Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 3331–3335, May 1986
Cell Biology

Specific binding of the mononuclear phagocyte colony-stimulating factor CSF-1 to the product of the v-*fms* oncogene

(colony-stimulating factor-1 receptor/growth factors/tyrosine kinases)

ROSALBA SACCA*, E. RICHARD STANLEY*, CHARLES J. SHERR†, AND CARL W. RETTENMIER†

*Department of Microbiology and Immunology, and Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY 10461; and †Department of Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, TN 38105

Communicated by Hidesaburo Hanafusa, January 22, 1986

ABSTRACT **Cells transformed by the McDonough strain of feline sarcoma virus (SM-FeSV) express a v-*fms*-encoded glycoprotein whose expression at the cell surface correlates with the transformed phenotype. The mouse mononuclear phagocyte growth factor CSF-1 specifically binds to SM-FeSV-transformed cells at high-affinity sites indistinguishable from those detected on normal feline macrophages. A monoclonal antibody to a v-*fms*-encoded epitope competed for CSF-1 binding to SM-FeSV-transformed cells, and chemical cross-linking demonstrated that murine CSF-1 bound to the v-*fms* gene product at the cell surface. Although SM-FeSV-transformed fibroblast lines were found to secrete CSF-1, the growth of transformed cells was not affected by antibodies to the v-*fms* gene product or to the growth factor. Tyrosine phosphorylation of the v-*fms* products in membranes was observed in the absence of CSF-1 and was not enhanced by addition of the murine growth factor. The data support the hypothesis that the c-*fms* protooncogene product is related, and possibly identical, to the CSF-1 receptor and suggest that the v-*fms*-encoded kinase functions in the absence of an exogenous growth factor.**

Murine colony-stimulating factor 1 (CSF-1) is a glycoprotein growth factor of 45–90 kDa that acts on cells of the mononuclear phagocyte lineage (1). CSF-1 is a heterodisperse dimer composed of two 14-kDa polypeptide chains that are glycosylated to varying extents (2, 3). Partial amino acid sequence determination suggests that the two polypeptide chains are identical (A. Boosman, J. E. Strickler, K. J. Wilson, and E.R.S., unpublished data). Iodinated CSF-1 binds to a single class of high-affinity receptors on mouse macrophages (4–6). The purified receptor is a 165-kDa glycoprotein consisting of a single polypeptide chain that exhibits CSF-1-dependent autophosphorylation on tyrosine (Y. G. Yeung, P. T. Jubinsky, and E.R.S., unpublished data). To date, only mononuclear phagocytes and their hemopoietic precursors have been shown to express CSF-1 receptors (4, 5, 7). Increased expression of the CSF-1 receptor on hemopoietic cells coincides with their determination to the mononuclear phagocytic lineage (7) and represents an early marker of monocytic differentiation.

The retroviral oncogene v-*fms* codes for an integral transmembrane glycoprotein of 140 kDa that shares critical properties with a class of receptors for polypeptide growth factors (8–10). The mature glycoprotein is oriented at the cell surface with its amino-terminal domain (≈450 amino acids) outside the cell and its carboxy-terminal portion (≈400 amino acids) in the cytoplasm (10). The cytoplasmic domain shares amino acid sequence homology with prototypic enzymes of the tyrosine kinase gene family (11) and undergoes autophosphorylation on tyrosine residues *in vitro* (12). The product of the feline c-*fms* protooncogene is also a glycoprotein with an associated tyrosine kinase activity (13). This normal cellular protein is primarily restricted in its expression to mononuclear phagocytes and was recently shown to be biochemically and antigenically related to the murine CSF-1 receptor (14). We now show that the feline v-*fms*-encoded transforming protein specifically binds murine CSF-1, supporting the hypothesis that the c-*fms* protooncogene product and the CSF-1 receptor are encoded by closely related and possibly identical genes.

MATERIALS AND METHODS

Cells and Cell Culture Conditions. Peritoneal-exudate macrophages were obtained after inoculation of cats with glycogen (14). Adherent monolayers of macrophages were obtained by plating 10^6 cells per 25-mm culture dish in Dulbecco's modified Eagle's medium (DMEM) plus 15% fetal bovine serum and removing cells that remained nonadherent after a 2-hr incubation at 37°C.

Subclones of mink CCL64, rat NRK, and mouse NIH 3T3 cells transformed with the McDonough strain of feline sarcoma virus (SM-FeSV) or with the Gardner–Arnstein strain of FeSV (GA-FeSV), expressing the v-*fms* and v-*fes* oncogene products, respectively, were grown in DMEM containing 10% fetal bovine serum, antibiotics, and 0.29 mg of glutamine per ml; SM-FeSV-transformed NRK and NIH 3T3 cells were cultured in 0.4% Noble agar (Difco) in the same medium. A CSF-1-dependent subclone (2F5) of the murine macrophage cell line BAC1 (15) was grown in alpha minimal essential medium containing 15% fetal bovine serum, 0.29 mg of glutamine per ml, 0.02 mg of asparagine per ml, 50 μM 2-mercaptoethanol, and 3000 units of stage-1 L-cell CSF-1 per ml. The CSF-1-independent mouse macrophage line J774.2 was maintained in the same medium without CSF-1.

Antisera and Monoclonal Antibodies. The rat monoclonal antibodies SM 5.15.4 and SM 2.6.3 are directed against v-*fms*-encoded polypeptide epitopes expressed on the surface of transformed cells and were purified to homogeneity as described (16). Rabbit antiserum to a recombinant v-*fms*-encoded polypeptide expressed in bacteria ($bp81^{v\text{-}fms}$) reacts with feline v-*fms*- and c-*fms*-encoded proteins and also with c-*fms*-encoded molecules from other species (14). Rabbit antisera to purified mouse L-cell CSF-1 were as described (17).

Binding of ^{125}I-Labeled CSF-1 to Cells. Mouse L-cell CSF-1 was purified (2, 17) and its concentration determined by radioimmunoassay (18) and by radioreceptor assay (17). Purified CSF-1 was iodinated with carrier-free ^{125}I (Amersham), with full retention of biological activity to a specific radioactivity of ≈2 × 10^5 cpm/ng of protein (2, 6). For binding assays, ^{125}I-labeled CSF-1 was incubated with

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Abbreviations: CSF-1, colony-stimulating factor 1; SM-FeSV, McDonough strain of feline sarcoma virus; GA-FeSV, Gardner–Arnstein strain of FeSV.

cell monolayers for 24 hr at 2°C in the presence or absence of a 20-fold excess of unlabeled CSF-1 (4, 6). For antibody-competition experiments, cells were incubated with monoclonal antibody at 50 μg/ml or with an isotype-matched control antibody at the same concentration. Binding of ^{125}I-labeled CSF-1 in the presence of excess unlabeled CSF-1 represented <0.3% of the input radioactivity.

Chemical Crosslinking of ^{125}I-Labeled CSF-1 to Cells. Chemical crosslinking was carried out by a modification (19) of the method of Pilch and Czech (20). Cells were incubated with ^{125}I-labeled CSF-1 (0.5 nM) for 2 hr at 2°C, washed, and incubated in 0.2 mM disuccinimidyl suberate for 45 min at 2°C. The crosslinking reaction was quenched by addition of 50 mM Tris Cl (pH 7.4). After a 10-min incubation in dissociation buffer (150 mM NaCl/70 mM sodium acetate, pH 4.0), the cells were disrupted with detergent, and clarified lysates were treated with rabbit antiserum to bp81$^{v\text{-}fms}$ (14). Approximately 7% of the specifically bound ^{125}I-labeled CSF-1 was crosslinked to the cells under the conditions used.

Binding of Monoclonal Antibodies to Cells. Purified monoclonal antibody SM 2.6.3, iodinated to a specific activity of ≈0.5 × 10^4 cpm/ng, was incubated with 10^5 cells per 16-mm culture dish for 3 hr at 2°C. Binding medium was DMEM containing 25 mM Hepes (pH 7.4), 0.2% bovine serum albumin, and 0.02% bovine gamma globulin, either with or without a 50-fold excess of unlabeled monoclonal antibody. Cells were washed with binding medium and then lysed, and the bound radioactivity was determined. Background binding in the presence of unlabeled antibody represented <7% of the total radioactivity bound to cells in the presence of labeled antibody alone.

Assay for CSF-1-Induced Membrane Phosphorylation. Cell membranes were prepared as described (14). Membranes (30–60 μg of protein) were incubated for 8 min at 4°C in 25 mM Hepes buffer (pH 7.4) containing 50 mM NaCl, 100 nM purified CSF-1, 15 mM $MnCl_2$, 8 mM $MgCl_2$, and 20 μM [γ-^{32}P]ATP (3000 Ci/mol, Amersham; 1 Ci = 37 GBq), in a final reaction volume of 50 μl. A 1-hr preincubation with CSF-1 prior to addition of [γ-^{32}P]ATP and divalent cations did not alter the results. The reaction products were analyzed by polyacrylamide gel electrophoresis under denaturing conditions, as described (8).

RESULTS

SM-FeSV-Transformed Cells Specifically Bind CSF-1. Because a mature form of the v-*fms*-encoded glycoprotein, gp140$^{v\text{-}fms}$, is transported to the plasma membrane of SM-FeSV-transformed cells (8–10, 21), radiolabeled monoclonal antibodies directed against epitopes in the extracellular domain can be used to estimate the number of molecules at the cell surface. Fig. 1*A* shows that, at 2°C, SM-FeSV-transformed mink and rat cells specifically bound an ^{125}I-labeled monoclonal antibody (SM 2.6.3) directed against a v-*fms*-encoded epitope, whereas nontransformed mink and rat cells or subclones transformed by GA-FeSV did not. Specific binding of ^{125}I-labeled SM 2.6.3 to SM-FeSV-transformed cells was completely inhibited by a 50-fold excess of unlabeled antibody, and no binding was observed with a control monoclonal antibody. Based on the specific activity of the iodinated antibody, the amount bound at saturating concentrations (≈10 nM) corresponded to about 2 × 10^5 and 1 × 10^5 molecules per cell for SM-FeSV-transformed mink and rat cells, respectively.

The v-*fms*-encoded glycoprotein includes the extracellular domain of the c-*fms* protooncogene product (13). Because of the relationship of the latter molecules to the CSF-1 receptor (14), we reasoned that cells transformed by SM-FeSV might specifically bind CSF-1. Fig. 1*B* shows that SM-FeSV-transformed mink cells bound ^{125}I-labeled murine CSF-1,

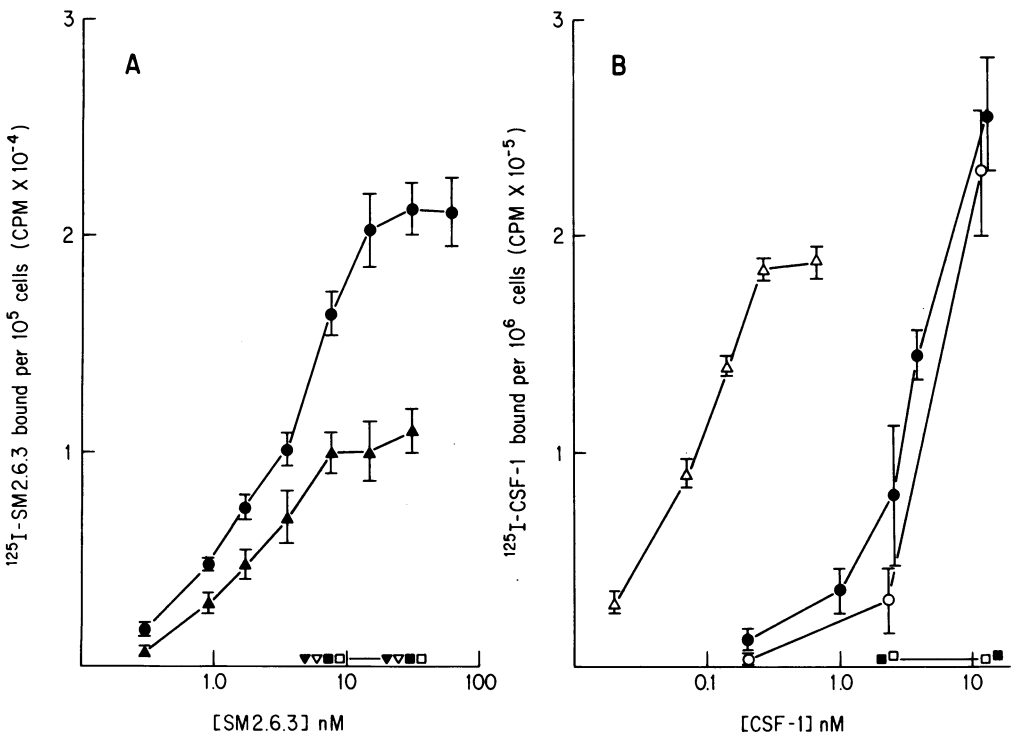

FIG. 1. Binding of ^{125}I-labeled monoclonal antibody (*A*) or ^{125}I-labeled CSF-1 (*B*) to SM-FeSV-transformed cells. Binding equals the total ^{125}I bound minus the ^{125}I bound in the presence of unlabeled antibody (*A*) or unlabeled CSF-1 (*B*). The error bars indicate ± 1 SD for triplicate determinations. Symbols: ●, SM-FeSV-transformed mink cells; ▲, SM-FeSV-transformed rat cells; ■, nontransformed mink cells; □, GA-FeSV-transformed mink cells; ▼, nontransformed rat cells; ▽, GA-FeSV-transformed rat cells; △, mouse J774.2 macrophages; ○, cat peritoneal-exudate macrophages.

whereas nontransformed mink cells or a GA-FeSV-transformed subclone did not. Similar results were obtained using SM-FeSV- or GA-FeSV-transformed rat NRK and mouse NIH 3T3 cell lines (data not shown), indicating that binding was specific and independent of the host-cell species.

With murine J774.2 macrophages, saturation of binding sites ($\approx 2 \times 10^4$ per cell) was obtained at CSF-1 concentrations of about 0.2 nM (Fig. 1*B*). The binding of CSF-1 to mouse macrophages is essentially irreversible, precluding analysis by equilibrium methods (6), and the binding affinity (K_d), estimated by kinetic measurements at 2°C, is $\leq 10^{-13}$ M. In contrast, saturation of binding sites on SM-FeSV-transformed mink cells was not obtained at CSF-1 concentrations as high as 12 nM, suggesting that the binding to transformed cells was of lower affinity. SM-FeSV-transformed mink cells bound 2.5×10^4 molecules per cell at the maximum CSF-1 concentration tested, corresponding to 12% of the number of v-*fms*-encoded molecules expressed per cell. Higher concentrations could not be used due to the limited availability of the purified ligand. Although relatively high concentrations of murine CSF-1 were needed to demonstrate significant receptor occupancy, SM-FeSV-transformed cells, like J774.2 cells, bound CSF-1 irreversibly at neutral pH, and no dissociation of the bound ligand was detected after 6 hr with the binding conditions used.

Since CSF-1 was purified from mouse cells and the v-*fms* gene product was derived from a feline protooncogene, binding of murine CSF-1 to normal cat peritoneal-exudate macrophages was also assessed. Binding of ^{125}I-labeled murine CSF-1 to cat macrophages was indistinguishable from that observed with SM-FeSV-transformed mink cells (Fig. 1*B*) and was again irreversible (data not shown). Therefore, the 100- to 1000-fold difference in the apparent affinity of murine CSF-1 for the feline CSF-1 receptor is due to a slower on-rate, and cells transformed by v-*fms* bind murine CSF-1 at affinities comparable to that of cat macrophages. Consistent with this reduced affinity of binding, cat macrophages cannot be maintained in culture by addition of relatively high concentrations (up to 22 nM) of the mouse growth factor.

CSF-1 Binds to the v-*fms* Gene Product. To determine whether the binding of ^{125}I-labeled CSF-1 to SM-FeSV-transformed cells involved a specific interaction with the v-*fms* gene product, two separate experiments were carried out. First, two monoclonal antibodies directed against epitopes in the extracellular domain of the v-*fms*-encoded glycoprotein were tested for their ability to compete with ^{125}I-labeled CSF-1 for binding to SM-FeSV-transformed mink cells. At 2 nM ^{125}I-labeled CSF-1 (see Fig. 1*B*), one of the monoclonal antibodies (SM 5.15.4) inhibited ^{125}I-labeled CSF-1 binding as effectively as a 20-fold excess of unlabeled CSF-1, whereas the other (SM 2.6.3) did not. An irrelevant isotype-matched antibody also had no effect on CSF-1 binding.

In a second experiment, SM-FeSV-transformed mink cells were incubated at 2°C with radioiodinated CSF-1, washed to remove unbound ligand, and treated with the chemical crosslinking agent disuccinimidyl suberate. After a brief incubation at pH 4 to remove labeled CSF-1 that was not crosslinked to receptors, the cells were washed and lysed with detergent, and the clarified lysates were incubated with an antiserum to a recombinant v-*fms*-encoded polypeptide that precipitates the CSF-1 receptor (14). Immune and preimmune control precipitates were then subjected to $NaDodSO_4$/PAGE.

Fig. 2 shows that the preparation of ^{125}I-labeled dimeric CSF-1 used in these experiments had an apparent molecular mass of 76 kDa (lane 1) and was comprised of 40-kDa subunits (lane 2). After ^{125}I-labeled CSF-1 was crosslinked to cells, no polypeptides were seen in control precipitates obtained with preimmune serum run under nonreducing (lane 3) or reducing (lane 4) conditions. In contrast, labeled polypeptides were detected in immunoprecipitates obtained with antiserum to the v-*fms*-encoded glycoproteins. In the absence of reducing agent (lane 5), the apparent size of one crosslinked species (220 kDa) suggested that it was formed by the crosslinking of one molecule of ligand to the mature, cell-surface form of the transforming glycoprotein ($gp140^{v\text{-}fms}$). A specifically precipitated crosslinked form of higher molecular mass was also seen, possibly corresponding to two receptors complexed to a single dimeric ligand. Consistent with this interpretation, a reduction in the apparent size of the crosslinked species (to 185 kDa) and the appearance of a species with the molecular mass of a single CSF-1 subunit was observed after reduction (lane 6). Since the efficiency of crosslinking is relatively low, only one subunit of CSF-1 would be crosslinked to its receptor in most cases, yielding single free subunits after reduction. Thus, the binding of CSF-1 to SM-FeSV-transformed cells is mediated through the v-*fms* gene product.

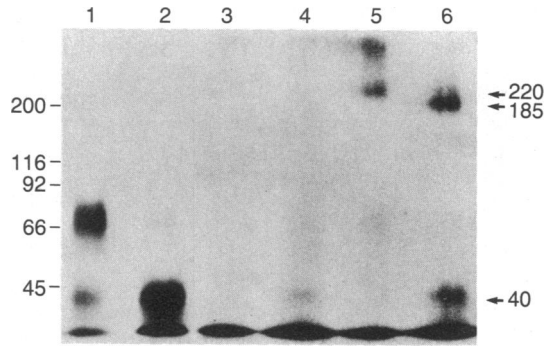

FIG. 2. Immunoprecipitation of ^{125}I-labeled CSF-1 chemically crosslinked at the surface of SM-FeSV-transformed mink cells. Autoradiographs of ^{125}I-labeled CSF-1 (lanes 1 and 2) and precipitates from solubilized cells obtained with preimmune serum (lanes 3 and 4) or antiserum (lanes 5 and 6) were composited from the same $NaDodSO_4$/7% polyacrylamide gel. Samples were separated by electrophoresis under nonreducing (lanes 1, 3, and 5) or reducing conditions (lanes 2, 4, and 6). The apparent molecular masses (in kDa) of marker proteins (left) and of the crosslinked species (right) are indicated.

Phosphorylation of v-*fms*-Encoded Molecules. Binding of CSF-1 to its receptor activates an associated protein kinase that phosphorylates the receptor on tyrosine residues (14). With membranes from the CSF-1-dependent murine macrophage cell line BAC1.2F5, enhanced phosphorylation of the 165-kDa murine CSF-1 receptor was observed in the presence of the growth factor (Fig. 3*A*, lanes 3 and 4). The 165-kDa phosphoprotein was precipitated with antiserum to a recombinant v-*fms*-encoded polypeptide (Fig. 3*B*, lanes 3 and 4) and was phosphorylated *in vitro* only on tyrosine (14). In similar experiments performed with membranes from cat peritoneal-exudate macrophages, *in vitro* phosphorylation of the homologous feline c-*fms*-encoded polypeptide was not observed, even at concentrations of murine CSF-1 as high as 200 nM (data not shown). Since cat peritoneal-exudate macrophages express CSF-1 receptors (Fig. 1*B*), the lack of *in vitro* phosphorylation, even in the presence of the growth factor, may reflect the decreased affinity of the feline receptor for murine CSF-1.

In contrast, phosphorylation of three SM-FeSV-encoded glycoproteins, $gP180^{gag\text{-}fms}$, $gp120^{v\text{-}fms}$, and $gp140^{v\text{-}fms}$, was observed in the presence or absence of the growth factor (Fig. 3*A*, lanes 1 and 2), and the immunoprecipitated polypeptides (Fig. 3*B*, lanes 1 and 2), contained similar amounts of radiolabeled phosphotyrosine whether hormone was added or not (phospho amino acid analysis not shown). The relative amounts of the three phosphorylated viral glycoproteins detected in membrane preparations were similar to their

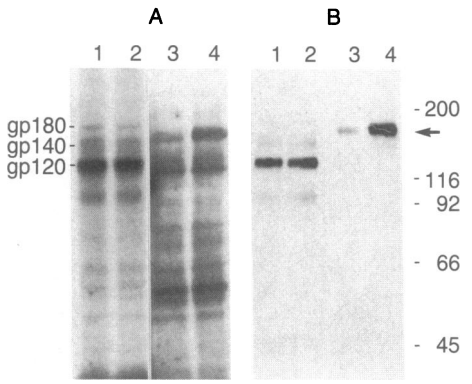

FIG. 3. Phosphorylation of v-*fms*-encoded glycoproteins and the murine CSF-1 receptor in membrane preparations. Membranes (30 μg of protein) from SM-FeSV-transformed mink cells (lanes 1 and 2) and from the BAC1.2F5 murine macrophage cell line (lanes 3 and 4) were incubated with [γ-^{32}P]ATP in the presence (lanes 2 and 4) or absence (lanes 1 and 3) of 100 nM purified murine CSF-1. Aliquots of the membrane preparations were treated with detergent and subjected to gel electrophoresis (*A*) or were immunoprecipitated with antiserum to a v-*fms*-encoded polypeptide prior to electrophoresis (*B*). Because the levels of v-*fms*-encoded proteins in transformed cells are 10- to 20-fold higher than those of the c-*fms* product in macrophages, approximately equal quantities of radioactivity, loaded in each lane of *A*, were taken for immunoprecipitation in *B*. The positions of protein standards of known molecular mass (kDa) are shown at right, and the positions of the three v-*fms* products are indicated at left. The arrow indicates the 165-kDa murine CSF-1 receptor.

steady-state levels in transformed mink cells (16, 21). The 180-kDa polyprotein encoded by the fused viral *gag* and v-*fms* genes is cleaved to generate gp120$^{v\text{-}fms}$, which is modified during intracellular transport to generate the mature, cell-surface form of the glycoprotein, gp140$^{v\text{-}fms}$ (21). The carboxyl-terminal domains of these membrane-bound molecules are sensitive to proteolytic attack when incubated *in vitro* (10), apparently generating the specifically precipitable 95-kDa band seen in Fig. 3*B*, lanes 1 and 2. Since gp120$^{v\text{-}fms}$ is inefficiently processed to mature molecules in transformed cells, it represents the major form detected at steady state (8, 16, 21). The ability to detect tyrosine phosphorylation of the v-*fms* products under conditions in which phosphorylation of the feline c-*fms* products was not observed suggests that the viral transforming protein may function constitutively as a kinase.

Does CSF-1 Affect the v-*fms*-Induced Transformed Phenotype? A clone of SM-FeSV-transformed NRK cells that forms continuously growing colonies in agar medium containing 10% fetal bovine serum failed to form colonies when the serum concentration was lowered to 1%. Under these conditions, the cells remain viable and can be stimulated to form colonies by addition of serum several days later. Cells plated in 1% serum with purified CSF-1 concentrations as high as 4.4 nM did not show an increased colony-forming efficiency. Therefore, the requirement of transformed cells for serum factors cannot be overcome by the addition of murine CSF-1 alone.

The inability of murine CSF-1 to stimulate colony formation by SM-FeSV-transformed cells could reflect the production of a related growth factor by the cells themselves. By use of a competition radioreceptor assay, CSF-1 activity was detected in conditioned media from SM-FeSV-transformed mink and rat cells as well as from the nontransformed parental cell lines. Since the relative affinities of mink and rat CSF-1 for the murine receptor are unknown, CSF-1 activity could not be quantitated. However, competition radioreceptor and radioimmunoassays indicated that medium conditioned for 3 days by confluent cultures of mouse NIH 3T3 cells or their transformed derivatives contained 0.07 ± 0.1 nM CSF-1 (mean ± SEM, $n = 6$).

To test for stimulation of a functional v-*fms*-encoded receptor by exogenous CSF-1, SM-FeSV-transformed rat cells were seeded in agar medium containing 10% fetal bovine serum, in the presence or absence of the v-*fms*-specific monoclonal antibody SM 5.15.4. Since this antibody inhibits CSF-1 binding to SM-FeSV-transformed cells, it might have affected their colony-forming efficiency. Concentrations of purified monoclonal antibody as high as 100 μg/ml did not inhibit colony formation. In each case, the cloning efficiency of the cells in agar was ≈25%, and the sizes of colonies were similar. In analogous experiments, SM-FeSV-transformed NIH 3T3 cells formed colonies at equal efficiency when plated in the presence or absence of antiserum to murine CSF-1. Thus, although transformed fibroblasts produce CSF-1, transformation induced by the v-*fms* gene product is not dependent on an exogenous source of the growth factor.

DISCUSSION

The v-*fms* oncogene, like its c-*fms* progenitor, codes for a protein with two functional domains: an extracellular ligand-binding moiety, which can specifically interact with CSF-1, and an intracellular portion, which functions as a tyrosine kinase. Our previous study (14) showed that the feline c-*fms* gene product was primarily restricted in its expression to cat macrophages and that antiserum to a recombinant v-*fms*-encoded polypeptide specifically precipitated the murine CSF-1 receptor from mouse macrophage cell lines. However, antigenic crossreactivity between the feline and murine polypeptides could have been due to shared epitopes mapping within the kinase domains of both molecules. In contrast, the functional relatedness of these molecules, as reflected by their ability to bind CSF-1, depends upon an extracellular ligand-binding domain not shared with other known oncogene products. Although sequencing of the CSF-1 receptor will provide unequivocal data, these results strengthen the hypothesis that v-*fms* was derived by recombination of feline leukemia virus with a CSF-1-receptor gene.

In the case of the murine CSF-1 receptor, ligand binding activates the receptor-associated kinase, apparently providing a signal for cell growth. In contrast, the v-*fms*-encoded glycoprotein exhibited tyrosine kinase activity *in vitro* in the presence or absence of exogenous CSF-1, suggesting that its kinase could act constitutively. The addition of murine CSF-1 to SM-FeSV transformants did not overcome their serum requirement for colony formation in agar. Moreover, antiserum to CSF-1 or a monoclonal antibody that was active in inhibiting CSF-1 binding to the v-*fms* gene product did not inhibit the growth of transformed cells in semisolid medium. Taken together, the results suggest that the feline v-*fms* gene product behaves as a receptor with uncoupled ligand-binding and enzymatic functions. Since the v-*fms* gene product binds murine CSF-1 in a manner indistinguishable from the receptor on feline macrophages, the apparent defect in kinase regulation is most likely determined by alterations in the enzymatic domain. In this respect, the viral oncogene product may be functionally analogous to the v-*erbB*-encoded glycoprotein which represents a truncated form of the epidermal growth factor receptor (22, 23).

The assays for v-*fms*-encoded tyrosine kinase activity were performed with purified murine CSF-1 rather than the feline growth factor. The murine growth factor binds with significantly lower affinity to feline macrophages (saturation >12 nM) than to murine macrophages (saturation at 0.2 nM) and does not support the growth of feline macrophages in culture. Attempts to stimulate tyrosine kinase activity in membranes prepared from feline macrophages by using concentrations of

murine CSF-1 as high as 200 nM did not result in enhanced phosphorylation of the feline c-*fms* gene product. These results leave open the possibility that the v-*fms*-encoded glycoproteins might show enhanced kinase activity in the presence of feline CSF-1. Cell lines susceptible to SM-FeSV transformation produced CSF-1. Despite the fact that the growth of transformed cells in semi-solid medium was not dependent on the exogenous growth factor, an effect of CSF-1 on the transformed phenotype may not require its release from cells. If this were the case, transduction of the v-*fms*-encoded receptor into fibroblasts that produce CSF-1 (24) could potentiate the ability of SM-FeSV to induce fibrosarcomas in cats (25).

We thank Claudia Saez, Sheila McParlin, Ann Palestroni, Hana Gilat, Shawn Kramer, and Jean Elmendorf for excellent technical assistance and Lenore Grollman and Peggy Burdick for help in preparation of the manuscript. This work was supported by Grants CA26504 (to E.R.S.) and CA38187 (to C.J.S.) from the National Cancer Institute; by the AECOM Cancer Center Core Grant (Albert Einstein College of Medicine), and by the American Lebanese Syrian Associated Charities of St. Jude Children's Research Hospital. R.S. was supported by National Institutes of Health Training Grant CA09173, E.R.S. is a recipient of an Irma T. Hirschl Career Scientist Award, and C.W.R. was supported by a Biomedical Research Support Grant (RR-05584-21) from the National Institutes of Health.

1. Stanley, E. R., Guilbert, L. J., Tushinski, R. J. & Bartelmez, S. H. (1983) *J. Cell. Biochem.* **21,** 151–159.
2. Stanley, E. R. & Heard, P. M. (1977) *J. Biol. Chem.* **252,** 4305–4312.
3. Das, S. K. & Stanley, E. R. (1982) *J. Biol. Chem.* **257,** 13679–13684.
4. Guilbert, L. J. & Stanley, E. R. (1980) *J. Cell Biol.* **85,** 153–159.
5. Byrne, P. V., Guilbert, L. J. & Stanley, E. R. (1981) *J. Cell Biol.* **91,** 848–853.
6. Stanley, E. R. & Guilbert, L. J. (1981) *J. Immunol. Methods* **42,** 253–284.
7. Bartelmez, S. H. & Stanley, E. R. (1985) *J. Cell. Physiol.* **122,** 370–378.
8. Roussel, M. F., Rettenmier, C. W., Look, A. T. & Sherr, C. J. (1984) *Mol. Cell. Biol.* **4,** 1999–2009.
9. Manger, R., Najita, L., Nichols, E. J., Hakomori, S.-I. & Rohrschneider, L. (1984) *Cell* **39,** 327–337.
10. Rettenmier, C. W., Roussel, M. F., Quinn, C. O., Kitchingman, G. R., Look, A. T. & Sherr, C. J. (1985) *Cell* **40,** 971–981.
11. Hampe, A., Gobet, M., Sherr, C. J. & Galibert, F. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 85–89.
12. Barbacid, M. & Lauver, A. V. (1981) *J. Virol.* **40,** 812–821.
13. Rettenmier, C. W., Chen, J. H., Roussel, M. F. & Sherr, C. J. (1985) *Science* **228,** 320–322.
14. Sherr, C. J., Rettenmier, C. W., Sacca, R., Roussel, M. F., Look, A. T. & Stanley, E. R. (1985) *Cell* **41,** 665–676.
15. Schwarzbaum, S., Halpern, R. & Diamond, B. (1984) *J. Immunol.* **132,** 1158–1162.
16. Anderson, S. J., Furth, M., Wolff, L., Ruscetti, S. K. & Sherr, C. J. (1982) *J. Virol.* **44,** 696–702.
17. Stanley, E. R. (1985) *Methods Enzymol.* **116,** 564–587.
18. Stanley, E. R. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 2969–2973, and correction (1979) **76,** 5411.
19. Morgan, C. J. & Stanley, E. R. (1984) *Biochem. Biophys. Res. Commun.* **119,** 35–41.
20. Pilch, P. F. & Czech, M. R. (1979) *J. Biol. Chem.* **254,** 3375–3381.
21. Anderson, S. J., Gonda, M. A., Rettenmier, C. W. & Sherr, C. J. (1984) *J. Virol.* **51,** 730–741.
22. Downward, J., Yarden, Y., Mayes, E., Scrace, G., Totty, N., Stockwell, P., Ullrich, A., Schlessinger, J. & Waterfield, M. D. (1984) *Nature (London)* **307,** 521–527.
23. Ullrich, A., Coussens, L., Hayflick, J. S., Dull, T. J., Gray, A., Tam, A. W., Lee, J., Yarden, Y., Libermann, T. A., Schlessinger, J., Downward, J., Mayes, E. L. V., Whittle, N., Waterfield, M. D. & Seeburg, P. H. (1984) *Nature (London)* **309,** 418–425.
24. Tushinski, R. J., Oliver, I. T., Guilbert, L. J., Tynan, P. W., Warner, J. R. & Stanley, E. R. (1982) *Cell* **28,** 71–81.
25. Hardy, W. D., Jr. (1981) *J. Am. Hosp. Assoc.* **17,** 981–996.