Expression of the Human c-fms Proto-oncogene Product (Colony-stimulating Factor-1 Receptor) on Peripheral Blood Mononuclear Cells and Choriocarcinoma Cell Lines


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Abstract

The c-fms gene product is related, and possibly identical, to the receptor for the mononuclear phagocyte colony stimulating factor, CSF-1. Using antiserum to a recombinant v-fms–coded polypeptide, glycoproteins encoded by the human c-fms locus were detected in mononuclear cells from normal peripheral blood and in promyelocytic HL-60 cells 24 hours after induction of monocytic differentiation with phorbol ester. The 150-kD human c-fms–coded glycoprotein was expressed at the cell surface, was active as a tyrosine-specific protein kinase in vitro, and shared primary structural features with the product of the feline retroviral v-fms oncogene. A biochemically indistinguishable glycoprotein was detected in human choriocarcinoma cell lines. Like peripheral blood mononuclear cells and phorbol ester-treated HL-60 cells, the choriocarcinoma cells expressed high affinity binding sites for human CSF-1. In addition to serving as a lineage specific growth factor in hematopoiesis, CSF-1 may play a role in normal trophoblast development.

Introduction

The colony-stimulating factor, CSF-1, is a hematopoietic growth factor required for the proliferation, differentiation, and survival of cells of the mononuclear phagocyte series (1). Cells of this lineage (including determined precursors, monoblasts, monocytes, monocytes, and macrophages) express a single class of high affinity receptors for CSF-1 (2–4). Although the CSF-1 receptor is expressed by less mature bone marrow progenitors, the number of receptors per cell increases about 10-fold as the cells become committed to the mononuclear phagocyte lineage (5). Thus, circulating peripheral blood monocytes express relatively high numbers of CSF-1 receptors, and the populations of CSF-1–binding cells in spleen, lymph nodes, and inflammatory exudates correspond in their number and morphology to macrophages (2, 6). By these criteria, the expression of the CSF-1 receptor represents a differentiation specific marker of cells of the mononuclear phagocyte lineage.

The purified murine CSF-1 receptor is a 165-kD glycoprotein with an associated tyrosine-specific protein kinase activity (7, 8), and is similar in its properties to the product of a feline proto-oncogene, c-fms (9). Indeed, the c-fms proto-oncogene product is preferentially expressed at high levels in feline and murine macrophages, specifically binds CSF-1, and, in the presence of the growth factor, is phosphorylated on tyrosine in membrane preparations (10). Moreover, the product of the feline retroviral oncogene, v-fms, retains a CSF-1 binding domain so that v-fms–transformed fibroblasts express high affinity binding sites for the growth factor (11). These results strongly suggest that the product of the c-fms proto-oncogene and the CSF-1 receptor are closely related, and possibly identical, molecules (10).

Transcripts of c-fms RNA have been detected in spleen, lymph nodes, liver (9), bone marrow and peripheral blood mononuclear cells (12, 13) and in human tumors of various histologic types (14). This pattern of gene expression is consistent with the tissue distribution of normal CSF-1 receptor-bearing cells of the mononuclear phagocyte lineage and with infiltration of tumor tissues by inflammatory cells. However, c-fms transcripts have also been detected at comparatively high levels in placental tissues (15) and in a human choriocarcinoma cell line established from malignant placental trophoblasts (16). The latter observations prompted us to examine the c-fms gene product in choriocarcinoma cell lines and to compare its properties to the c-fms–coded glycoprotein expressed in normal human peripheral blood mononuclear cells. The c-fms gene products in both cell types are biochemically indistinguishable, and the cells express high affinity binding sites for human CSF-1.

Methods

Cell lines. The derivation and properties of mink lung cells nonproductively transformed by the Susan McDonough strain of feline sarcoma virus (SM-FeSV) have been previously described (17). The human BeWo cells (18) and JEG-3 (19) choriocarcinoma cell lines were obtained from the American Type Culture Collection, Rockville, MD. BeWo cells secrete human chorionic gonadotropin (HCG), human placental lactogen, and steroid hormones, and JEG cells produce HCG, steroid hormones, and human chorionic somatomammotrophin. Both cell lines have the morphologic appearance of undifferentiated trophoblasts, and JEG-3 cells form choriocarcinomas when injected into nude mice. BeWo cells were maintained in RPMI 1640 medium containing 15% fetal bovine serum. JEG-3 and the promyelocytic leukemia cell line, HL-60 (20) were maintained in Iscove’s modified Eagle’s minimal essential medium (MEM) (Gibco, Grand Island, NY) containing 20% fetal calf serum. HL-60 cells were induced to monocyte differentiation by treatment with 12-O-tetradecanoyl-phorbol-13-acetate (TPA) (21) or to granulocytic differ...
tiation by dimethylsulfoxide (DMSO) (22). TPA and DMSO were purchased from Sigma Chemical Co. (St. Louis, MO). Human leukemic cell lines of erythroid (K562), myeloid (KG-1 and KG-1a) and monocytic (U937) lineages, and leukemic lymphoid cells of the pre-B (NALM-1), B (Daudi, Raji) or T (MOLT-4) series were provided by Dr. J. Mirro of St. Jude Children’s Research Hospital.

Peripheral blood cell fractions. Fresh peripheral blood mononuclear cells, granulocytes, and erythrocytes were prepared as described by others (23, 24). Blood from normal volunteers was collected by venipuncture in the presence of heparin and mixed with an equal volume of Hanks’s balanced salts solution (HBSS) at 4°C. The samples were divided into 40-ml aliquots, each of which was layered over 10 ml Ficol-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) in 50-ml polypolyethylene tubes, and then centrifuged at 1,250 rpm for 30 min at 4°C. Mononuclear cells were collected from the interface, washed in HBSS, and examined morphologically by cytopsin preparations; this fraction consisted primarily of lymphocytes with typically 5–10% monocytes and 1% or fewer granulocytes. For CSF-1 binding assays, the mononuclear cells were used directly or were seeded into 35-mm culture dishes (4 x 10^6 cells/dish) and cultured at 37°C in α-MEM; after 2 h, the nonadherent cells were removed and the adherent cells cultured for an additional 17 h. Erythrocytes and granulocytes recovered from the Ficol-Hypaque pellets were resuspended in HBSS containing 4% Dextran and subjected to unit gravity sedimentation at 4°C for 2 h. The granulocyte-rich supernatant was then collected, centrifuged into a pellet, washed twice with 10 mM KHCO₃ (pH 7.4) containing 155 mM NH₄Cl and 0.1 mM EDTA to lyse erythrocytes, resuspended in HBSS and examined morphologically; this fraction typically consisted of 90–95% granulocytes and up to 10% lymphocytes. Erythrocytes were recovered from the pellet of the unit gravity sedimentation step. A platelet fraction was prepared by differential centrifugation (25).

Cell surface radioiodination. Cells were washed twice with phosphate-buffered saline (PBS) and incubated in 1.0 ml of PBS containing 5 mM D-glucose, 1.0 mM carrier-free Na¹²⁵I (Amersham Corp., Arlington Heights, IL), 75 μg lactoperoxidase and 8.8 μg glucose oxidase (Sigma Chemical Co., St. Louis, MO) for 15 min at 22°C with occasional gentle mixing. The cells were then washed twice in ice-cold PBS and lysed in RIPA buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 20 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS]) containing 2% Aprotinin and 1 μM phenylmethylsulfonyl fluoride as protease inhibitors. Immune precipitation with rabbit antisera to a recombinant v-fms-coded polypeptide (10) was carried out as previously described (26).

Other analytical methods. The procedures for metabolic radiolabeling of cell lines, preparation of cell lysates, the immune complex kinetic assay, tryptic peptide analysis (26), digestion of immune precipitates with glycosidases enzymes, and phosphoamino acid analysis (9) have been previously described in detail elsewhere. For phosphoamino acid analysis, ³²P-labeled polypeptides were hydrolyzed in 6 N HCl by heating for 2 h in a boiling water bath. The products were separated by electrophoresis in two dimensions and detected by autoradiography. During hydrolysis, some ³²P-labeled protein is degraded to inorganic phosphate, whereas incompletely hydrolyzed phosphorylated peptides can also be detected. To quantify the positions of individual phosphoamino acids the autoradiogram spots were aligned with internal standards run on the same chromatogram and visualized by ninhydrin staining.

Binding of¹²⁵I-human CSF-1 to cells. Stage V human urinary CSF-1 was purified, iodinated, and further purified by immunoaffinity chromatography as described (27). The specific radioactivity of the purified ¹²⁵I-CSF-1 used for the binding studies was 1.5 x 10⁶ cpm/ng protein. Binding studies with either nonadherent or adherent cells were carried out at 2°C in 25 mM Hepes, 0.02% bovine serum albumin in α-MEM lacking bicarbonate, pH 7.4 (6, 27). Cells were incubated with 10 nM ¹²⁵I-CSF-1 for 4 h with or without a 2-h preincubation with 10 nM CSF-1. Separation of cell-bound ¹²⁵I-CSF-1 from free ¹²⁵I-CSF-1 in incubations with nonadherent cells (400 μl, 4 x 10⁵ cells/ml) was achieved by centrifugation (400 g, 15 min, 4°C) of 325 μl of the incubation mixture through 175 μl of horse serum. For incubations with adherent cells, the supernatant medium was removed and the monolayer washed five times with ice-cold PBS and solubilized with 5% SDS in 50 mM Tris-HCl, pH 7.4. The cell-associated radioactivity was determined in a gamma counter. Autoradiography of the nonadherent and adherent cells was carried out as described (2, 6).

Results

Human c-fms products are expressed in peripheral blood mononuclear cells and in TPA-induced HL-60 cells. Rabbit antisera to a recombinant v-fms coded polypeptide synthesized in bacteria reacted specifically with v-fms-coded gene products expressed in normal feline and murine macrophages and precipitated the bona fide murine CSF-1 receptor (10). The same antisera were found to precipitate antigenically crossreactive molecules from human peripheral blood cells. Fig. 1A shows the results of immune complex kinase assays prepared from lysates of fractionated normal human blood cells. Two polypeptides of 150 kD and 130 kD were phosphorylated in immune complexes prepared from peripheral blood mononuclear cells (lane 2) but were not

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**Figure 1.** Detection of the human c-fms proto-oncogene product by an immune complex kinase reaction. Detergent lysates of the indicated cells were incubated with either preimmune rabbit serum or antisem to a recombinant v-fms-coded polypeptide (bp81-v-fms) expressed in Escherichia coli. Washed immunoprecipitates were assayed for endogenous protein kinase activity, and the products were separated by SDS polyacrylamide gel electrophoresis and detected by autoradiography. (A) Normal human peripheral blood cell fractions were assayed for c-fms associated kinase activity: mononuclear cells (lanes 1 and 2), granulocytes (lanes 3 and 4), erythrocytes (lanes 5 and 6), and a platelet fraction (lanes 7 and 8). Immune complexes were prepared using preimmune serum (lanes 1, 3, 5, and 7) or antisem to bp81-v-fms (lanes 2, 4, 6, and 8). Reactions in lanes 1–6 were performed with lysates of 1 x 10⁵ fresh cells from each of the indicated fractions. Exposure time for autoradiography was 60 min. (B) HL-60 leukemic cells, either untreated (lane 1) or treated with TPA for 24 (lane 2) and 48 h (lane 3), were assayed for c-fms associated kinase activity in immune complexes prepared with antisem to bp81-v-fms. Reactions in each lane were prepared from 1.5 x 10⁵ cells. Exposure time, 120 min. (C) Human choriocarcinoma cell lines were assayed in immune complexes prepared with preimmune serum (lanes 1 and 3) or antisem to bp81-v-fms (lanes 2 and 4). Reactions were performed with lysates of 4 x 10⁵ BeWo cells (lanes 1 and 2) or 2 x 10⁵ JEG-3 cells (lanes 3 and 4). Exposure time, 30 min. The electrophoretic mobilities of the human c-fms-coded glycoproteins, gp150 and gp130 (left margin), and protein standards of known molecular weight (right margin) are noted.
detected in precipitates prepared with nonimmune serum (lane 1). These polypeptides were not observed in immune complex kinase assays performed with granulocytes (lanes 3 and 4), erythrocytes (lanes 5 and 6) or platelets (lanes 7 and 8). Extensive manipulation of fresh human or feline mononuclear cells leads to the loss of c-fms-coded kinase activity. When adherent human peripheral blood cells were studied, there was no enrichment of immunoprecipitable kinase activity as compared to that in the total mononuclear cell population. These results appear to reflect the instability of the kinase, but based on these experiments, we cannot exclude the presence of the c-fms gene product in the nonadherent population. Two-dimensional phosphoamino acid analysis showed that both polypeptides were phosphorylated on tyrosine. (See Fig. 2 A for representative results with the 150 kD polypeptide.) The detection of these polypeptides with specific antiserum to the v-fms gene product, their activity as substrates for an associated tyrosine kinase in vitro, and their restricted expression in peripheral blood mononuclear cells suggested that they represented products of the human c-fms gene.

By analogy with the feline v-fms-coded glycoproteins and c-fms-coded products of cats and mice, we suspected that the 130-kD and 150-kD polypeptides might represent two forms of the same glycoprotein that differed in their composition of asparagine-linked oligosaccharides (9, 28). The retroviral v-fms-coded gene products and the putative human c-fms-coded proteins from peripheral blood mononuclear cells were radiolabeled in immune complex kinase reactions, digested with glycosidic enzymes, and separated by electrophoresis. Like the immature forms of the viral glycoproteins, gp180gag-fms and gp120v-fms (Fig. 3 A), the human 130-kD polypeptide was resistant to neuraminidase but sensitive to endoglycosidase H treatment (Fig. 3 B), showing that it contained mannose-rich oligosaccharide chains characteristic of intracellular glycoproteins. By contrast, the mature cell surface form of the viral glycoprotein, gp140v-fms (Fig. 3 A), and the human 150-kD polypeptide were sensitive to neuraminidase, demonstrating the presence of terminal sialic acid residues. The human 150-kD glycoprotein also showed a slight decrease in apparent molecular weight after endoglycosidase H treatment (Fig. 3 B, lane 2), indicating that at least some oligosaccharide chains were not processed to endo H-resistant structures. Taken together, the results are consistent with the possibility that the 130-kD glycoprotein is an immature glycosylated precursor of the mature 150-kD molecule. The estimated molecular weight of the deglycosylated polypeptide derived by endo H treatment of the human 130-kD glycoprotein was about 10 kD higher than that of the corresponding form derived from gp120v-fms (see arrows in the margins of Fig. 3). This suggests that the polypeptide chain of the human c-fms gene product is longer than that of the viral transforming protein.

The human promyelocytic cell line, HL-60, differentiates to adherent macrophage-like cells after treatment with phorbol esters (21) and to granulocytic cells after treatment with DMSO (22). Transcripts of the c-fms gene have been detected approximately 12 h after TPA treatment when the cells attach to culture dishes and begin to assume a macrophage morphology; by contrast, c-fms RNA is not induced by DMSO (12, 13). Control and TPA-treated HL-60 cells were tested for expression of the presumptive c-fms gene products using the immune complex kinase reaction. As shown in Fig. 1 B, the 130-kD and 150-kD polypeptides were detected in HL-60 cells both 24 h (lane 2) and 48 h (lane 3) after TPA treatment. Neither of the polypeptides was detected after induction of granulocytic differentiation with DMSO (data not shown). By comparison to the levels of
c-fms-coded polypeptides in normal peripheral blood mononuclear cells, the levels of the 130-kD and 150-kD polypeptides in HL-60 cells were significantly lower. Moreover, the 130-kD polypeptide was the major form detected in HL-60 cells throughout the duration of treatment with TPA. The same result was observed in the human U937 histiocytic lymphoma cell line (29) when induced to monocytic differentiation (data not shown). The predominance of the immature form of the c-fms gene product could reflect the failure of these cells to differentiate completely in culture, or alternatively, could be due to the continued presence of an inducing stimulus throughout the duration of the experiments. Neither the 130- nor 150-kD polypeptide was detected in immune complex kinase assays performed with established lines of erythroid or granulocytic leukemic cells or with several human B or T lymphoid lines (see Methods).

Detection of the c-fms-coded products in choriocarcinoma cell lines. By Northern blotting analysis using a v-fms-specific probe and by nuclease protection experiments performed with a probe derived from the human c-fms locus, we confirmed previously published results of others that a prototypic ~4-kb c-fms polyadenylated RNA is expressed at high levels in placenta and in choriocarcinoma cell lines (15, 16). We therefore used the immune complex kinase reaction to screen two human choriocarcinoma cell lines, BeWo and JEG-3, for expression of c-fms-related gene products. Fig. 1 C shows that the 150-kD and 130-kD polypeptides could be specifically precipitated from both cell lines and were active as substrates for an associated tyrosine kinase in vitro. These molecules were the same size as those precipitated from peripheral blood cells (Fig. 1 A) and were phosphorylated on tyrosine residues (Fig. 2 B). Digestion with glycosidic enzymes demonstrated that both forms were glycoproteins indistinguishable from the analogous products expressed on peripheral blood mononuclear cells (see Fig. 3 B).

To compare regions of primary structure adjacent to sites of phosphorylation in vitro, 32P-labeled molecules phosphorylated in immune complex kinase reactions were digested with trypsin, and the resulting peptides were separated in two dimensions by electrophoresis and chromatography. As shown in Fig. 4, A and B, the labeled tryptic phosphopeptides of the human 150 kD proteins from both peripheral blood mononuclear cells and choriocarcinoma cells were identical. The 130-kD proteins from both sources yielded the same labeled peptides (data not shown). Moreover, several of the phosphopeptides comigrated with those derived from the v-fms-coded glycoprotein, gp140v-fms (Fig. 4 C). A major 32P-labeled tryptic peptide unique to the retrowiral protein (see schematic diagram in Fig. 4 D) maps to its extracellular aminoterminal domain (Rettenmier, C. W., unpublished data), whereas the location of the major tryptic phosphopeptide unique to the human protein has not been determined.

A comparison of [35S]methionine-labeled tryptic peptides from the 150-kD glycoprotein expressed in BeWo cells (Fig. 5 A) with those derived from virus-coded gp140v-fms (Fig. 5 B) also suggested that the proteins had related primary structures. A mixing experiment (Fig. 5 C) confirmed the presence of a subset of comigrating peptides as well as unique methionine-containing peptides derived from each molecule (see diagram in Fig. 5 D). The peptides unique to the viral product were not resolved in the peptide maps because they did not migrate in the chromatography solvent; these peptides are derived from the extracellular domain of gp140v-fms and are probably glycosylated (Rettenmier, C. W., unpublished data). Based on their structural and biochemical properties, we conclude that the 150-kD proteins expressed in choriocarcinoma cell lines are authentic c-fms gene products.

Human gp150c-fms is expressed at the cell surface. The mature form of the retroviral glycoprotein, gp140v-fms, is expressed at the surface of transformed cells, where it can be detected by peroxidase- or fluorescent-labeled antibody conjugates (26, 28, 30, 31). To test for expression of c-fms-coded molecules on the surfaces of human cells, live peripheral blood mononuclear cells and BeWo cells were enzymatically radioiodinated, and after detergent lysis, labeled molecules from the plasma membrane were precipitated with antiserum. Fig. 6 (lanes 1 and 2) shows that when this technique was applied to virus-transformed cells, only [125I]-labeled gp140v-fms was detected (28, 30). A radiolabeled
product of 150 kD was specifically precipitated from human peripheral blood mononuclear cells (lane 4) and from BeWo cells (lane 6), but was not detected with preimmune serum (lanes 3 and 5, respectively). This polypeptide was not evident in immunoprecipitates prepared from TPA-treated HL-60 cells (data not shown), as predicted from the low levels of its expression seen in the immune complex kinase assay (Fig. 1 B). Also consistent with the results of metabolic labeling and immune complex kinase assays, the human c-fms-coded glycoproteins were detected at significantly lower levels than their viral counterparts. The human c-fms-coded glycoprotein of 130 kD was not detected at the cell surface in agreement with the previous findings that it lacked mature N-linked oligosaccharide chains (Fig. 3).

**Human peripheral blood monocytes and choriocarcinoma cells bind CSF-1.** To determine whether cells producing the human c-fms gene product also expressed receptors for CSF-1, binding studies with the radiolabeled growth factor were performed. Human CSF-1 was partially purified from urine, radiolabeled with 123I, and the labeled growth factor was recovered by affinity chromatography using columns prepared with specific antibodies to CSF-1. Due to the limited amount of 123I-CSF-1 obtained by this procedure, the binding experiments were carried out at sub saturating CSF-1 concentrations. Autoradiographic examination of the total peripheral blood mononuclear cell population (5-10% monocytes; see Methods) indicated that only 3.5% of the cells were heavily labeled with 123I-CSF-1. The fraction of cells binding the growth factor was only slightly lower than the fraction of CSF-1 binding cells in mouse peripheral blood mononuclear cell preparations (~7.5%; see reference 3). When adherent peripheral blood mononuclear cells were isolated and tested for ligand binding using 10 nM CSF-1, these cells specifically bound ~40,000 123I-CSF-1 molecules per cell, and the bound 123I-CSF-1 did not dissociate during a 6-h incubation at 2°C in the absence of the growth factor.

Similar results were obtained with BeWo cells, which bound ~32,000 123I-CSF-1 molecules per cell. Again, binding was irreversible, and no dissociation of the bound ligand was detected after 6 h at 2°C in the absence of free CSF-1. In contrast, only about 1,500 CSF-1 binding sites per cell were detected under the same conditions using TPA-induced, adherent HL-60 cells. Thus, the extent of binding of CSF-1 correlated with the level of expression of gp150c-fms in the different cell populations.

**Discussion**

Antisera to a recombinant v-fms-coded polypeptide reacted with glycoproteins of 150 kD and 130 kD in lysates of human peripheral blood monocytes, TPA-induced HL-60 cells, and two human choriocarcinoma cell lines. Based on their antigenic crossreactivity with the v-fms gene product, their activities as substrates for an associated tyrosine kinase in immune complexes, and comparative analyses of methionine-containing and phosphorylated tryptic peptides, both polypeptides were shown to be products of the human c-fms gene. Like the retroviral gene products, gp120v-fms and gp140v-fms, gp130c-fms and gp150c-fms differ in their composition of asparagine-linked oligosaccharide chains, and even the larger species is expressed at the cell surface. These results suggest that gp130c-fms represents an immature form of the glycoprotein which is processed during intracellular transport to yield the mature cell surface glycoprotein, gp150c-fms. The molecular weight of the deglycosylated human c-fms polypeptide appears to be about 10 kD higher than the analogous viral protein. Recent nucleotide sequence analysis predicts that the human c-fms gene product differs from the virus-coded glycoprotein at its carboxyterminal end and contains additional C-terminal amino acids (32; Browning, P., H. F. Bunn, and A. W. Nienhuis, unpublished data). The carboxyterminal truncation of the v-fms gene product may be sufficient to account for the apparent molecular weight difference between it and the c-fms-coded polypeptide. However, since gp140v-fms is generated by proteolytic cleavage of a viral polyprotein precursor (gp180gag-fms), the extent of am inoterminal amino acid sequence homology between gp140v-fms and gp150c-fms also depends on post-translational processing events.

Expression of the c-fms gene product on the surfaces of human peripheral blood mononuclear cells was expected, based on the tissue-specific expression of c-fms in the feline system, and the recently defined relationship between the murine c-fms-coded glycoprotein and the receptor for CSF-1 (10). Similarly, c-fms transcripts were previously detected in TPA-treated HL-60 cells after they had differentiated to postmitotic, adherent macrophage-like cells (12, 13). Unlike the results obtained with normal peripheral blood mononuclear cells, reduced levels of gp150c-fms relative to gp130c-fms were seen in TPA-treated HL-60 cells. These results might reflect the failure of HL-60 cells to differentiate completely in culture or the possibility that phorbol esters downregulate expression of CSF-1 receptors, reducing the binding of CSF-1 to macrophages (33). Taken together, the above results are consistent with the conclusion that c-fms expression in adult tissues is restricted to cells of the mononuclear phagocyte lineage. However, c-fms transcripts (16) and protein products were also detected in choriocarcinoma cell lines derived from placental trophoblasts. Although expression of the c-fms gene products in choriocarcinoma cell lines could be related to transformation of trophoblastic cells, c-fms RNA is expressed at relatively high levels in normal placental tissues as well (15). Therefore, it seems more likely that the c-fms gene product plays a physiologic role in the placenta.
The relationship of the murine c-fms gene product to the CSF-1 receptor (10) and the recent demonstration that the v-fms oncogene product can itself bind murine CSF-1 (11) together suggested that human cells expressing gp150c-fms at their surface would specifically bind the growth factor. Purified murine CSF-1 does not bind to high affinity receptors on human mononuclear phagocytes nor support their growth in culture (1), and the limited availability of human CSF-1 precluded detailed studies of its binding to the different human cell populations. However, a comparison of the number of human CSF-1 molecules bound per adherent monocyte (~40,000 binding sites per cell) with the number of binding sites for murine CSF-1 on mouse bone marrow-derived macrophages (~50,000 per cell; see reference 4) suggested that almost all the CSF-1 sites on human peripheral blood monocytes were occupied under the binding conditions used. Furthermore, the binding was irreversible at 2°C, as observed for the binding of CSF-1 to murine cells (4), consistent with an interaction of the ligand with a high affinity receptor. Under the same conditions, the degree of 125I–CSF-1 binding by BeWo and TPA-induced HL-60 cells correlated with the levels of expression of gp150c-fms, with BeWo cells expressing a relatively high number of binding sites and HL-60 cells expressing very few. Although these correlative data strongly suggest that human gp150c-fms is related and possibly identical to the human CSF-1 receptor, the limited availability of the purified human hormone precluded more definitive biochemical assays. For example, purified murine 125I–CSF-1, when bound to its receptor, was precipitated with antibodies to the murine c-fms product and was shown to enhance phosphorylation of the immunoprecipitable receptor on tyrosine (10). Our attempts to generate similar results in the human system were unsuccessful. Since the human CSF-1 gene has been recently cloned and expressed (34), the availability of recombinant human CSF-1 may facilitate such studies.

A clear implication of our data is that CSF-1 could play a role in placental development. Murine CSF-1 levels have been shown to be elevated in uterine tissues during pregnancy (35), and conceivably, uterine CSF-1 could regulate early stages of development with specificity for cells of the fetal placenta that elaborate the receptor. Hence, CSF-1 may stimulate embryonic cells that are not committed to the mononuclear phagocytic lineage.

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Note added in proof. Woolford et al. (36) have recently identified the human c-fms gene product in BeWo choriocarcinoma cells and phorbol ester–treated ML-1 cells.

References


