

REGULATION OF COLONY-STIMULATING FACTOR 1  
DURING PREGNANCY

BY A. BARTOCCI, J. W. POLLARD,\* AND E. R. STANLEY

*From the Departments of Microbiology and Immunology, and Cell Biology, Albert Einstein College of Medicine, Bronx, New York 10461; and the \*Medical Research Council Group in Human Genetic Diseases, Department of Biochemistry, Kings College, London W8 7AH, United Kingdom*

Colony-stimulating factor 1 (CSF-1) is a homodimeric glycoprotein growth factor that has been shown to regulate the survival, proliferation, and differentiation of mononuclear phagocytes (reviewed in 1). Its receptor is a 165 kd tyrosine kinase, closely related or identical to the *c-fms* protooncogene product (2).

Extensive studies in vitro (1, 3), and preliminary in vivo experiments (E. R. Stanley, and T. R. Bradley, unpublished observations) indicate that CSF-1 selectively regulates mononuclear phagocyte production. However, other possible effects of CSF-1 were suggested by a study (4) in which high levels of colony-stimulating activity were found in pregnant uterus and fetal tissues. Furthermore, recent observations (5) have shown the existence of the CSF-1 receptor and the *c-fms* gene product in human choriocarcinoma cell lines. In this paper, we find that pregnancy induces a 1,000-fold increase in the murine uterine CSF-1 concentration, which appears to be regulated by chorionic gonadotrophin (CG). These observations suggest a novel role for CSF-1 in pregnancy.

Materials and Methods

Endotoxin-unresponsive 8–12-wk-old C3H/HeJ mice (The Jackson Laboratory, Bar Harbor, ME) were used exclusively. Females were mated, and day zero of pregnancy was established when a vaginal plug was observed. Ovariectomy was performed via a dorsal incision as described (6). Purified human CG (HCG, CR123, 9,000 IU/mg protein; Dr. Canfield, Center for Population Research, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD), was administered i.p. (1,000 IU/mouse) at daily intervals. At various times after the treatments described in the text, animals were decapitated, bled, and tissues were processed as described below.

Extraction of tissues for CSF-1 determination was carried out at 4°C. The tissue was suspended in four volumes (wt/vol) of  $\alpha$ -MEM (KC Biological, Lenexa, KS) containing 25 mM Hepes (Gibco Laboratories, Grand Island, NY), 0.2% (wt/vol) BSA (Sigma Chemical Co., St. Louis, MO), and 0.02% NaN<sub>3</sub>, pH 7.3, and homogenized by 20 strokes of an Eberbach (7265; Fischer Scientific Co., Pittsburgh, PA) homogenizer. The homogenate was adjusted to 50 mg wet weight of tissue per milliliter and centrifuged at 800 *g* for 10 min. The supernatant fluid was heated to 56°C for 30 min, centrifuged at 800 *g* for 10 min, and dilutions of the supernatant were assayed in duplicate in the CSF-1 RIA (3). 1 U of CSF-1 represents ~0.44 fmol (7).

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TABLE I  
Tissue CSF-1 Concentrations in Male, Female, and Pregnant Mice

| Tissue             | CSF-1 concentration (U/mg tissue)* |                          |                          |
|--------------------|------------------------------------|--------------------------|--------------------------|
|                    | Male                               | Female                   | Pregnant                 |
| Submaxillary gland | 8.70 ± 0.13                        | 4.80 ± 0.92 <sup>‡</sup> | 7.60 ± 1.81 <sup>‡</sup> |
| Lung               | 2.40 ± 0.22                        | 2.20 ± 0.30              | 4.70 ± 0.10 <sup>‡</sup> |
| Spleen             | 2.20 ± 0.19                        | 1.50 ± 0.09 <sup>‡</sup> | 2.03 ± 0.20 <sup>‡</sup> |
| Kidney             | 1.90 ± 0.19                        | 2.30 ± 0.21              | 5.80 ± 0.91 <sup>‡</sup> |
| Lymph nodes        | 1.43 ± 0.19                        | 1.26 ± 0.48              | 2.46 ± 0.25 <sup>‡</sup> |
| Brain              | 1.10 ± 0.10                        | 0.90 ± 0.32              | 0.85 ± 0.12              |
| Liver              | 0.66 ± 0.22                        | 0.30 ± 0.20              | 0.66 ± 0.15 <sup>‡</sup> |
| Testis             | 0.35 ± 0.20                        | —                        | —                        |
| Ovary              | —                                  | 0.72 ± 0.09              | 1.06 ± 0.20              |
| Urine (U/μl)       | 0.73 ± 0.02                        | 0.64 ± 0.10              | 0.86 ± 0.04 <sup>‡</sup> |
| Serum (U/μl)       | 1.27 ± 0.16                        | 0.99 ± 0.11              | 1.43 ± 0.05 <sup>‡</sup> |

There was no significant change in CSF-1 concentration in any tissue at day 5 of pregnancy; 10-d pregnant mice were used for these experiments. At term, whole organ wet weights were determined. Increases were observed for liver (100%), kidney (23%) and spleen (13%). No change was observed in ovary or lung.

\*  $n = 3$ , mean ± SD.

<sup>‡</sup> Significant differences, male vs. female,  $p < 0.05$ .

<sup>‡</sup> Significant difference pregnancy vs. female  $p < 0.05$  (student t-test).

Colony formation (>40 cells/colony) by CSF-1-dependent colony-forming units (CFU-C) in single-cell suspensions of bone marrow and spleen was carried out in agar culture in the presence of mouse L cell CSF-1 (300 U/ml) as described elsewhere (7).

## Results

*Tissue CSF-1 Concentration in Adult Male and Female Mice.* CSF-1 concentrations were determined by an RIA that has been shown (3) to specifically measure biologically active CSF-1. The use of this assay obviated problems associated with tissue-derived inhibitors of biological assays (8). Several methods of CSF-1 extraction from tissues were tested, including freeze-thawing and heating at different temperatures. The selected method was the most efficient for extraction of CSF-1, and known amounts of L cell CSF-1 added to tissue extracts before homogenization were quantitatively recovered (data not shown).

Before studies with pregnant animals, tissue CSF-1 levels in male and female C3H/HeJ mice were determined (Table I). With the exception of the submaxillary salivary gland and spleen, in which higher levels were observed in males, no sex difference was observed. The highest CSF-1 concentrations were found in submaxillary glands, lung, spleen, and kidney, respectively.

*Effect of Pregnancy on CSF-1 and CSF-1-responsive Cells.* CSF-1 concentrations in all tissues except ovary and brain increased during pregnancy (Table I). This increase was two- to threefold in most tissues, and less marked (1.4-fold, see Table I) in serum. Correlated with the increased concentration of CSF-1 in the serum, there was a marked increase (5.2-fold) in the concentration of circulating monocytes, and a slight increase in the concentration of neutrophils (1.7-fold). The effects were selective, in that the eosinophil concentration did not change. The increase in proportion of monocytes and neutrophils occurred primarily at the expense of a decrease in the concentration of lymphocytes. In addition, the hematocrit was lower in pregnant mice (Table II).

Because of a marked increase in monocyte concentration, the concentrations of mononuclear phagocyte precursor cells (CFU-C) in the bone marrow and

TABLE II  
*Differential Peripheral Cell Count and Hematocrit During Pregnancy*

| Mice                        | White blood cells | Lymphocytes         | Monocytes         | Granulocytes        |                  | Hematocrit  |
|-----------------------------|-------------------|---------------------|-------------------|---------------------|------------------|-------------|
|                             |                   |                     |                   | Neutrophils         | Eosinophils      |             |
|                             |                   |                     |                   |                     |                  |             |
|                             |                   |                     | <i>cells/ml</i>   |                     |                  | <i>%</i>    |
| Nonpregnant<br>(n = 3)      | 4,850 ± 570       | 2,764 ± 222 (57.6)  | 161 ± 28 (3.4)    | 1,600 ± 167 (33.3)  | 274 ± 55 (5.7)   | 51.0 ± 1.8  |
| 16-20-d pregnant<br>(n = 4) | 5,950 ± 370‡      | 1,770 ± 173* (30.5) | 826 ± 220* (14.3) | 2,699 ± 487* (46.6) | 501 ± 140‡ (8.6) | 41.0 ± 2.9* |

Mean ± SD of triplicate counts of ≥200 cells each, per animal. Figures in parentheses are percentages. No basophilic granulocytes were observed in either pregnant or control animals.

\* Statistically significant ( $p < 0.05$ ) difference between pregnant and control (student's *t* test).

‡ No statistically significant difference.

TABLE III  
*Bone Marrow and Splenic CFU-C During Pregnancy*

| Days of pregnancy | CFU-C           |                 |
|-------------------|-----------------|-----------------|
|                   | Bone marrow     | Spleen          |
| Nonpregnant       | 1.0 ± 0.05*     | 1.0 ± 0.05      |
| 5-10              | 1.27 ± 0.03 (3) | 2.61 ± 0.15 (2) |
| 11-15             | 1.50 ± 0.29 (4) | 2.10 ± 0.42 (2) |
| 16-20             | 1.30 ± 0.25 (5) | 1.47 ± 0.18 (2) |

CFU-C (colonies >40 cells) were calculated as the mean of individual estimates from single animals (mean of triplicate determinations per animal) per  $7.5 \times 10^4$  bone marrow and  $7.5 \times 10^5$  spleen cells, and expressed as a ratio of the controls set at 1.0. Numbers in parentheses indicate the number of animals (*n*).

\* SD (bone marrow) or range (spleen). Average control numbers were 61 colonies for bone marrow and 35 colonies for spleen. Cluster formation (<40 cells) displayed the same pattern of effects as observed for colonies. The effect of pregnancy was not significant for bone marrow CFU-C, but was significant ( $p < 0.001$ ) for spleen CFU-C.

spleen were determined. By day 5 of pregnancy, CFU-C concentrations were elevated 2.6-fold in the spleen. This increase was sustained throughout pregnancy (Table III). In contrast, pregnancy had little effect on bone marrow CFU-C. These findings, together with the differential white cell counts, indicate that monocytopoiesis is selectively elevated at relatively early stages of pregnancy.

*Marked Elevation of Concentration of Uterine CSF-1 During Pregnancy.* In contrast to the roughly twofold increase in CSF-1 concentration in most tissues and serum (Table I and Fig. 1) there was a dramatic and sustained rate of increase in the uterine CSF-1 concentration throughout pregnancy (Fig. 1). In fact, at term, the 1,000-fold increase in concentration, coupled with the ~10-fold increase in uterine weight resulted in a total uterine CSF-1 content of  $\sim 4 \times 10^5$  U. The elevation in uterine CSF-1 concentration was accompanied by a slight increase in the level of fetal CSF-1, while the placental CSF-1 concentration remained constant (Fig. 1).

In mice, the luteal phase is only begun after fertilization of the ovum, by the production of murine CG (9). To determine whether CG would mimic the effects of early pregnancy on uterine CSF-1 concentration, human CG (HCG) was administered to normal females over a 5-d period, and the uterine and serum CSF-1 concentrations were determined. There was five- to sixfold increase in uterine CSF-1 concentration (Table IV), to concentrations that closely matched the levels observed at day 5 of pregnancy. Because the primary site of action of

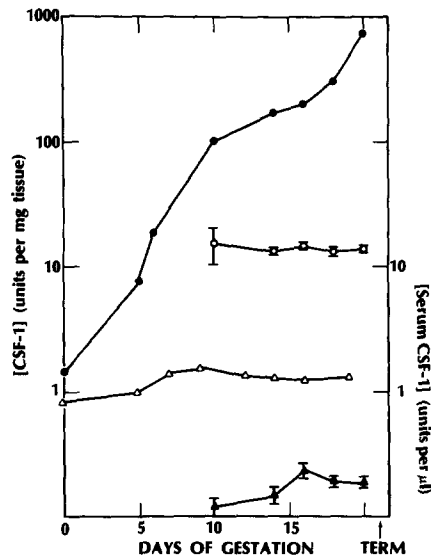


FIGURE 1. CSF-1 concentration in the uterus, placenta, fetus, and serum during pregnancy. Points represent the means of triplicate determinations of individual animals for uterine (●) and maternal serum (Δ) CSF-1 concentrations. Placental (○) and fetal (▲) CSF-1 concentrations are the means of determinations on three animals performed in triplicate  $\pm$ SD.

TABLE IV  
*Effect of HCG on Uterine CSF-1 Concentration*

| Animals               | Treatment        | CSF-1 concentration |                | Uterine weight (mg) |
|-----------------------|------------------|---------------------|----------------|---------------------|
|                       |                  | Serum (U/ $\mu$ l)  | Uterus (U/mg)  |                     |
| Normal female         | Saline           | 1.4 $\pm$ 0.2*      | 2.5 $\pm$ 0.5  | 54.2 $\pm$ 11.1     |
|                       | HCG <sup>†</sup> | 1.9 $\pm$ 0.2       | 13.8 $\pm$ 0.9 | 68.9 $\pm$ 1.9      |
| Ovariectomized female | Saline           | 1.6 $\pm$ 0.2       | 2.0 $\pm$ 0.3  | 33.3 $\pm$ 3.2      |
|                       | HCG              | 1.7 $\pm$ 0.2       | 2.2 $\pm$ 0.4  | 29.3 $\pm$ 1.15     |

\*  $n = 3$ , mean  $\pm$  SD.

<sup>†</sup> HCG (1,000 U/mouse) was injected i.p. at daily intervals before killing on day 5. In two other independent experiments, the effect of this dose of HCG over the control untreated female was 2.7- and 3.4-fold. Maximum effects of HCG were also observed at 1 U/mouse/d. The effect of HCG was significant  $p < 0.001$  by student's  $t$  test.

CG is the ovary (9), we looked at the effect of ovariectomy on these responses to HCG. Using a comparable regime for the administration of HCG, ovariectomy completely inhibited the elevation of uterine CSF-1 concentrations (Table IV). These data suggest that the early changes in CSF-1 concentrations in pregnancy are regulated by CG via its influence on ovarian function.

### Discussion

The method developed for the measurement of tissue CSF-1 provided a reliable means of measuring biologically active CSF-1. The relative distribution of CSF-1 in the tissues of normal male and female mice paralleled to a large degree the previously reported (8) distribution of colony-stimulating activity. However, colony-stimulating activity determined by in vitro bioassay could have resulted from the activities of several distinct CSFs, including GM-CSF (granu-

locyte/macrophage CSF), G-CSF (granulocyte CSF), and IL-3 (reviewed in 10) that are not detected by the CSF-1 RIA (3).

The concentrations of nonuterine tissue and serum CSF-1 were significantly elevated between days 5 and 10 of pregnancy. During this period, there was an increase in the concentration of splenic CFU-C. This effect was correlated with monocytosis and granulocytosis, although the kinetics of these increases were not determined in the present study. These results are consistent with an *in vivo* role of CSF-1 in the regulation of mononuclear phagocyte production. Similar changes in the differential white cell count and hematocrit have been reported for studies of pregnancy in humans (11, 12).

In contrast to the other tissues examined, the kinetics of the increase in uterine CSF-1 concentration was more rapid and far greater in extent. A 5-fold increase in the CSF-1 concentration was already apparent at day 5, reaching 1,000-fold by term. In fact, by term, the total CSF-1 content of the uterus ( $\sim 5 \times 10^5$  U) far exceeded the total CSF-1 content of the other tissues. At present, it is not clear whether the increase in uterine CSF-1 contributes to, or results from, the increase in serum CSF-1, or is, alternatively, a local phenomenon. The data indicate that increases in uterine CSF-1 are under the control of CG, mediated by the ovary. Ovarian involvement could be due either to the production of progestins and estrogens, known to be regulated by CG, or to as yet uncharacterized ovarian factors.

In view of the presence of *c-fms* mRNA in human placenta (13), and its occurrence, together with CSF-1 receptor, in choriocarcinoma cell lines with trophoblastic morphology (5), it has been suggested that CSF-1 may play a role in placental development. Our data are consistent with this possibility. The placenta is composed of both fetally-derived trophoblasts and maternally-derived decidual cells. Interestingly, it has been shown (14, 15) that at least some of the decidual cells arise from bone marrow precursors. Uterine CSF-1 may also cause local proliferation and differentiation of cells of the mononuclear phagocytic lineage, giving rise to mature macrophages. Macrophages have been identified on the maternal side of the fetal-maternal border of the placenta (16), where they may contribute to the immunosuppression of the maternal response to the allogeneic fetus (17). These questions may be approached by identification of CSF-1-producing and -responding cells *in situ*.

### Summary

Pregnancy results in an elevation in serum and tissue concentrations of the mononuclear phagocytic growth factor, CSF-1 (colony-stimulating factor 1). These increases are associated with an increase in the number of monocytes in the circulation, and with increases in the number of splenic macrophage precursors. In contrast to the  $\sim 2$ -fold elevation of the CSF-1 concentrations in serum and most tissues, pregnancy results in a 1,000-fold increase in the concentration of uterine CSF-1. The roughly fivefold elevation in uterine CSF-1 concentration observed at day 5 of pregnancy could be mimicked by administration of chorionic gonadotrophin in intact but not ovariectomized mice. These dramatic changes in uterine CSF-1 concentrations may indicate a role for CSF-1 in the regulation of nonmononuclear phagocytic cell types.

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