Distribution of interleukin-6 in maternal and embryonic tissues during the first trimester

Eric Jauniaux\(^1\), Beatrice Gulbis\(^2\), Liliane Schandene\(^3\), Julien Collette\(^4\) and Jean Hustin\(^5\)

\(^1\)Academic Department of Obstetrics and Gynaecology, University College London Medical School, London, UK. 
\(^2\)Departments of 2Clinical Chemistry and 3Immunology, Academic Hospital Erasme, Free University of Brussels (ULB), Brussels. 
\(^4\)Laboratory of RadiolImmunology, University of Liege (ULG), Liege and \(^5\)Institute of Pathology, Loverval, Belgium.

Interleukin-6 (IL-6) distribution was investigated in coelomic fluid, amniotic fluid, maternal serum, decidua and placental villous tissue collected from 16 normal pregnancies between 7 and 12 weeks of gestation. IL-6 levels in fluids and tissues were measured by an immunoenzymatic assay and mouse monoclonal antibodies specific for IL-6 were used to localize immunohistochemically IL-6 in decidual and placental tissue. IL-6 was detected in all samples of coelomic and amniotic fluids and in most extracts of placental and decidual tissues. IL-6 concentration was significantly \((P < 0.005)\) higher in the coelomic fluid than in amniotic fluid and was positively correlated with gestational age. Immunostaining for IL-6 was present in both syncytiotrophoblast and extra-villous trophoblast. IL-6 was in significantly \((P < 0.001)\) higher concentration in decidual than in placental tissues. These data indicate that IL-6 is normally present in coelomic and amniotic fluids of early pregnancy and that IL-6 concentrations mainly result from the accumulation of trophoblastic-derived IL-6. During the first trimester IL-6 could play a role in tissue remodelling associated with placenta but also in the haematopoiesis function of the secondary yolk sac and in the generation of new vessels in placental villous tissue.

Key words: coelomic fluid/early placenta/first trimester/interleukin-6/pregnancy

Introduction

Interleukins are immunomodulatory peptides of low molecular weight secreted in very small amounts by a variety of cell types, including activated macrophages or monocytes, fibroblast and endothelial cells (Chantry and Feldmann, 1991; Daiter et al., 1992; Simon et al., 1994). Human interleukin-6 (IL-6) is a 22–29 kDa molecule, originally described as a lymphokine which is now regarded as a major mediator of host response to infection and tissue damage (Kishimoto, 1989). Well-defined biological activities of IL-6 include the induction of B-lymphocyte differentiation into antibody-forming cells and the stimulation of acute-phase proteins in hepatocytes (Kishimoto, 1989).

In the lower genital tract of non-pregnant women, IL-6 is mainly produced by the epithelial cells of the glandular endometrium (Laird et al., 1994) and may play a role in changes that prepare this tissue for implantation and menstrual shedding (Tabibzadeh et al., 1995). During pregnancy, IL-6 continues to be produced by the decidua and its localization in the trophoblast has been demonstrated by Kameda et al. (1990). IL-6 is normally present in low concentrations in the amniotic fluid and fetal serum of non-labouring pregnant women in the second and third trimester (Opsjon et al., 1993; Silver et al., 1993). Activation of IL-6 release stimulates prostaglandin synthesis by uterine tissues, which may mediate parturition in the setting of intrauterine infection (Menon et al., 1995; Yoon et al., 1995). It has also been suggested that IL-6 is involved in growth and development of the normal gestational sac (Silver et al., 1993). In early pregnancy, it is likely that IL-6 is implicated in decidua remodelling during placentation. The aim of this study was to examine the distribution of IL-6 in the decidua, placenta and in the maternal, exocoelomic and amniotic compartments during the first trimester of pregnancy.

Materials and methods

Patients and samples

Placenta, decidua, coelomic fluid, amniotic fluid and maternal venous blood were obtained from 16 healthy patients undergoing elective termination of pregnancy between 7 and 12 weeks of gestation. All pregnancies had been uncomplicated and the fetal heart rate was within normal range at the time of the procedure. Gestational age was determined from the menstrual history and confirmed by an ultrasound scan.

After written informed consent, coelomic and amniotic fluid samples of 1.0 ml minimum were aspirated from the corresponding cavities as previously described (Jauniaux et al., 1991). Coelomic fluid was first aspirated using a 20-gauge needle. Subsequently, a new 20-gauge needle was reintroduced through the guide to aspirate amniotic fluid. The first 0.2 ml of each sample was discarded. Maternal blood samples were collected from an antecubital vein and immediately centrifuged. Placental and decidual tissues were separated.
using a dissecting microscope and small pieces of both tissues were snap frozen in liquid nitrogen. Serum, fluids and tissues were stored at -70°C without preservative until analysed.

**Preparation of tissue samples**

Samples of decidua and placenta were homogenized individually in 3 volumes of Tris-HCl buffer (0.1 M, pH 7.6). The homogenate was centrifuged at 10 000 g for 20 min at 4°C. The supernatant was the source of IL-6.

**IL-6 assay**

Total (free and soluble receptor bound) IL-6 levels in the different samples were determined by a human IL-6 enzyme amplified sensitivity immunoassay following the methodology described by the manufacturer (Medgenix IL-6-EASIA: Fleurus, Belgium). The detection limit of this multiple-site sandwich immunoassay is >3 pg/ml of IL-6. The inter- and intra-assay coefficients of variation were <8%. In tissue, data are expressed as amounts of IL-6/mg protein of the crude homogenate. The protein assay was performed according to the method of Bradford (1976).

**Immunohistochemistry**

Serial frozen sections (4–6 μm thick) were cut and mounted on poly-l-lysine coated glass slides. Six series of samples were selected (one for each gestational week) for immunostaining. The first and last sections of each sample were stained with haematoxylin and eosin for conventional histological examination. Sections were air dried, followed by fixation in cold acetone (100%) for 20 min and washed twice in phosphate-buffered saline (PBS) for 5 min each time. Single immunostaining procedures were performed using two monoclonal antibodies which react with human recombinant IL-6. The first antibody (Anti-IL-6; Cat No 1271121: Boehringer, Mannheim, Germany) was derived from mouse–mouse hybrid cells (clone IL-6–8), whereas the second (Anti-IL-6; Cat No P66301M: Biodesign, Kennebunk, USA) was purified from E. coli. The second antibody was applied at a 1/1000 dilution for 16 h at 4°C. After rinsing with PBS, the sections were incubated for 30 min with a horseradish peroxidase–mouse antihorseradish peroxidase in PBS. After two further washes with PBS, the slides were flooded with a diaminobenzidine–hydrogen peroxide solution and counterstained lightly with Harris haematoxylin. PBS and a mouse IgG (Dako, Copenhagen, Denmark) were used alone to incubate step sections of both tissues and decidual tissues as negative controls for human IL-6. Cell type identification was performed on step sections using monoclonal antibodies against cytokeratine 8 (CAM 5.2; Becton-Dickinson, Eirenbodegem, Belgium) and vimentin (Dako, Copenhagen, Denmark).

**Statistical analysis**

Differences in median IL-6 concentrations in the different compartments were tested by the Kruskal–Wallis rank test. Relationships between IL-6 concentration in fluids and tissues and gestational age were assessed by regression equations which were calculated by the least square methods and their slopes tested for significance by the F-ratio test. Results were considered statistically significant at \( P < 0.05 \).

**Results**

**IL-6 concentrations**

IL-6 was detected in all samples of coelomic and amniotic fluids and extracts of placental and decidual tissues. Only two

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Median (pg/mg protein)</th>
<th>Range (pg/mg protein)</th>
<th>Interquartile range (pg/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decidua</td>
<td>30.3</td>
<td>1.8–164.3</td>
<td>19.1–85.9</td>
</tr>
<tr>
<td>Placenta</td>
<td>4.7</td>
<td>0.9–15.7</td>
<td>5.2–8.1</td>
</tr>
<tr>
<td>Coelomic fluid</td>
<td>87.5</td>
<td>10–340</td>
<td>23–192</td>
</tr>
<tr>
<td>Amniotic fluid</td>
<td>16.5</td>
<td>5–201</td>
<td>12–26.5</td>
</tr>
</tbody>
</table>

**Figure 1. Distribution of IL-6 individual values with gestation age in coelomic fluid.**

samples of maternal serum demonstrated IL-6 concentrations (11 and 98 pg/ml respectively) above the detection limit of the IL-6 assay. Significantly \( (P < 0.001) \) higher levels of IL-6 were found in decidua compared to placental villous tissue (Table I). The IL-6 concentration was also significantly \( (P < 0.005) \) higher in the coelomic fluid than in amniotic fluid. A significant exponential \( (r = 0.61, n = 16, F = 8.4; P = 0.011) \) correlation was found between gestational age and coelomic fluid IL-6 concentration (Figure 1). No trend was observed for IL-6 concentrations between the different compartments.

**Immunohistochemistry**

Syncytiotrophoblast stained intensely for IL-6, whereas the villous cytotrophoblast and the villous stroma were mostly negative (Figure 2A). There was no staining of the endothelium of villous vessels and Hofbauer cells. In the decidua, immunostaining was located in the extravillous trophoblast (Figure 2B). There was a weak immunostaining of the decidual cells, macrophages and the endothelial cells of maternal vessels. In control experiments, no immunostaining was found when primary antibody was omitted. The extravillous trophoblast stained intensely for CK 8 (Figure 2C) whereas decidual and endothelial cells stained for vimentin (Figure 2D).

**Discussion**

There is increasing evidence sustaining a role for cytokines in regulating cell function in human placentation (Tabibzadeh,
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Figure 2. Immunostaining of adnexial tissue at 8 weeks of gestation. 

A Villous tissue showing the staining of the syncytiotrophoblast and syncytial sprouts for IL-6 (arrows) and the absence of staining of the cytotrophoblast (C) and villous stroma (S). B Decidual tissue from the implantation site showing the strong staining of extravillous trophoblastic cells for IL-6 (arrows) around a uteroplacental vessel (V). Note the weak immunostaining of decidual cells and endothelial cells. C Decidual tissue showing the strong staining of the extravillous trophoblast for CK8 (arrows). D Decidual tissue showing the strong staining of the decidual and endothelial cells stained for vimentin. Harris Haematoxylin counterstain (Original magnification: A ×75; B ×400; C ×400; D ×450).

1991; Starkey, 1993). This study indicates that IL-6 is present in greater concentration in the exocoelomic cavity compared to the amniotic cavity or to maternal circulation during the first trimester of normal pregnancies.

IL-6 is produced in vitro by stromal and epithelial cells of the human endometrium in response to stimulation by IL-1, placental protein 14 and tumour necrosis factor (TNF) and has been found to inhibit the proliferation of glandular cells (Tabibzadeh et al., 1989; Laird et al., 1994). In vivo, the architecture of the placental bed and the spatial relationship between the different cell types and the matrix may influence their specific biological role and their interaction. IL-6 has been shown to support the development of haematopoietic progenitor cells in fetuses and newborns (Gardner et al., 1990) and to have a role in angiogenesis (Motro et al., 1990). In-vitro immunohistochemical investigations have also revealed
that trophoblast-derived IL-6 interacts with IL-6 receptors on the trophoblast, stimulating human chorionic gonadotropin release (Nishino et al., 1990).

Our immunohistochemical data, which are consistent with the work of other investigators (Kameda et al., 1990; Nishino et al., 1990), indicate that IL-6 is produced by syncytiotrophoblastic cells as early as 6 weeks of gestation. In addition, we also demonstrated that in the decidua tissue at the implantation site there is a preferential presence of IL-6 in the extravillous trophoblast as defined by their associated immunostaining by CK 8 antibodies (Figure 2). It is difficult to ascertain by immunostaining whether IL-6 production by the extravillous trophoblast is sufficiently high to explain the important difference in its production by the placental and so-called decidual tissues.

Opsjon et al. (1993), using a bioassay, found little or no IL-1, IL-6 or TNF-α in the fluid compartments of the first trimester pregnancy and only found IL-6 in second and third trimester amniotic fluid samples. All our coelomic and amniotic fluid samples demonstrated IL-6 concentrations above the detection limit of the IL-6 assay and the IL-6 coelomic concentration increased with advancing gestational age. Using commercially available assays (ELISA), we also measured the concentration of IL-1, TNF-α, transforming growth factor β2 (TGF) and granulocyte macrophage-colony-stimulating factor (GM-CSF) in embryonic fluids and found no IL-1 (n = 5), TGF (n = 16), GM-CSF (n = 16) in both fluids, whereas TNF-α values in coelomic fluid samples (n = 5; mean = 53 pg/ml; range 10–82 pg/ml) were all above the detection limit of the corresponding assay. The present data suggest that IL-6 levels in coelomic fluid mainly result from the accumulation of placental/decidual-derived IL-6 through unidentified pathways. We have reported previously that the coelomic fluid composition results from an ultrafiltrate of maternal serum with an important contribution of placental and secondary yolk sac specific bioproducts (Jauniaux et al., 1991, 1993; Gulbis et al., 1992). Two-dimensional gel electrophoresis of proteins under 100 kDa indicated no significant qualitative differences between the coelomic and amniotic compartments except for low molecular weight proteins (below 30 kDa), which probably correspond to interleukins or to other small proteins such as relaxin (Jauniaux et al., 1993; Johnson et al., 1994). We suggest that coelomic fluid IL-6 mainly originates from the implantation site where the levels are elevated and that a concentration gradient exists between the implantation site and the early villous tissue.

High IL-6 concentrations have been observed in the amniotic fluid of labouring parturients and of patients in preterm labour with microbial invasion of the amniotic cavity (Yoon et al., 1995). IL-6 is in lower concentration in the amniotic fluid and in higher concentration in the maternal serum of pregnancies complicated by pre-eclampsia (Silver et al., 1993; Greer et al., 1994; Vince et al., 1995). IL-6 has been detected in lower amounts in the serum of neonates than their mother, both when delivered by elective Caesarean section and after spontaneous labour (Opsjon et al., 1993). The mechanism which operates in the elevation of amniotic fluid IL-6 during labour is unknown. The presence of IL-6 in the exocoelomic cavity suggests that in addition to its role in tissue remodelling associated with placental and fetal growth, IL-6 might play a role in the haematopoiesis function of the secondary yolk sac and in the generation of new vessels in placental villous tissue.

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