Oncostatin M is produced during pregnancy by decidual cells and stimulates the release of HCG

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Oncostatin M (OSM) is a member of the interleukin-6 superfamily and a multifunctional cytokine that affects the growth and differentiation of many different cell types. OSM concentrations in the sera of pregnant women were found to be significantly higher than those of non-pregnant women. Western blot analysis revealed that the OSM protein was present in the decidua and chorionic tissue in each trimester. Throughout pregnancy, the amount of the OSM protein in the decidua was larger than that in the chorionic tissue. Immunohistochemistry using an anti-OSM monoclonal antibody demonstrated that OSM was mainly localized in the decidual glands and stroma. OSM transcripts in the decidua and the chorionic tissue were detected during each trimester by reverse transcription–polymerase chain reaction (RT–PCR). The regulation of human chorionic gonadotrophin (HCG) release by the placenta in first trimester stimulated with recombinant OSM was also investigated. Stimulation of the placenta by OSM augmented HCG release in a time- and dose-dependent manner. HCG release induced by recombinant human OSM was completely blocked by antibodies against OSM and the signal transducer, gp130, but only partially inhibited by antibodies against the leukaemia inhibiting factor (LIF) receptor. These results suggest that OSM molecules produced by decidual glands and stromal cells during pregnancy have an important role in placental endocrine function.

Key words: chorion/decidua/HCG/oncostatin M/pregnancy

Introduction

Oncostatin M (OSM) is a 196 amino acid glycoprotein (relative molecular weight 28 000 kDa) which is produced by activated T cells and phorbol ester-treated human U937 histiocytic leukaemia cells (Zarling et al., 1986). OSM was identified by its ability to inhibit the growth of human A375 melanoma cells and a variety of other tumour cell lines. However, OSM induces the proliferation of normal fibroblasts (Zarling et al., 1986) and is a potent mitogenic factor for cells derived from the AIDS-related Kaposi’s Sarcoma (Cai et al., 1986). It has previously been reported that the placenta secretes several cytokines, e.g. IL-6 (Kameda et al., 1990; Nishino et al., 1990), tumour necrosis factor (TNF)-α (Li et al., 1992), IL-1 (Masuhiro et al., 1991), transforming growth factor (TGF)-β (Matsuzaki et al., 1992), IL-8 (Shimoya et al., 1992, 1999), monocyte chemotactic activating factor (MCAF) (Shimoya et al., 1998) and LIF (Sawai et al., 1995a), and that these cytokines played an important role in endocrine and immunological functions in the human placenta. We have also reported that the chorionic tissue in the first trimester mainly produces IL-6, while the decidua produces LIF (Sawai et al., 1995a). Little information, however, is available regarding OSM-mediated modification of normal human placental functions. In the present study, we

The human placenta has multiple functions which are regulated by cytokines, growth factors and hormones. We have previously reported that the placenta secretes several cytokines, e.g. IL-6 (Kameda et al., 1990; Nishino et al., 1990), tumour necrosis factor (TNF)-α (Li et al., 1992), IL-1 (Masuhiro et al., 1991), transforming growth factor (TGF)-β (Matsuzaki et al., 1992), IL-8 (Shimoya et al., 1992, 1999), monocyte chemotactic activating factor (MCAF) (Shimoya et al., 1998) and LIF (Sawai et al., 1995a), and that these cytokines played an important role in endocrine and immunological functions in the human placenta. We have also reported that the chorionic tissue in the first trimester mainly produces IL-6, while the decidua produces LIF (Sawai et al., 1995a). Little information, however, is available regarding OSM-mediated modification of normal human placental functions. In the present study, we

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investigated the nature of OSM production by the human placenta and decidual tissue. We first measured OSM concentrations in the sera of pregnant women; then we examined the localization of OSM in human pregnant tissue by immunohistochemical analysis and assessed the mechanism of OSM production in human pregnant tissue. Finally, we examined whether or not OSM was involved in endocrine functions, e.g., production of human chorionic gonadotrophin (HCG).

### Materials and methods

#### Samples

A total of 60 pregnant women (20 women in each trimester) and 20 non-pregnant women were monitored for serum OSM concentration. Pregnant tissues were obtained from different patients. Fresh decidual and chorionic tissues in the first trimester (8–11 weeks) and the second trimester (16–19 weeks) were obtained from women undergoing elective Caesarean section with no labour. Three chorionic and three decidual tissues from each trimester were used for RNA extraction, Western blot analysis and tissue culture. None of these tissues showed histological signs of infection or other complications. To observe the effect of OSM on HCG production, recombinant human OSM was added at the start of culture (day 0) of chorionic tissues. For these experiments, five fresh chorionic tissues were obtained from legal abortions at 7–9 weeks gestation. All fresh samples were thoroughly washed with Roswell Park Memorial Institute (RPMI) 1640 medium. Chorionic tissues were carefully separated from decidual tissues by macroscopic method. Informed consent was obtained from all patients.

#### Reagents

Purified *Escherichia coli*-derived recombinant human (rh) OSM and anti-human OSM monoclonal antibodies were purchased from R&D Systems (Minneapolis, MN, USA). A control mouse immunoglobulin G (IgG) for the control of histochemical analysis was purchased from Zymed Laboratories (San Francisco, CA, USA). An anti-human LIFR antibody and anti-human gp130 antibody (*E. coli* expressed) were purchased from R&D Systems.

#### Tissue culture and OSM assays

Decidual and chorionic tissues from each trimester were thoroughly washed, separated from the connective tissue, and minced on ice. Samples were tissue blocks of ~5 mm in diameter. The samples were plated in 24-well plates and cultured in 1 ml RPMI 1640 medium plus 15% FCS in 5% CO₂ at 37°C for 12, 24 and 48 h. After each period in culture, the OSM concentrations of the supernatants were determined as described below. The determined titres were divided by the tissue block dried weight. Triplicate separate experiments were performed.

To determine the concentration of OSM in the serum and supernatant of the tissue culture, an enzyme-linked immunosorbant assay (ELISA) kit using a monoclonal antibody specific for OSM (R&D Systems) was used. The OSM concentration detection limit of this kit was 15.6 pg/ml. The intra- and inter-assay coefficients of variation were 3.4–5.1 and 5.2–9.7% respectively.

#### Western blot analysis

The homogenizing buffer for protein extraction from the chorionic and decidual tissues consisted of 0.5 mol/l Tris–HCl (pH6.8), 10% sodium dodecyl sulphate (SDS), 6% β-mercaptoethanol, and 1% Bromophenol Blue (BPB). The chorionic and decidual tissues were homogenized in a 2 ml volume. Homogenates were centrifuged at 4°C for 30 min at 1400 g to remove debris. Following protein determinations, the samples were divided into aliquots, and the proteins (20 μg) were subjected to polyacrylamide gel electrophoresis (PAGE).

Protein concentrations were determined with Bio-Rad Protein Determination Reagent (Hercules, CA, USA), according to the method of Bradford (Bradford, 1976). The samples were electrophoresed on a 15% SDS–polyacrylamide gel and transferred onto nitrocellulose membranes (0.45 μm; Schleicher and Schuell, Dassel, Germany). The membranes were blocked overnight at 4°C in phosphate-buffered saline.
To determine the localization of OSM in the chorionic tissue and decidua, we performed immunohistochemical staining using an avidin–biotin peroxidase complex kit (Omititags Universal Streptavidin/Biotin affinity Immunostaining Systems, Lipshaw, Pittsburgh, PA, USA). Fresh frozen sections of the tissue were bleached in 0.3% hydrogen peroxide to block endogenous peroxidase and covered with 2% mouse IgG to minimize non-specific binding. An appropriately diluted mouse monoclonal anti-OSM antibody (R&D Systems) or control mouse IgG for the control was applied at room temperature and left for 1 h. After rinsing with phosphate-buffered saline solution, the sections were further incubated for 30 min with biotin-labelled anti-mouse IgG, followed by the addition of the avidin–peroxidase complex at 4°C. Peroxidase activity in the section was visualized with 0.1% 3,3-diaminobenzidiname-tetrahydrochloride containing 0.02% hydrogen peroxide in 0.1 mol/l Tris buffer (pH 7.2). The slides were counterstained with Mayer’s haematoxylin.

Effects of recombinant OSM on HCG production

To observe the effect of OSM on HCG production, rhOSM was added at the start of culture (day 0) of blocked chorion. At the indicated times and doses, the culture supernatants were collected and assayed for HCG. Each experiment was repeated at least three times, with identical results.

To characterize the signal transduction pathway for HCG release in the chorionic tissues activated by rhOSM, the chorionic tissues were stimulated with rOSM in the presence or absence of 0.5 μg/ml anti-OSM antibody, 40 μg/ml anti-LIFR antibody or 25 μg/ml anti-gp130 antibody. The tissues were cultured for 30 min, followed by washing with fresh culture medium. All of the treated groups were stimulated with 0.5 ng/ml rhOSM with antibodies or a control medium for 6 h. Culture supernatants were collected for assay of HCG with an enzyme immunosassay kit (Tosoh, Tokyo, Japan). The HCG concentration detection limit was 0.5 mIU/ml and the intra- and inter-assay coefficients of variation were <5%. Each experiment was repeated at least three times, with identical results.

Statistical analysis

Data were assessed by analysis of variance, followed by Student’s t-test or Duncan’s new multiple range test for multiple comparisons. P < 0.05 was considered to be statistically significant.

Results

We determined the OSM concentration in the sera of pregnant and non-pregnant women using a specific ELISA for OSM. As shown in Table I, the mean OSM titer in the sera of non-pregnant women was 4.6 ± 1.8 pg/ml. The average of the OSM concentrations from pregnant women in the first trimester, the second trimester, and the third trimester were 20.3 ± 3.9, 25.4 ± 4.6 and 11.8 ± 2.6 pg/ml respectively. OSM concentrations in the sera of pregnant women in each trimester were significantly higher than those of non-pregnant women (P < 0.01). OSM concentrations in the first and second trimesters were significantly higher (P < 0.05) than those in the third trimester. To verify the presence of the OSM protein in the
Figure 3. (a) Reverse transcriptase–polymerase chain reaction (RT–PCR) analysis of oncostatin M mRNA expression in first trimester chorionic tissue. Lane 1 = DNA size marker 100 bp ladder. Lane 2 = mock reaction with first trimester chorionic tissue. Lane 3 = cDNA from first trimester chorionic tissue. Lane 4 = cDNA (Lane 3) digested by Pst-I. (b) Immunohistochemical staining of oncostatin M (OSM)-producing cells in chorionic tissue in the first trimester. Sections of chorionic tissue in the 1st trimester were stained using the avidin–biotin complex method with a mouse monoclonal anti-OSM antibody (original magnification ×100).

Figure 4. For details see facing page.
placenta and decidual tissue, we performed Western blot analysis. Figure 1 shows that the OSM protein was detected as a 32 kDa band in the chorionic and the decidual tissues in each trimester. The intensity of OSM in the decidual tissue was stronger than that in the chorionic tissue.

To examine the expression of the OSM gene in the chorionic tissue and decidual tissue during pregnancy, we performed RT–PCR using specific primers for OSM. As shown in Figure 2, OSM transcripts were determined in the decidual tissue in each trimester. As shown Figure 3a, OSM transcripts were also present in the chorionic tissue in the first trimester. OSM transcripts were also detectable in the second and the third trimesters (data not shown). To identify the origin of this large amount of OSM, we performed immunohistochemical staining of chorionic and decidual tissues in each trimester, using an anti-OSM monoclonal antibody. As shown in Figure 3b, trophoblasts were stained in the first trimester. The trophoblasts in the second and the third trimesters were also positively stained (data not shown). However, the intensity of staining in the trophoblasts was fainter than in the decidual cells. Figure 4 shows the staining of decidual cells in each trimester. To quantify OSM production by the chorionic and the decidual tissue, we performed a block culture to determine the OSM concentrations of the supernatants. As shown in Figure 5, the OSM produced by the chorionic and the decidual tissues increased in a time-dependent manner in each trimester. The OSM concentrations of the culture supernatants of the decidual tissue were significantly higher than those of the chorionic tissue in each trimester.

To determine the function of the OSM molecule in the pregnant tissue, we examined the effect of OSM on HCG release by the chorionic tissue in the first trimester. Figure 6 represents the time- and dose-dependent stimulative effect of OSM on HCG release by the chorionic tissue in the first trimester. RhOSM stimulated HCG production by the chorionic tissue in a time- and dose-dependent manner. To characterize the signal transduction pathway for HCG release by chorionic tissues activated by rhOSM, the chorionic tissues were stimulated with rhOSM in the presence or absence of 0.5 µg/ml anti-OSM antibody, 40 µg/ml anti-LIFR antibody or 25 µg/ml anti-gp130 antibody. As shown in Figure 7, HCG release induced by 0.5 ng/ml rhOSM was completely blocked by the anti-OSM antibody (P < 0.005 compared with rhOSM only) and anti-gp130 antibody.

**Figure 5.** Time-dependent oncostatin M (OSM) production in the decidual tissue (△) and the chorionic tissue (○) in (A) first trimester, (B) second trimester, and (C) the third trimester. The values are given as the mean ± SEM (n = 3) of the OSM concentration. Statistically significant difference, *P < 0.05, **P < 0.01; data were assessed using Duncan’s new multiple range test. Triplicate separate experiments showed identical results.
(P < 0.0001 compared with rhOSM only). However, HCG release was only partially inhibited by the anti-LIFR antibody (P < 0.05 compared with rhOSM only). The addition of only the anti-OSM antibody, anti-LIFR antibody, or anti-gp130 antibody had no effect on HCG release by the chorionic tissue.

Discussion

Cytokines of the IL-6 family play important roles in implantation and the maintenance of pregnancy. We have previously reported that IL-6 and LIF stimulate HCG release by trophoblasts and that LIF enhances trophoblast differentiation (Nishino et al., 1990; Sawai et al., 1995a,b). OSM is one of the cytokines in this family (Rose and Bruce, 1991). The present study demonstrated that pregnant women showed a significantly higher titre of OSM than non-pregnant women. The chorionic tissue and decidua appear to be the major organs which induce the difference in OSM titres between pregnant and non-pregnant women, because the chorionic tissue and the decidua actively produced OSM in vitro. The production of OSM by the pregnant tissues may be regulated by steroid hormones. The OSM production by the decidua in the third trimester in vitro was almost the same as that by the decidua in the second trimester. However, serum OSM concentrations in pregnant women in the third trimester were significantly lower than those in the first and second trimesters. The reason why the OSM titres in the third trimester were lower is not clear. One possibility is that the maternal blood volume increases markedly during pregnancy. The blood volumes average ~40–45% higher than non-pregnant amounts. This increase occurs especially after 20 weeks of gestation (Cunningham et al., 1997). The OSM concentrations of pregnant women in the third trimester may be diluted by this increased blood volume.

The chorionic tissue and decidua expressed detectable levels of OSM mRNA and protein concentrations in each trimester. The chorionic tissue produced significantly lower amounts of OSM than the decidua. We have previously reported that IL-6 production by chorionic villi is apparently higher than that in the decidua. However, LIF production by chorionic villi is significantly lower than that by the decidua (Sawai et al., 1995a). The pattern of OSM production by the placenta during pregnancy was similar to that of LIF production. We have demonstrated that LIF production by decidual cells was increased by cytokines, e.g. IL-1 and TNF-α, and oestradiol (Sawai et al., 1997). However, the regulation mechanism of OSM production by decidual cells remains to be clarified in further studies.

Figure 6. Time- and dose-dependent effect of recombinant human (rh) oncostatin M (OSM) on human chorionic gonadotrophin (HCG) production by the chorionic tissue in the first trimester. Various concentrations of rhOSM were added simultaneously to block cultures. At each indicated (A) time and (B) dose, the culture media were removed and assayed for HCG using an enzyme immunoassay assay. The values are presented as the mean ± SEM (n = 3) of the HCG titres. Triplicate separate experiments showed identical results. Statistically significant difference, *P < 0.05; **P < 0.01.

Figure 7. Blocking of the release of human chorionic gonadotrophin (HCG) from chorionic tissue with 0.5 µg/ml anti-oncostatin M (OSM) antibody, 40 µg/ml anti-leukaemia inhibiting factor receptor (LIFR) antibody and 25 µg/ml anti-gp130 antibody. The chorionic tissues were cultured with antibodies or a control medium for 6 h in the presence or absence of 0.5 ng/ml recombinant human (rh) OSM. The supernatants were collected for the assay of HCG using an enzyme immunoassay assay. The values are the mean ± SEM (n = 3) of the HCG titres. Neg = no addition of rhOSM. Triplicate separate experiments showed identical results. Statistically significant difference, *P < 0.05; **P < 0.01.
HCG is called the ‘pregnancy hormone’ and plays a critical role in the maintenance of pregnancy (Cunningham et al., 1997). HCG production has been shown to be regulated by a number of agents, e.g. gonadotrophin-releasing hormone (GnRH) (Kohdr and Siler-Kohder, 1980) and steroids (Iwashita et al., 1989), as well as cytokines. Placental cytokines such as IL-1 (Masuhiro et al., 1991), TNF-α (Li et al., 1992), TGF-β (Matsuzaki et al., 1992), and IL-6 (Nishino et al., 1990) form a cytokine-mediated regulatory network controlling HCG. IL-1 and TNF-α stimulate IL-6 production, leading to a subsequent IL-6-induced HCG production by trophoblasts (Masuhiro et al., 1991; Li et al., 1992). TGF-β down-regulates IL-1-, TNF-α-, and IL-6-mediated IL-6 production (Matsuzaki et al., 1992). We have reported that LIF (which is mainly derived from the decidua), plays an important role in HCG production (Sawai et al., 1995a). Although OSM has multiple biological activities in various tissues and cell types, the present study further expands the list of OSM-mediated biological functions by demonstrating its ability to induce HCG production via chiorion in the first trimester.

OSM has structural and functional similarities to other members of the IL-6 family of cytokines, all of which signal a variety of responses via the binding of different heterodimeric receptor complexes involving the β subunit of gp130. OSM initiates signalling through two receptor complexes, LIFR-gp130 and OSMβ-gp130, and can produce a wide range of activities in vitro, including modulation of cell growth (Liu et al., 1992, 1994). We have demonstrated the expression of gp130 on the human chorial membrane (Sawai et al., 1995a). To characterize the signal transduction pathway for HCG release by the chorial membrane stimulated by rhOSM, chorial tissues were stimulated with rhOSM in the presence or the absence of anti-OSM antibodies, anti-LIFR antibodies or anti-gp130 antibodies. HCG release induced by rhOSM was completely blocked by the anti-OSM antibody and anti-gp130 antibody, but was only partially inhibited by the anti-LIFR antibody. These results suggest that half of HCG release induced by rhOSM was involved in signalling through LIFR-gp130 and other half was involved in signalling through OSMβ-gp130.

Studies of gp130-mediated signal transduction pathways have been reported (Kishimoto et al., 1994). We have reported that LIF-mediated HCG production is dependent on tyrosine kinases sensitive to genistein, but resistant to protein kinase C inhibitors (Sawai et al., 1995a). IL-6 can also induce HCG production by trophoblasts through activation of tyrosine kinase, but not of protein kinase C. cAMP-dependent kinase, or calcium-calmodulin-dependent kinase (Matsuzaki et al., 1995). These tyrosine kinases subsequently phosphorylate a family of transcription factors called signal transducers and activators of transcription 3 (STAT3). Recently, it was reported that OSM stimulated endothelin-1 production in cultured human umbilical vein endothelial cells (Saijonnmaa et al., 1998). Tyrosine-phosphorylated proteins were involved in OSM-stimulated endothelin-1 production by endothelial cells (Saijonnmaa et al., 1998). Experiments using protein kinase inhibitors will be necessary to determine the signal transduction pathways induced by OSM and which augment HCG production by chorionic tissue in the first trimester.

Acknowledgements
The authors thank Drs. M.Nagamatsu, S.Araki, and T.Ide (Kaizuka City Hospital, Kaizuka, Osaka, Japan) for providing specimens used in the present study. The authors also thank N.Goto and J.Nakano for their technical support.

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The role of OSM in pregnancy


Received on February 16, 2000; accepted on May 15, 2000