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THE GENE EXPRESSIONS OF MACROPHAGE COLONY-STIMULATING FACTOR (MCSF) AND MCSF RECEPTOR IN THE HUMAN MYOMETRIUM DURING PREGNANCY: REGULATION BY SEX STEROID HORMONES

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Summary—We investigated the biological effect of sex-steroid hormones, secreted from the corpus luteum and placenta, on the induction of mRNA encoding macrophage colony-stimulating factor (MCSF) and c-fms proto-oncogene (MCSF receptor) in the human uterine myometrium. Poly(A)$^+$RNA was extracted from the myometrium of pregnant and non-pregnant uterine myometrium and then Northern blot analysis was performed on poly(A)$^+$RNA. The myometrium of non-pregnant women expressed neither mRNA of macrophage colony-stimulating factor (MCSF) nor any transcript related to the c-fms proto-oncogene. On the other hand the myometrium of pregnant women expressed MCSF mRNA (4.7 kb) and two kinds of transcript related to the c-fms proto-oncogene (3.9 and 1.3 kb). The mRNAs of both MCSF and c-fms proto-oncogene were induced in the uterine myometrium of non-pregnant women under pseudopregnant therapy of mestranol and norethindrone. These results indicate that sex steroid hormone secreted from the corpus luteum of pregnancy and/or placenta may be deeply involved in the hypertrophic change of uterus during pregnancy by inducing MCSF and MCSF receptor (c-fms proto-oncogene protein product) in the myometrium.

INTRODUCTION

Since the very early stage of human pregnancy, perhaps even before the day of nidation, human chorionic gonadotropin (hCG) synthesized in the trophoblast [1, 2] maintains luteal function, transforms this organelle into the corpus luteum in pregnancy and continuously stimulates progesterone and estrogen synthesis for at least an additional several weeks until the placenta becomes the main organ for supplying these hormones. These hormones secreted from the corpus luteum and placenta are responsible for not only the secretory change of endometrium but also the uterine change during pregnancy. In normal intrauterine pregnancy, the almost solid uterus with a cavity of 10 ml or less is converted into a relatively thin-walled muscular container with sufficient capacity to house the fetus, placenta and amniotic fluid, and by the end of pregnancy the uterus has achieved a 500–1000 times greater capacity. Correspondingly the weight of the uterus has reached about 1000 g, as compared to about 70 g in the non-pregnant state. These changes of the uterus involve both stretching and marked hypertrophy of pre-existing muscle cells and at parturition a single myometrial cell is about 500 μm in length with the nucleus placed in the thickest part of the cell. The contribution of new muscle cells is limited. This hypertrophic change of myometrium, during the first few months of gestation, is assumed to be chiefly due to the stimulation by estrogen and progesterone secreted from the corpus luteum in pregnancy. During this period, the corpus luteum is the main organ for the supplying of these hormones. The mechanical distension of myometrium as a result of conception within the uterus does not contribute to the change of uterus, because similar uterine change can be observed when the ovum is implanted in the fallopian tube or in the ovary. At a further stage of pregnancy, during which the placenta is the main organ for supplying these hormones, the uterine change may be due to the hormones secreted from the placenta and

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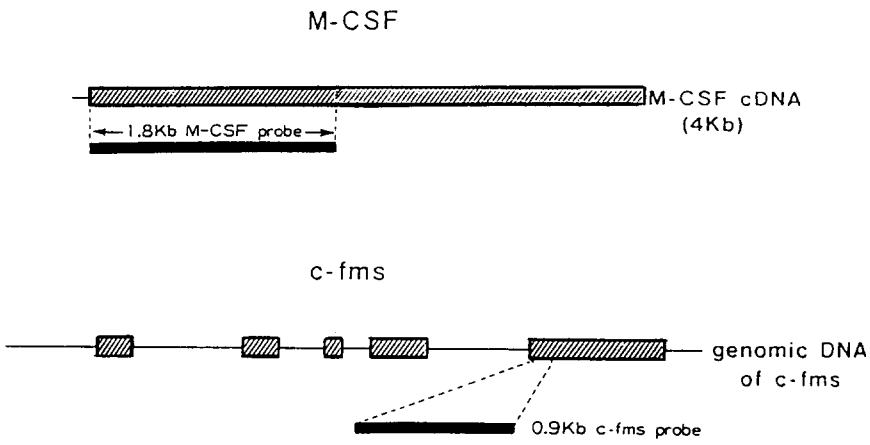

Fig. 1. The probes for the blot analysis. The probe of macrophage colony stimulating factor (MCSF) of 1.8 kb includes the whole coding region and a small part of the 3′-non-coding region of total MCSF cDNA. The probe of c-fms of 0.9 kb is derived from the 5′-region of exon 5 of genomic DNA of c-fms.

mechanical distension of the myometrium as a result of conception within the uterus. However the direct effects of these steroid hormones on the myometrium at the cellular level has not been completely revealed.

In order to investigate the relationship between the sex steroid hormones secreted from the corpus luteum or placenta and hypertrophic change of myometrium during pregnancy, we extracted poly(A)$^+$RNA from the myometrium of pregnant and non-pregnant women, and performed Northern blot analysis. Results of the Northern blot analysis revealed that sex-steroid hormones secreted from the ovary and placenta influence the hypertrophic change of myometrium during pregnancy via a mechanism of action involving local production of MCSF and its receptor.

METHODOLOGY

Tissue isolation

Human placenta was obtained at normal vaginal delivery. The myometrium of pregnant uterus was obtained during Caesarian section and therapeutic simple total hysterectomy. The myometrium of non-pregnant uterine myometrium was obtained from women receiving no hormonal treatment, or therapy with Norluten-D (Shionogi Co. Ltd, Osaka, Japan—a mixture of 0.05 mg mestranol and 5 mg norethindrone), 2 tablets/day for 4 weeks before the operation to induce pseudopregnancy. Informed consent was obtained from all patients before undertaking the study. Each material was washed repeatedly with cold phosphate buffered saline and the endometrium was completely isolated from the myometrium. All samples were kept at −80°C until RNA extraction.

RNA extraction

RNA was isolated by the method of Chirgwin [3]. The frozen samples were homogenized in 10 volumes of 4 M guanidine thiocyanate, 0.5% sodium *N*-laurylsarcosine, 25 mM sodium citrate (pH 7.0) and 0.1 M 2-mercaptoethanol. Cesium chloride was added to the homogenate at 1 g/2.5 ml and layered on a cushion of 5.7 M cesium chloride in 0.1 M EDTA. After centrifugation at 85,000 *g* for 18 h, the RNA pellet was resuspended in 4 mM EDTA (pH 7.4), extracted once with phenol–chloroform and twice with chloroform. The RNA was precipitated with ethanol and resuspended in 2 mM EDTA. Poly(A)$^+$RNA was isolated by an oligo (dT) affinity procedure as described previously [4]. The concentration of recovered poly(A)$^+$RNA was measured spectrophotometrically at 260 nm.

Preparation of probes

A 1.8 kb MCSF cDNA, corresponding to the open reading frame flanked by short regions of 5′- and 3′-untransrated RNA was kindly provided by Dr Wong [5]. Genomic DNA (0.9 kb) complementary to c-fms proto-oncogene mRNA was purchased from Oncogene Science Inc. (Manhasset, NY, U.S.A.) (Fig. 1). The β-actin cDNA probe was purchased from Wako Pure Chemical Ind. Ltd (Osaka, Japan). Multiprimer DNA labeling kit was purchased from Takara-Shuzo (Kyoto, Japan) and used according to the manufacturer's recommendations. 30 ng of each DNA probe were labeled with

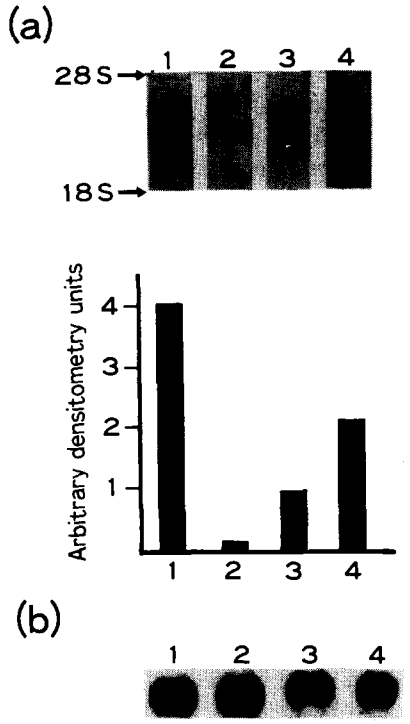

Fig. 2. Northern blot analysis of macrophage colony-stimulating factor (MCSF) mRNA from samples of placenta and myometrium and densitometric analysis of mRNA signals (a). Northern blot analysis of mRNA rehybridized to β-actin cDNA probe (b). Each lane represents 5 μg poly(A)$^+$RNA. Lane 1, term placenta, lane 2, myometrium of non-pregnant uterus; lane 3, myometrium of first trimester of gestation; lane 4, myometrium of third trimester of gestation.

[^{32}P]dCTP (Amersham International plc, Amersham, Bucks., U.K.) by multiprimer labeling kit to a high specific activity (10^9 dpm/μg).

Northern blot hybridization

Identical amounts (5 μg) of poly(A)$^+$RNA were denatured in 50% formamide, 2.2 M formaldehyde, 40 mM 3-[*N*-morpholino] propanesulfonic acid (pH 7.0) by heating at 55°C for 15 min, and then loaded onto 1.0% agarose/2.2 M formaldehyde gels and electrophoresed for 3 h at 50 V. After electrophoresis, the RNA was transferred to a 0.4 μm pore size nitrocellulose filter (Schleicher & Schell, Dassel, Fed. Rep. Germany) by blotting according to the method of Thomas [6]. The filter was then baked in a vacuum oven at 80°C for 3 h. ^{32}P-labeled probe (30 ng) was hybridized to the filter at 60°C for 12 h. The hybridization was performed in Denhardt's solution, 1 M NaCl, 50 mM Tris–HCl (pH 7.4), 10 mM ethylendiaminetetracetic acid (EDTA), 0.1% sodium dodecyl sulfate (SDS) and 0.1 mg/ml sonicated salmon sperm DNA. After hybridization reaction, the filter was washed. Washing conditions were as follows; once at room temperature in 2 × SSC (SSC is 0.1 M NaCl–0.015 M Na citrate), 0.1% sodium dodecyl sulfate (SDS) and twice at 60°C in 2 × SSC, 0.1% SDS. Autoradiography was performed using Kodak XRP-1 film (Kodak, Rochester, NJ, U.S.A.) and a Lighting Plus intensifying screen (DuPont, Boston, U.S.A.) at −80°C for 48 h. Autoradiography signals were analyzed densitometrically. After autoradiography, the filter was washed with 0.01 M Tris–HCl (pH 8.0) at 95°C for 2 min to remove the hybridized probe, rehybridized to ^{32}P-labeled β actin cDNA probe and then autoradiographed to confirm that the RNA was intact. All the reagents used for RNA extraction and Northern blot hybridization were purchased form Sigma Chemical Co. (St Louis, MO, U.S.A.).

RESULTS

Expression of MCSF mRNA by human myometrium was investigated by Northern blot analysis. A single strong signal of 4.7 kb corresponding to MCSF mRNA could be detected in the placental RNA, which was thus consistent with a result of a previous study [5]. The transcript of MCSF gene of 4.7 kb could be detected in the myometrium of pregnant uterus. The densitometric analysis showed that the signal of myometrium of third trimester of gestation was stronger than that of first trimester of pregnancy. However they were not as strong as that of term placenta. Any signal related to the MCSF could not be detected in the myometrium of non-pregnant uterus (Fig. 2). As the poly(A)$^+$RNAs subjected to the blot analysis were confirmed to be intact by the blot analysis using a β-actin cDNA probe, these results indicated that the mRNA of MCSF detected in the myometrium was a newly induced one during pregnancy (Fig. 2).

MCSF can function via binding to its cell surface receptor, the protein product of c-fms proto-oncogene [7], which is mainly expressed on the cell surface of phagocytic cells and on placental cells [5]. The expression of mRNA of c-fms proto-oncogene in the myometrium of pregnant uterus was compared to that of the placenta. Figure 3 shows the results of Northern blot analysis. Both the placenta and myometrium of pregnant uterus expressed the mRNA of c-fms proto-oncogene of 3.9 kb. The densitometric analysis revealed that the signal of

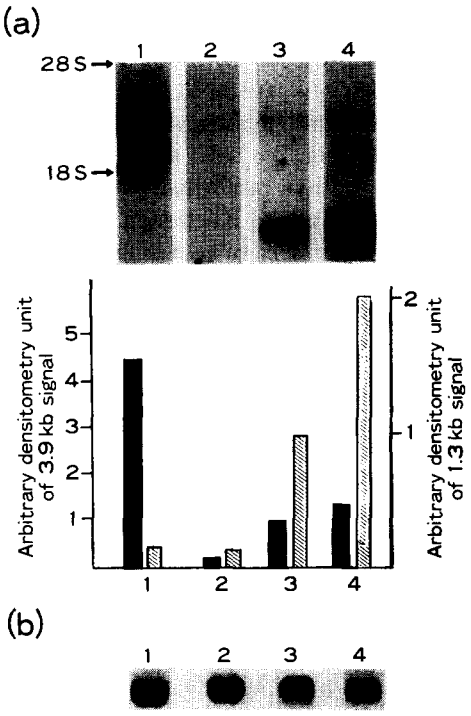

Fig. 3. Northern blot analysis of c-fms mRNA from samples of placenta and myometrium and densitometric analysis of mRNA signals (a). Northern blot analysis of mRNA rehybridized to β-actin cDNA probe (b). Each lane represents 5 μg poly(A)$^+$RNA. Lane 1, term placenta; lane 2, myometrium of non-pregnant uterus; lane 3, myometrium of first trimester of gestation; lane 4, myometrium of third trimester of gestation.

myometrium was rather weak compared to that of the placenta. In addition to the signal of 3.9 kb, a very strong signal of 1.3 kb which could not be shown in the placenta was detected in the myometrium of pregnant uterus. Both the 3.9 kb and 1.3 kb signals in the myometrium of third trimester of gestation were stronger than those of first trimester of gestation. The myometrium of non-pregnant uterus expressed neither mRNA of 3.9 kb nor mRNA of 1.3 kb.

In order to investigate the relationship between the induction of mRNAs of MCSF and c-fms proto-oncogene in the myometrium during pregnancy and the effects of sex-steroid hormones secreted from the corpus luteum and placenta, poly(A)$^+$RNA was extracted from the myometrium of non-pregnant women under pseudopregnant therapy by norethindrone and mestranol for 4 weeks and then subjected to Northern blot analysis. Figure 4 shows the transcripts of MCSF of 4.7 kb and c-fms proto-oncogene of 3.9 and 1.3 kb were induced in the myometrium of non-pregnant uterus. The level of each signal of myometrium was almost comparable to that of pregnant myometrium. The results indicated that the expression of these mRNAs in the myometrium during pregnancy were due to the effects of progesterone and estrogen secreted from the corpus luteum in pregnancy and/or placenta.

There has been increasing evidence to indicate that sex steroid hormones secreted from the corpus luteum and placenta regulate the gene expressions of some specific proteins. Progesterone induces the expression of mRNA of uteroglobin gene and the synthesis of uteroglobin in the endometrium [8, 9]. The proenkephalain gene expression in the rodent uterus is regulated by progesterone, and injection of progesterone stimulates the proenkephalin mRNA [10, 11]. Some of the mitogenic effects by autocrine and paracrine acting growth factors such as epithelial cell growth factor (EGF) are regulated by estrogen [12–14].

The experiments reported here were designed to investigate the effects of sex steroid hormones secreted from the corpus luteum and/or placenta on the induction of mRNAs of both MCSF and c-fms proto-oncogene in human myometrium. The results of Northern blot analysis revealed the following: first, the mRNA of MCSF was expressed in the myometrium of pregnant uterus; second, two kinds of transcripts rerated to c-fms proto-oncogene, 3.9 kb mRNA and 1.3 kb mRNA, were expressed in the myometrium of pregnant uterus; third,

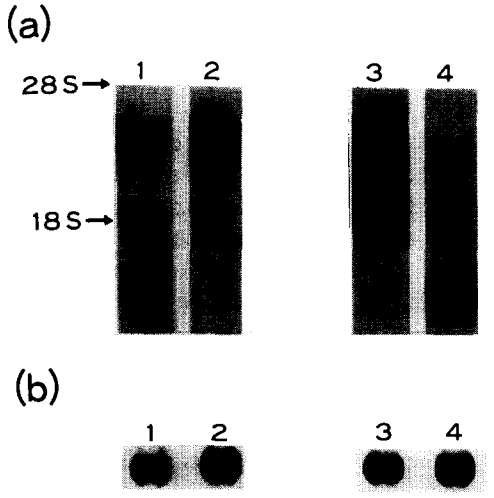

Fig. 4. Northern blot analysis of macrophage colony-stimulating factor (MCSF) mRNA and c-fms mRNA from samples of placenta and myometrium of non-pregnant women under pseudopregnant therapy for 4 weeks (a). Northern blot analysis of mRNA rehybridized to β-actin cDNA probe (b). Each lane represents 5 μg poly$^+$RNA. Lanes 1 and 3, term placenta; lanes 2 and 4, myometrium of non-pregnant women receiving hormonal therapy by norethindrone and mestranol for 4 weeks.

exogenous sex steroid hormones could induce mRNAs of both MCSF and c-fms proto-oncogene in the myometrium of non-pregnant women.

MCSF is a glycoprotein growth factor first identified as a growth factor which selectively regulates the proliferation or differentiation of mononuclear phagocytic cells [15]. MCSF can function via binding to its cell surface receptor, the c-fms proto-oncogene protein product. MCSF receptor, which consists of an extracellular ligand-binding domain, a single membrane spanning segment and an intracellular tyrosin kinase domain [7], is closely related in its amino acid sequence to the platelet-derived growth factor receptor and c-kit proto-oncogene product [16–18]. MCSF receptor is mainly expressed in the blood cells of the mononuclear phagocytic lineage and placental cells [5, 15]. Recently, in addition to the effect of MCSF on the stimulation of growth of phagocytic cells, other possible effects of MCSF on such as the formation and maintenance of murine placenta have been reported. The radioimmunoassay shows a 1000-fold increase of MCSF in the murine uterus during pregnancy, but only a 2-fold increase in other tissues [19, 20]. Exogenous MCSF can stimulate murine placental cell proliferation [21].

We had investigated the biological effects of progesterone and estrogen on the human uterus and already reported that MCSF and c-fms mRNAs were induced in human uterine endometrium by these sex-steroid hormones [22]. In the present study we have shown that the mRNA of MCSF and two kinds of transcripts related to c-fms proto-oncogene were expressed in the myometrium of pregnant women, and that exogenous sex-steroid hormones induced the mRNAs in the myometrium of non-pregnant uterus, thus implying that the expression of MCSF and MCSF receptor in the myometrium during pregnancy was regulated by estrogen and progesterone secreted from the corpus luteum in pregnancy and/or placenta. The expression of mRNAs of MCSF and c-fms proto-oncogene could be detected in the pregnant uterus of first trimester of gestation, that is during when the corpus luteum is the main organ for supplying progesterone and estrogen. In the second and third trimesters of gestation, the placenta is the main organ for supplying these hormones. Therefore the gene expressions of MCSF and c-fms in the myometrium mainly depend on the hormones secreted from the corpus luteum during the first trimester of gestation and on the hormones from placenta at further stage of pregnancy. The quantity of steroid hormones secreted from the placenta during the second or third trimester is far more than that secreted from the corpus luteum in pregnancy. This increment in hormones and the mechanical stimulation to the uterine muscle as a result of conception may cause expressions of MCSF mRNA and c-fms mRNA in the myometrium of third trimester stronger than that of first trimester or of pseudopregnant uterus.

The co-expression of mRNAs of MCSF and MCSF receptor (c-fms proto-oncogene protein product) in the myometrium during pregnancy indicates that MCSF is deeply involved in the physiological change of myometrium with autocrine and/or paracrine fashion. Recent studies have reported that the proliferating murine skeletal muscle cells and muscular stem cells express the mRNA of c-fms and c-fms related gene [23, 24]. However, the change of uterine myometrium during pregnancy or pseudopregnancy is hypertrophy without cell proliferation. Our results revealed that the transcripts related to c-fms proto-oncogene were induced in the muscular tissues with hypertrophic change. As the materials subjected to Northern blot analysis in our experiments were poly(A)$^+$RNA purified from total cellular RNA passed through oligo (dT) column, the transcript of both MCSF and c-fms proto-oncogene were functional mRNA. The protein products of truncated c-fms mRNA of 1.3 kb, detected by DNA probe derived from the fifth exon of c-fms gene, corresponds to the cytoplasmic space of MCSF receptor involving tyrosine kinase domain. This protein product cannot react with MCSF because it lacks the extracellular ligand-binding domain. The relationship between this tyrosine kinase protein and hypertrophic change of uterine myometrium is a matter of deep interest in subsequent studies. However for the time being, it is not clear whether both of the 3.9 and 1.3 kb transcripts related to c-fms proto-oncogene are derived from the gene closely related or identical to the c-fms proto-oncogene or from two distinct gene that share kinase coding sequences. In order to clarify this, an experiment for studying the gene sequence of the truncated mRNA related to the c-fms proto-oncogene using a cDNA library constructed in our laboratory is under way.

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