The cytokine receptor gp130 and its soluble form are under hormonal control in human endometrium and decidua

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The transmembrane protein gp130 plays a central role in cytokine action as a signal transducing receptor subunit common to all interleukin-6 type cytokines. Endometriat tissue obtained from women with a normal menstrual cycle and decidua obtained from women in the first or second trimester of pregnancy were assessed for gp130 by western blotting, immunohistochemistry and enzyme-linked immunosorbent assay (ELISA) analysis. By immunoblotting, two forms of gp130 were detected: one—the soluble form—of ~100 kDa and a larger membrane-bound form of ~150 kDa. The latter became clearly visible in the mid to late secretory phase and was more pronounced in decidual tissue of second trimester compared to first trimester. Immunohistochemically, gp130 was located in glandular epithelial cells during the mid to late secretory phase, whereas staining in the proliferative phase was rather weak. In first and second trimester decidua, glandular cells were also positively stained. In addition, the invading trophoblast cells were gp130 positive. Soluble gp130 release was measured in the supernatants from primary endometrial and decidual cell cultures by ELISA and reached maximum values in cell cultures without addition of hormones. In cultured endometrial epithelial cells obtained during the proliferative phase of the cycle, the soluble gp130 release increased significantly under combined estradiol/progesterone supplementation which mimics the secretory phase conditions compared to estradiol supplementation alone. In cultured epithelial cells derived from decidual tissue of first trimester of pregnancy, similar effects of hormonal regulation were observed. Our results suggest that the balance between soluble gp130 and its membrane-bound form may play an important role in regulating cytokine action necessary for blastocyst implantation and for further interaction between the decidualized endometrium and the invading trophoblast.

Key words: human endometrium/decidua/soluble gp130/IL-6 type cytokines

Introduction

The cytokine receptor gp130 is the common receptor subunit of the interleukin-6 (IL-6) type cytokines. Besides IL-6 itself, IL-11, ciliary neurotrophic factor (CNTF), cardiotrophin (CT-1), oncostatin M (OSM), leukaemia inhibitory factor (LIF), and cardiotrophin-like cytokine (CLC) (Elson et al., 2000) belong to this group of cytokines (Heinrich et al., 1998). IL-6 and IL-11 signal via gp130 homodimers, whereas CNTF, LIF, CT-1, CLC and OSM form heterodimers of gp130 and LIF-R; alternatively, OSM can also signal through heterodimers of gp130 and OSMR (Mosley et al., 1996). IL-6, IL-11 and CNTF first bind to a specific α-receptor subunit not involved in the intracellular signal-transduction cascades.

The significance of IL-6 type cytokines and their receptor molecules for reproduction first became evident in studies by Stewart et al. (1992) showing that embryos of LIF-deficient mice failed to implant due to a maternal defect although viable blastocysts were produced. Mice deficient in IL-11-αα appeared phenotypically normal, but became infertile because of defective decidualization after implantation (Robb et al., 1998). Gene targeting of the common cytokine receptor gp130 and LIF-R resulted in even more severe defects already evident at the embryo development stage. The homozygous gp130 knockout embryos obtained by intercrossing gp130 +/+ heterozygotes died in utero between days 15.5 and 18.5 post coitum as a result of abnormal heart and placenta development and disturbed haematopoiesis (Akira et al., 1995). Gene targeting of the LIF-R (Ware et al., 1995) resulted in perinatal death and severe placenta defects. Targeted disruption of the STAT 3 (signal transducer and activator of transcription 3) gene activated by the IL-6 family of cytokines resulted in a very early embryonic lethality (day 7 post coitum) which might be due to a loss of a combined effect of two or more of these receptor-mediated signals (Takeda et al., 1997).

The functional redundancy of the gp130 cytokines can be explained by the shared use of gp130 as signal transducing element. The extracellular region of gp130, which represents the soluble form, consists of six domains, an amino-terminal Ig-like domain (D1), one cytokine-binding module (CBM) consisting of two fibronectin type III (FNIII)-like domains (D2, D3) and three membrane proximal FNIII-like domains (D4, D5, D6) (Hibi et al., 1990).

Besides the membrane-bound receptors, soluble receptors have been found for several cytokines which interfere with the cytokine response by ligand-binding, thereby acting as antagonists and in rare cases as agonists (Müller-Newen et al., 1996). Soluble human gp130 (sgp130) was identified as a 100 kDa protein, whereas the membrane-bound receptor has a molecular mass of 130–150 kDa. The soluble form has been detected in human serum (Narazaki et al., 1993; Müller-Newen et al., 1998) at a concentration of ~300–400 ng/ml. In general, sgp130 can be generated by translation from an alternatively spliced mRNA or by proteolytic cleavage (Müllberg et al., 1993; Sharkey et al., 1995; Diamant et al., 1997). In human
endometrium it is most likely produced by proteolytic cleavage (Sherwin et al., 2002). The physiological role of sgp130 was suggested to be modulation of IL-6 type cytokine signalling by competing with membrane-anchored gp130 for cytokine binding (Narazaki et al., 1993; Müller-Newen et al., 1998).

The aim of our study was to elucidate the possible role of gp130 and its soluble form in preparing the human endometrium for implantation (Sanchez-Cuenca et al., 1999) and its significance during first trimester pregnancy. For this reason, endometrial tissue from women with a normal menstrual cycle and decidua from women in the first and second trimester of pregnancy were assessed by western blotting and immunohistochemistry. In addition, supernatants from endometrial and decidual cell cultures were analysed by ELISA to investigate a possible hormonal regulation of soluble gp130 release.

Materials and methods

Human tissue samples

Endometrial tissue was obtained throughout the menstrual cycle from normal fertile women undergoing hysterectomy due to benign uterine diseases in collaboration with the Departments of Gynaecology and Obstetrics of Marienhospital, Aachen, Luisenhospital Aachen and St Antonius Hospital, Eschweiler. Dating of each specimen was done by menstrual history, histological examination (Noyes et al., 1950) and hormonal assessment for 17β-estradiol (E₂), progesterone and LH on the day of hystereectomy by routine laboratory diagnostics performed by the Department of Gynaecological Endocrinology and Reproductive Medicine, University Hospital, RWTH Aachen. All patients had a regular menstrual cycle and did not receive hormones for ≥6 months before surgery.

Decidual tissues were collected from legal termination of normal healthy pregnancies in collaboration with the Bourgogneklinik Maastricht, The Netherlands. The informed consent of the patients was obtained.

Patients included in this study were proven to be pregnant by hCG serum test and sonographic control immediately before surgery.

Gestational age was determined by the duration of amenorrhoea, analysis of individual cycle data and by the caput–rump length (CRL) determined by an Elwein potter by adding 400–800 µl ammonium hydroxide [0.15 mol/l NH₄(HCO₃)].

The tissue samples were snap-frozen in liquid nitrogen and stored at −80°C until processed. Tissue blocks were embedded in Tissue-Tek OCT (Miles, USA). Cryostat sections (8–10 µm) were cut on a cryostat Reichert Jung 2800 Picrocut E, mounted on APES (aminopropyltriethoxy-silan)-coated slides and fixed for 10 min in acetone (4°C). Immunohistochemical staining was performed by a streptavidin–biotin–peroxidase method (Histostain®-Plus Kit; Zymed Laboratories Inc., USA) at room temperature. The sections were rehydrated in phosphate-buffered saline (PBS) and the activity of endogenous peroxidase was blocked by incubation with 3% H₂O₂/methanol for 25 min. After blocking with 10% non-immune goat serum (undiluted-10 min), sections were incubated for 1 h with a specific monoclonal mouse antibody B-P4 (Wijdenes et al., 1995) against the membrane-proximal ectodomains (D4-D6) of gp130 (Kurth et al., 2000) which specifically recognizes an epitope in D4 under both denaturing and native conditions (Pflanz et al., 2001).

The antibody B-P4 was provided by Dr John Wijdenes, Diaclon, Besançon, France, but also commercially obtained at Cell Sciences, Inc., USA. B-P4 (1 µg/ml) was diluted 1:50 in the case of decidual tissue and 1:100 in the case of endometrial tissue with PBS/bovine serum albumin (BSA) 1.5%. After washing in PBS, the sections were incubated with a secondary biotinylated goat anti-mouse antibody (Zymed-Kit, undiluted) for 10 min. Streptavidin–peroxidase complex was allowed to bind for 10 min and the antigenic sites were visualized by the chromogen aminoethylcarbazole (AEC). To stain cells of epithelial origin, especially to detect trophoblast cells, an immunohistochemical staining with a monoclonal mouse antibody (clone KL1; Coulter Immunotech, Germany) against cytokeratin 2, 6, 8, 10, 11, 18 and 19 (dilution 1:50, incubation for 1 h at room temperature) was performed on serial sections according to von Rango et al. (2001).

The immunohistochemical procedure was controlled by replacing the primary antibody with non-immune mouse IgG at the same concentration. None of the controls revealed a positive staining. The reproducibility of the results was confirmed by repeating immunohistochemistry twice for each specimen.

The staining intensity for gp130 in the tissue sections was scored by two independent investigators (I.C.L.; U.v.R.). The predominantly epithelial staining was scored as follows: no staining = 0; weak staining and/or single cells strongly stained = 1; moderate staining and/or <50% cells strongly stained = 2; strong staining of >50% cells = 3. The mean values of the scoring for the different phases of the menstrual cycle and first and second trimester of pregnancy were used for statistical analysis.

Protein isolation

Endometrial tissue obtained throughout the menstrual cycle (n = 28; 14 proliferative phase, 14 secretory phase) and decidua tissue fragments from elected termination of normal healthy pregnancies (n = 22; 11 from first trimester, 11 from second trimester) were snap-frozen immediately after surgery in isotonic ammonium hydrogen carbonate [0.15 mol/l NH₄(HCO₃)]. For protein isolation the samples were quickly thawed and homogenized using an Elwein potter by adding 400–800 µl ammonium buffer containing the protease inhibitor “cocktail complete, TM mini” (Roche, Germany). After homogenization, the cells were centrifuged (10 000 × g for 10 min 4°C) and the supernatant was frozen in aliquots for further processing. The total protein concentration of each sample was measured by a Bio-Rad DC protein assay (Bio-Rad Laboratories, Germany).

SDS gel electrophoresis and western blot

The SDS–disc polyacrylamide gel electrophoresis (PAGE) was performed under reducing conditions (5% mercaptoethanol) according to Laemmli (1970). Gels (10%) were used to separate the proteins according to their molecular weights. Each lane was loaded with 30 µg protein. Molecular weight markers used were obtained from Amersham Biosciences, (Germany): marker 1 = ECL DualVue western blotting markers (15–150 kDa), marker 2 = Rainbow™ coloured protein molecular weight markers (14.3–220 kDa). The separated proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Immobilon P, pore diameter 0.45 µm; Millipore, Germany) by a semidry electrolablotting procedure (2 mA/cm²) for 40 min. The membranes were blocked at 4°C overnight in TBS (5 mmol/l Tris-buffered saline, pH 7.6) 0.1% Tween, 5% goat serum. The detection of the immobilized proteins was performed by incubating for 1 h at room temperature with the antibody B-P4 diluted 1:300 in TBS/0.1% Tween followed by 1 h incubation with horseradish peroxidase-conjugated secondary antibody (goat anti-mouse IgG, Santa Cruz, USA; 1:5000 in PBS/0.1% Tween). Immunoreactive proteins were detected by chemiluminescence using the enhanced chemiluminescence (ECL + ) kit (Amersham Pharmacia Biotech, Germany) following the manufacturer’s instructions.

The western blotting was carried out for each sample at least twice.
Cell culture
Epithelial cell culture was performed as described in detail before (Classen-Linke et al., 1997, 1998). The tissue, either endometrial tissue of the proliferative phase or decidual tissue of early pregnancy, was minced under a laminar flow in ~1 mm³ fragments and digested by shaking for 1 h in 0.025% type I A collagenase (470IU/mg; Sigma, Germany) at 37°C in Dulbecco’s modified Eagle’s medium/Ham’s F-12 (ccPro, Germany) without Phenol Red containing 1% streptomycin, penicillin and fungizone and 10% charcoal-treated steroid hormone-free fetal calf serum (ccPro).

Stromal and epithelial cells were separated by two filtration steps (Classen-Linke et al., 1997). The epithelial cell and smaller glandular fragments were digested in a Neubauer haemocytometer and seeded with a density of ~2–5 x 10⁵ cells/cm² on a transparent Biopore membrane with a membrane pore size of 0.4 μm (Millicell CM-filters, 12 mm; Millipore, Germany) coated with the extracellular matrix Matrigel (BD Biosciences, Germany) diluted 1:3 with culture medium without any additions in a dual-chambered Millicell system. From one endometrial specimen, different inserts were seeded with an equivalent amount of epithelial cells and glandular fragments in 100 μl each, after carefully shaking and distributing the cell suspension. One group (two inserts) was treated only with E₂ (Sigma; 10⁻⁸ mol/l); the other group (two inserts) with E₂ (10⁻⁴ mol/l) in combination with medroxyprogesterone acetate (MPA; Sigma, a metabolically stable progestin), at a concentration of 10⁻⁶ mol/l; both hormones diluted in ethanol (<0.1%) and a third group (two inserts) without hormones as a control. Every 2 days the culture medium was changed. Endometrial epithelial cells originating from proliferative phase endometrium were performed from eight different donors and epithelial cell cultures originating from decidua of the first trimester of pregnancy (5–10 weeks) were carried out from seven different donors. As has been shown by earlier immunohistochemical and electron microscopic studies (Classen-Linke et al., 1997; von Rango et al., 2003) the average purity of epithelial cells on Matrigel was ~95% after 10–12 days culture and the epithelial cells became polarized under the culture conditions used. The apical media of the cultured epithelial cells were collected after 6, 8, 10 and 12 days and frozen in aliquots before analysis for soluble gp130 by enzyme-linked immunosorbent assay (ELISA).

sgp130 ELISA
Analysis of the sgp130 in the cell culture supernatant was performed by an established ELISA as described in detail previously (Müller-Newen et al., 1998). The 96-well microtitre plates (F96 Maxisorp immunoplate; Nunc, USA) were coated overnight at room temperature with anti-sgp130 mAb B-4 (0.5 μg/well in PBS). After incubation with saturation buffer (0.1% (w/v) BSA in PBS), the samples or standards (in PBS, 1% BSA) and the biotinylated recombinant sgp130 (300 ng/ml) from baculovirus-infected insect cells was used as a standard. The standard curve was obtained by 2-fold serial dilutions of sgp130. All samples were investigated twice using two different dilutions. The detection limit of the sgp130 ELISA was <40 pg/ml; the inter-assay variation was 8.5% and the intra-assay variation 4.4%.

Statistics
Statistical analysis was performed with GraphPad PRISM version 3.00 for Windows (GraphPad Software, USA). For the ELISA data the mean and SEM were calculated. The relative increase or decrease of sgp130 release in cell culture compared to E₂ substitution alone was calculated for each cell culture as percentage. Although considerable variations between individual cell cultures occurred, the same tendency of change relative to E₂ substitution became obvious. Differences in sgp130 protein content were confirmed to be a normal distribution as assessed by Kolmogorov–Smirnov test. Therefore differences in sgp130 protein content were tested by the paired Student’s two-tailed t-test. Statistical analysis of the immunohistochemical scoring of gp130 in the first and second trimester of pregnancy was performed by the non-parametric Mann–Whitney test for two groups, and differences between the different phases during the menstrual cycle were analysed by the Kruskal–Wallis test. P < 0.05 were accepted as statistically significant.

Results
Immunohistochemical detection of gp130 in endometrial and decidual tissues
Using a monoclonal antibody against the ectodomain of gp130 (Wijdenes et al., 1995), a very intense staining in the endometrial glandular epithelial cells is detected during the mid to late secretory phase (days 19–26; Figure 1b, d, e), whereas the staining of the proliferative phase endometrium (days 6–14; Figure 1a) is rather weak (Figure 2). Localization of gp130 was at the apical and basolateral membrane as well as in the cytoplasm. No stromal staining could be detected (Figure 1).

The glandular epithelial cells are weakly stained in the decidua of first trimester (Figure 3a, c) but staining becomes more pronounced in the second trimester of pregnancy (Figure 3c, g and Figure 4). In addition, the invading trophoblast cells in the decidua basalis which are identified by cytokeratin staining on serial sections (Figure 3d, h) are positive for gp130 (Figure 3c, g).

Detection of soluble and membrane-bound gp130 by western blot
Western blots were performed using the supernatant of homogenized endometrial and decidual tissues. On western blots, two forms of gp130 were detected (Figures 5 and 6). One form of ~100 kDa representing the soluble form, and one form of ~150 kDa representing the membrane-bound form. The soluble gp130 which is ~100 kDa represents the glycosylated ectodomain and has the molecular mass expected for gp130 lacking the transmembrane and cytoplasmic parts which are ~30 kDa. The membrane-bound form of gp130 has an observed molecular mass of 130–150 kDa (Heinrich et al., 1998). A positive control was used (Figure 7) to verify that the monoclonal antibody B-P4 is specific for gp130. For this reason, insect cells were infected with a recombinant baculovirus encoding the human gp130 ectodomain (amino acids 1–606); the supernatant of these cells was subjected after immunopurification to SDS–PAGE 10% and immunoblotted using the monoclonal antibody B-P4. The recombinant soluble gp130 which was used as control is not or only weakly glycosylated and has a molecular mass of 65–75 kDa which corresponds to the molecular mass calculated from the amino acid sequence of the gp130 ectodomain (Müller-Newen et al., 1998). In mammalian cells, gp130 is highly glycosylated which accounts for the additional 25–35 kDa.

In human endometrium, in the proliferative phase, no or only a weak gp130 signal can be obtained (Figure 5). The membrane-bound and the soluble gp130 became clearly visible from day 19 onward until the late secretory phase (Figure 5) confirming the immunohistochemical results showing intense staining of the endometrial glands during mid to late secretory phase (Figure 1b, d, e).

In decidual tissue, both the short soluble form of ~100 kDa and the longer membrane-bound form (150 kDa) can be distinguished (Figure 6).

During the early first trimester pregnancy the soluble form predominates, whereas the membrane-bound form shows only weak staining until pregnancy week 10. At pregnancy week 11 the longer membrane-bound form increases. This increase proceeds during the second trimester (13–17 weeks after the last menstrual period) showing inter-individual variation. This result confirms the immunohistochemical data showing stronger immunoreaction of

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glands in the second trimester and the greater amount of invading cytotrophoblast cells in the decidua basalis expressing gp130.

**Hormonal regulation of sgp130 in epithelial cell culture**

By ELISA analysis of the supernatants of cultured epithelial cells originating either from proliferative endometrial tissue or from decidual tissue of first trimester of pregnancy, the release of soluble gp130 was measured. Absolute values were in the range of 600–6000 pg/ml. The amount of release varied under different hormonal treatment protocols. For endometrial epithelial cells the culture supernatant was measured by ELISA after 8, 10 and 12 days; for decidual epithelial cells after 6, 8 and 10 days. Different time points had been chosen to look for time-dependent differences. During the cell culture the cells and their viability were checked under an inverse microscope and no differences between the various treatments could be observed. In addition, the concentration of RNA extracted from the isolated cells after cell culture revealed similar values measured by absorbency at 260 nm in the different treatment groups. The highest amount of sgp130 secretion was assessed without hormones—a condition which is unphysiological in vivo—in the supernatant of decidual epithelial cells (after 10 days of culture up to 6230 pg/ml compared to 1824 pg/ml under E2 addition). In endometrial ($n = 10$) and in decidual epithelial cell culture ($n = 4$) the increase of sgp130 release in culture without hormonal substitution relative to E2 substitution was significant ($P = 0.0054$ and $P = 0.0365$ respectively, see Figures 8 and 9).

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**Figure 1.** Representative images of immunohistochemical detection of gp130 in human endometrium during the normal menstrual cycle. An intense staining in the glandular epithelium can be detected in the mid to late secretory phase (b, d, e; days 19–26); weak staining in the proliferative phase (a; day 9). No staining is observed in the stromal compartment. Control section (c) is a serial section to b (day 19) with non-immune mouse IgG instead of the primary antibody; no staining was revealed. Scale bar: a, c = 50 μm; d, e = 100 μm; b = 25 μm.
In addition, an increase of sgp130 release could be observed under E₂ (10⁻² mol/l) + MPA (10⁻⁶ mol/l) treatment compared to E₂ (10⁻³ mol/l) alone. The increase of sgp130 secretion in endometrial cell supernatants after 8–12 days of culture was proven to be statistically significant (P = 0.0445, n = 16, Figure 8). In decidual epithelial cells after 6 days of culture, sgp130 release under E₂ + MPA was weakly reduced (not significantly: P = 0.27, n = 5, Figure 9). After 8 days, and even more obviously after 10 days, sgp130 release started to increase under E₂ + MPA substitution in decidual cell culture comparable to the endometrial cell culture (n = 8, Figure 9).

Discussion

In this study, the transmembrane cytokine receptor gp130 and its soluble form were analysed in human endometrium and decidua during the menstrual cycle and in decidua of first and second trimester by immunohistochemical detection.
immunohistochemistry, western blotting and ELISA. Soluble gp130 protein was detected on western blots for the first time in homogenized endometrial and decidual tissue. The membrane-bound form of 150 kDa became clearly visible in samples of the mid to late secretory phase and was more pronounced in decidual tissue of second trimester compared to first trimester of pregnancy.

In vitro assays revealed secretion of soluble gp130 by cultured epithelial cells originating either from proliferative phase endometrium or from decidual tissue of first trimester pregnancy. The secretion of sgp130 by the endometrial and decidual epithelial cells was modulated by steroid hormones. In control experiments without hormones—which represents a rather unphysiological situation—soluble gp130 release reached maximum values. Epithelial cells supplemented with E₂ released less sgp130. Addition of E₂ + MPA—which mimics secretory phase conditions or pregnancy—increases the secretion of soluble gp130. This correlates with our results obtained by western blotting and immunohistochemistry, since in the progesterone-dominated secretory phase, the up-regulation of gp130 in its membrane-bound and the soluble form also becomes apparent.

An increase of sgp130 secretion from endometrial biopsies obtained between days 20 and 26 of the menstrual cycle compared with biopsies obtained during the proliferative phase has also been shown in a study by Sherwin et al. (2002). Interestingly, infertile patients showed a reduced secretion during this period, indicating a possible biological function of sgp130 in fertility. Thus the balance between soluble gp130 and the membrane-bound form seems to play an important role. As a physiological consequence of increased sgp130 in the secretory phase, high levels of circulating cytokines might be antagonized in their bioactivity.

Previous studies using northern blot analysis (Kojima et al., 1995) and northern blot analysis and in situ hybridization (Cullinan et al., 1996) revealed gp130 expression increasing during mid to late secretory phase and localization of gp130 transcripts to glandular and luminal epithelia. Other groups did not detect a marked variation in the expression of gp130 mRNA across the menstrual cycle by using RT–PCR (Yoshioka et al., 1999; Dimitriadis et al., 2000)
or RNAse protection assay (von Wolff et al., 2002). At the protein level, immunodetection of gp130 has been performed previously by using a rabbit polyclonal antibody (Tabibzadeh et al., 1995) and was reported to be present in endometrial glands throughout the menstrual cycle in all phases except the menstrual phase. In rhesus monkey uterus, gp130 was also mainly localized in the glandular epithelium during the menstrual cycle and in early pregnancy with only slight gp130 staining of the uterine stroma. The highest level of gp130 immunoreactivity was detected in mid-secretory phase of the menstrual cycle (Yue et al., 2000). In our study, we stained gp130 protein with a highly specific monoclonal antibody against the gp130 ectodomain (Wijdenes et al., 1995). The antibody B-P4 was further specified to be directed against the membrane-proximal part of gp130 consisting of three fibronectin-type-III-like domains D4, D5 and D6 (Kurth et al., 2000) and it specifically recognizes an epitope in D4 under both denaturing and native conditions (Pflanz et al., 2001). Mainly glandular epithelial cells were stained and a very intense staining was detected during the mid to late secretory phase whereas the staining of the proliferative phase endometrium was rather weak. The immunoreactivity was not only confined to the membrane but also to the cytoplasmic part of the cells, i.e. the endoplasmic reticulum and the Golgi vesicles, probably staining both, the membrane-bound as well as the soluble forms. In accordance with our in vitro results, in endometrium of the proliferative phase which is dominated by E2, no or only a weak staining has been observed. Confirming our results, Sherwin et al. (2002) found gp130 immunoreactivity also to be low in the proliferative phase and to increase in the secretory phase. Concerning human decidual tissue, gp130 was detected for the first time by immunohistochemistry in glands and cytotrophoblast cells. Steinborn et al. (1998) described immunoreactive gp130 during human pregnancy as well, but mainly in endothelial cells of fetal vessels within placental villi and expression of gp130 in the fetal trophoblast layer. In our study, in first trimester decidua, the glandular epithelial cells were weakly stained, but staining was more pronounced in the second trimester, similar to the data obtained by western blot. Earlier studies (Kojima et al., 1995) detected gp130 mRNA in placenta and decidua of first and second trimester by northern blot analysis. The levels of gp130 expression in second trimester placenta and term placenta were found by Kojima et al. (1995) to be higher than those in chorionic villi from the first trimester.

Several soluble receptors of the IL-6-type cytokines have been described besides gp130 (Narazaki et al., 1993; Sharkey et al., 1995; Diamant et al., 1997; Müller-Newen et al., 1998), i.e.

**Figure 7.** Insect cells were infected with a recombinant baculovirus encoding the human gp130 ectodomain (amino acids 1–606 rsgp130); the supernatant of these cells was subjected after immunoaffinity purification to SDS–PAGE 10% in a concentration of 20 ng, and immunoblotted using the monoclonal B-P4 antibody. M1: ECL DualVue western blotting marker; M2: rainbow coloured protein molecular weight markers.

**Figure 8.** Soluble gp130 release from epithelial cells derived from proliferative phase endometrium was measured in the supernatant by enzyme-linked immunosorbent assay after 8–12 days. sgp130 level under 17β-estradiol (E2) substitution was defined as 100% and in each experiment the percentage of increase or decrease under E2 + medroxyprogesterone acetate substitution \((n = 16, P = 0.0445)\) or without hormones \((n = 10; P = 0.0054)\) was calculated.
IL-6-Rα (Yoshioka et al., 1999), LIF-R (Layton et al., 1992; Owczarek et al., 1996; Metcalf et al., 2003) and CNTF-R (Davis et al., 1993). Soluble cytokine receptors retain remarkable affinities to their ligands in the nanomolar range (Müller-Newen et al., 1996). They can function as antagonists by binding the ligands and neutralizing their bioactivities or they may constitute a depot form of cytokines. Due to the higher molecular mass of ligand–soluble receptor complexes, they are less rapidly cleared from the circulation than the free ligands. The function and physiological role of soluble receptors to act as modulators for the bioactivity of their ligands has also been widely described for several other cytokine receptors such as the soluble form of tumour necrosis factor receptor sTNFR (Olsson et al., 1993; Fernandez-Botran, 2000), and of the vascular endothelial growth factor receptor sVEGFR-1 (Hornig and Weich, 1999; Neulen et al., 2001).

Soluble gp130, which occurs naturally in relatively high concentrations in human serum (Narazaki et al., 1993; Müller-Newen et al., 1998), can be translated from an alternatively spliced mRNA or produced by proteolytic cleavage (Müllberg et al., 1993; Sharkey et al., 1995; Diamant et al., 1997; Sherwin et al., 2002). As Sherwin et al. (2002) found, none of the known splice variants encoding sgp130 are present in endometrium. Therefore it is more likely that sgp130 in this tissue is produced primarily by proteolytic cleavage of the membrane-bound form instead of being translated by alternative splicing.

A role for regulation of proliferation and differentiation by sgp130 can be deduced from in vitro experiments performed in a human erythroleukaemic cell line (Narazaki et al., 1993). Addition of sgp130 resulted in inhibition of proliferation otherwise induced by the cytokines OSM, LIF and CNTF.

A dysregulation of cytokine-mediated processes can result in disorders of implantation as we know from knockout experiments. LIF is well known to be responsible for implantation failure when lacking in the mouse (Stewart et al., 1992), and the uterus of LIF-deficient mice fails to respond to the presence of embryos or to artificial stimuli by decidualization (Chen et al., 2000). IL-11 is also an important cytokine for the regulation of decidualization, as shown by gene targeting of IL-11-Rα (Robb et al., 1998) and by in vitro studies by Dimitriadis et al. (2002). Since gp130 as the common receptor is mainly localized in epithelial cells, the influence on decidualization of the stromal cells may most probably be achieved by paracrine signalling. Implantation failure in the case of LIF- or IL-11-Rα-deficient mice occurs in both cases due to disturbed decidualization, but at different times during the decidualization cascade. In the case of LIF gene targeting, no decidualization occurs and no implantation is possible. In the case of IL-11-Rα knockout mice, implantation can take place, but the postimplantation uterine decidual response is disturbed, i.e. the secondary decidual response which occurs in mice at 6 days post coitum, at the time of the maximal expression of IL-11 (Robb et al., 1998).

Possibly, also in human endometrium, in which the stromal cells decidualize spontaneously in the late secretory phase of the menstrual cycle (so-called predecidualization) and the decidualization proceeds during pregnancy, there might be different signals which trigger firstly predecidualization and secondly decidualization of pregnancy, comparable to the primary and secondary decidual response in mice (Robb et al., 1998). In the transition from first to second trimester the cytotrophoblast becomes less invasive (Benirschke and Kaufmann, 2000). This shift is thought to be caused partly by the maternal environment, i.e. the decidua, to control the invasiveness of the trophoblast possibly by reducing proliferation (Lala and Hamilton, 1996; Morrish et al., 1998; Bischof et al., 2000). This regulation may be influenced by the balance between the soluble and membrane-bound gp130. Hence, the change between first and second trimester decidua in expressing membrane-bound gp130 and its soluble form may have an impact on cytokine actions in further placentation events.

According to our results, epithelial cells under combined E₂/progesterone supplementation which mimics secretory phase conditions release more sgp130 than under E₂ supplementation alone. From our results we conclude that the release of sgp130 is under hormonal control and a regulatory role in modulating the cytokine...
action necessary for implantation and further trophoblast invasion might be suggested. As a working hypothesis we propose that sgp130 may control—by binding cytokine ligands—the proliferation of fibroblasts in the stroma and their differentiation to decidualized cells. Thus, the process of decidualization, transformation in a receptive endometrium, and implantation may be modulated by sgp130. As in the case of infertile women, the absence or reduction of sgp130 might be a sign of endometrial dysregulation (Sherwin et al., 2002). The significance of gp130 and its naturally occurring soluble form for the action of cytokine ligands, such as LIF and IL-11, remains to be further elucidated.

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