inspection of the chorionic and decidual surface, an intact cotyledon was selected, as defined by a single chorionic artery and vein with no anastomotic connection to vessels of a neighboring cotyledon and with the corresponding region of decidual surface being nontraumatized.

A fetal artery and corresponding vein from a single cotyledon were cannulated within 15–20 min of delivery. Following successful establishment of the fetal circulation, the placenta was mounted in a perfusion chamber and the maternal circulation simulated by placing four catheters into the intervillous space of the lobe, corresponding to the isolated perfused 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 consisted of M-199 cell culture medium [M-199 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 μg/ml) (Teva, Petah Tekva, Israel). The pH of the medium was adjusted to 7.4 with bicarbonate.

The two reservoirs, containing the perfusion medium for the maternal and fetal circuits, were placed into heated water baths at 37 °C and equilibrated with a pre-humidified gas mixture of 95% oxygen and 5% carbon dioxide on the maternal side and 95% nitrogen and 5% carbon dioxide on the fetal side. A perfusion pressure of 20–30 mmHg was established, giving a flow rate of 6–8 ml/min and 10–12 ml/min in the fetal and maternal circulations, respectively. The venous return could be recycled into the respective reservoir, giving a closed circuit perfusion. Five of term and four of preterm placentas were perfused with medium alone; another five term and four preterm placentas were perfused with medium containing LPS into the maternal reservoir which distributed to the both maternal and fetal compartments [1 μg/kg perfused placental tissue (Sigma)] for 10 h (Huleihel et al., 2004).

2.2. Examination of collected samples by ELISA

Perfusate samples from the fetal and maternal sides were collected in all experiments every 15 min until the end of the perfusion. These samples were stored at −70 °C until examined by ELISA using a specific pair antibodies and recombinant proteins, for TNF-α [mouse monoclonal anti-human TNF-α antibodies (first antibodies) and biotin-conjugated mouse monoclonal anti-human TNF-α antibodies (second antibodies); sen-