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Interleukin-6 and insulin-like growth factor system relationships and differences in the human placenta and fetus from the 35th week of gestation

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Abstract

The integrity of the insulin-like growth factor (IGF) system is essential for normal fetal growth. Cytokine and IGF–IGFBP relationships have been shown in specific tissues, but it is unknown whether these occur in the placenta. We aimed to assess possible differences in the IGF system depending on gestational age (GA) from week 35 to 40, and to study relationships of IL-6 with components of the IGF system in the placenta and newborn infant.

We followed 32 normal births and collected whole villous tissue and cord serum. Total RNA was extracted from the placenta samples, reverse transcribed and then real-time quantitative (TaqMan) RT-PCR was performed to quantify cDNA for IGF-I, IGF-II, IGFBP-1, IGFBP-2 and IL-6. The corresponding proteins were assayed in placenta lysates and cord serum using specific commercial kits. Two groups of subjects (Group 1, 35–37 weeks GA, $N = 12$ and Group 2, 38–40 weeks GA, $N = 20$) were studied. In placenta, IGF-I mRNA was more abundant than IGF-II mRNA at all times and together with IGFBP-1mRNA were less expressed at term. IGFBP-2 and IL-6 mRNAs were higher after week 37 GA. IL-6 and IGFBP-2 gene expression were closely related. The corresponding proteins showed similar differences to the genes but IGF-I was undetectable in the lysates, whereas IGF-II was abundant. IGFBP-2 concentrations were very high and greater than those of IGFBP-1. In the newborn, no difference was seen in any cord serum protein after week 35 GA. IGFBP-1 was negatively correlated with parameters of neonatal size.

In conclusion, this study reports new insights into IL-6, IGF–IGFBP relationships within the human placenta and shows the importance of comparing subjects with the same GA.

Keywords: Interleukin-6; Insulin-like growth factors; Insulin-like growth factor binding proteins; Placenta

1. Introduction

Fetal growth is driven mainly by the IGF system, which consists of two main peptides, IGF-I and IGF-II that have anabolic actions and modulate cell proliferation, differentiation and apoptosis and six principal binding proteins (BPs), which regulate IGF bioavailability.

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Experiments in knockout mice have shown the importance of IGFs for fetal growth as gene deletions for IGF-I and IGF-II are associated with a birth weight even 40% less than in the wild type [1]. Furthermore, similar experiments have suggested that IGF-II and the type I IGF receptor are the most important for fetal growth [2]. From human studies, we know that the IGF-I gene is important as its deletion causes intrauterine growth restriction (IUGR) [3]. IGF-I gene polymorphisms also have been related with birth size [4], and IGF-I receptor mutations have been reported in rare cases of IUGR [5,6].

Moreover, IGF-I knockout mice have a normal placenta, whereas IGF-II knockouts have modifications, and IGF-II receptor knockouts have placental hypertrophy [7]. In recent years, studies on IGFBP transgenic mice have suggested that these may have an effect on placental development too. Mice overexpressing IGFBP-1 have an increased placental mass [8], whereas IGFBP-2 knockout mice have a normal placenta [9].

It is well known that IGF-I and IGF-II are both synthesized in the placenta [2,10–15]. As in this tissue the localization of IGFs and IGFBPs was similar, it was suggested that the IGFBPs regulated IGFs within the placenta [16]. A further study [17] showed that IGF-II was more abundant than IGF-I during all pregnancy, and that all placenta cell types expressed IGFBP-1, IGFBP-2, IGFBP-4 and IGFBP-6 while only some expressed IGFBP-3 and IGFBP-5.

Cytokines may also play an important role in regulating placental formation and growth although they are still poorly studied. The placenta produces pro-inflammatory cytokines as IL-6, IL-8, IL-10 and TNF-α as they are normally present and increase in conditions as corio-amnionitis [18–21]. Human decidua cells, in vitro, secrete IL-6 which increases markedly after stimulation with IL-1α, IL-1β and TNF-α [22]. Very few data have been published in the fetus; blood cultures stimulated with LPS, show an increase in IL-6, IL-1β, TNF-α and G-CSF, [23].

In recent years, cytokine and IGF–IGFBP interactions and relationships have been reported. These relationships are cell- and tissue-specific, and could occur also within the placenta. We know that in transgenic mice overexpressing IL-6, growth is severely impaired, serum IGF-1 concentrations are very low [24,25]. We showed that in inflammatory bowel disease in relapse serum IL-6 and IGFBP-2 were very much increased and the two peptides were positively correlated [26]. In vitro, we also showed that both IL-1 and IL-6 reduced IGFBP-2 and IGFBP-4 concentrations in conditioned media [27]. Subsequently, we observed similar relationships in patients with cystic fibrosis suggesting an overall reduced IGF-I bioactivity [28] This study was undertaken to verify whether there were differences in the IGF system depending on gestational age (GA), from week 35 to 40, and study relationships of IL-6 with the IGF system in the human placenta and newborn at birth.

2. Materials and methods

2.1. Subjects

Thirty-two appropriate for gestational age (GA) births were followed. The definition of appropriate for GA was given based on a normal birth weight (<80th and >10th centile) with respect to the Italian standards of referral [29], a normal pregnancy and the absence of maternal risk factors. Ethnicity was not taken into account. All neonates were delivered by elective cesarean section (CS). No cases with increased blood pressure, gestational diabetes or reduced amount of amniotic fluid were included in the study.

At birth we collected the following information: age of the mother, weight at birth of both parents, body mass index of the mother before pregnancy and at delivery, previous gynecological history, medical history during pregnancy, fetal biophysical data (exact duration of pregnancy, growth trend) clinical data at delivery (indication for caesarean section, neonatal data as sex, weight, length, head circumference, APGAR score, acid–base equilibrium, perinatal data), weight and aspect of the placenta.

The main features are reported in Table 1.

2.2. Collection of biological material

The cord blood was delivered to the laboratory within 20 min, centrifuged (2000 g/min for 10 min at 4 °C) and the sera aliquoted and stored at −80 °C until assayed. Four fragments of perifunicular villous tissue of approximately 5 mm^3 were taken close to the fetal plate, rinsed repeatedly in sterile saline solution at

Table 1
Main features of mothers, placentas and neonates

	Group 1 (35–37 weeks GA)	Group 2 (38–40 weeks GA)
N° (Males/females)	12 (4/8)	20 (8/12)
Age of mothers (yr)	34.5 ± 0.9	34.0 ± 1.1
Weight of placenta (g)	642.7 ± 39.8	590.0 ± 21.9
Weight at birth	2.7 ± 0.1*	3.3 ± 0.1
Length at birth	48.6 ± 0.6	51.1 ± 0.6
Head circumference at birth	33.5 ± 0.4*	35.4 ± 0.4
Ponderal index (g/cm^3)	2.3 ± 0.1	2.4 ± 0.04
Weight newborn/weight placenta (gr)	3.2 ± 9.2.	2.6 ± 2.1

Data are mean ± SEM.
GA, gestational age.
* $P < 0.05$ vs Group 2.