Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 5613–5617, August 1990
Cell Biology

Macrophage-colony-stimulating factor (CSF-1) induces proliferation, chemotaxis, and reversible monocytic differentiation in myeloid progenitor cells transfected with the human c-*fms*/CSF-1 receptor cDNA

JACALYN H. PIERCE*, EDDI DI MARCO*, GEORGE W. COX†, DANIELA LOMBARDI*, MARCO RUGGIERO*, LUIGI VARESIO†, LING MEI WANG‡, G. GHOSH CHOUDHURY‡, ALAN Y. SAKAGUCHI‡, PIER PAOLO DI FIORE*, AND STUART A. AARONSON*

*Laboratory of Cellular and Molecular Biology, National Cancer Institute, Building 37, Room 1E24, Bethesda, MD 20892; †Laboratory of Molecular Immunoregulation, National Cancer Institute-Frederick Cancer Research Facility, Frederick, MD 21701-1013; and ‡Department of Cellular and Structural Biology, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78284

Communicated by William E. Paul, April 13, 1990

ABSTRACT **The c-*fms* protooncogene encodes the receptor for macrophage-colony-stimulating factor (CSF-1). Expression vectors containing either normal or oncogenic point-mutated human c-*fms* genes were transfected into interleukin 3 (IL-3)-dependent 32D cells in order to determine the effects of CSF-1 signaling in this murine clonal myeloid progenitor cell line. CSF-1 was shown to trigger proliferation in association with monocytic differentiation of the 32D-c-*fms* cells. Monocytic differentiation was reversible upon removal of CSF-1, implying that CSF-1 was required for maintenance of the monocyte phenotype but was not sufficient to induce an irrevocable commitment to differentiation. Human CSF-1 was also shown to be a potent chemoattractant for 32D-c-*fms* cells, suggesting that CSF-1 may serve to recruit monocytes from the circulation to tissue sites of inflammation or injury. Although c-*fms* did not release 32D cells from factor dependence, point-mutated c-*fms*[S301,F969] (Leu-301 → Ser, Tyr-969 → Phe) was able to abrogate their IL-3 requirement and induce tumorigenicity. IL-3-independent 32D-c-*fms*[S301,F969] cells also displayed a mature monocyte phenotype, implying that differentiation did not interfere with progression of these cells to the malignant state. All of these findings demonstrate that a single growth factor receptor can specifically couple with multiple intracellular signaling pathways and play a critical role in modulating cell proliferation, differentiation, and migration.**

Accumulating evidence indicates that genetic alterations subverting growth factor-regulated signaling pathways play key roles in malignancy. However, it is not clear whether susceptibility to transformation correlates with a particular stage in differentiation and whether transformation necessarily blocks further maturation. To address these questions it is necessary to understand the mechanisms that regulate differentiation, commitment, and maturation of cells to the postmitotic state. One model suggests that irreversible commitment and maturation may occur at random after a certain number of cell divisions or may be triggered in a preprogrammed cell by the withdrawal of an external proliferative signal. An alternative model is that differentiation is at least partially controlled by external physiological stimuli.

Certain hematopoietic cytokines, in particular the colony-stimulating factors (CSFs), are thought to be involved in the normal progression of hematopoietic progenitor cells to a terminally differentiated state (reviewed in ref. 1). One CSF whose actions have been implicated in the differentiation process is macrophage-CSF (CSF-1) (reviewed in ref. 2). CSF-1 interacts with a single class of high-affinity receptors expressed specifically on cells of the monocytic lineage (3, 4). The receptor for CSF-1 is identical to the c-*fms* protooncogene product and is endowed with intrinsic tyrosine kinase activity that is activated by ligand binding (5). It has been shown that two point mutations at amino acids 301 and 969 in the human c-*fms* gene confer constitutive tyrosine kinase activity on the receptor and activate its transforming potential for NIH 3T3 cells (6).

One successful approach to the study of biological and biochemical effects of growth factor–receptor interaction has utilized factor-dependent hematopoietic cell lines. One such line, 32D, is strictly dependent on interleukin 3 (IL-3) for growth, possesses a normal diploid karyotype, and is nontumorigenic in nude mice (7, 8). Although these cells maintain an immature myeloid phenotype when propagated in IL-3, they can be programmed to differentiate to mature neutrophils when exposed to granulocyte-CSF (G-CSF) (8). The fact that 32D is a clonal myeloid precursor cell line has raised the question as to whether signals transduced by certain activated receptor kinases might interact with substrates capable of promoting a sustained differentiation as well as a proliferation signal. If so, it might be possible to eventually identify the specific biochemical pathways involved. In the present study, we inserted expression vectors containing either the normal or activated human c-*fms* genes into 32D cells to provide a model system for determining the effects of CSF-1 on biological signaling pathways in a homogeneous clonal population of myeloid progenitor cells.

MATERIALS AND METHODS

Cells, Expression Vectors, and Transfection Assays. The 32D cell line (7) and the mouse macrophage cell line P388D_1 (3) (ATCC) have been described. The WEHI-3 and MIA-PaCa-2 cell lines (both from ATCC) were used as a source for conditioned medium containing murine IL-3 or human CSF-1, respectively. The LTR-2/c-*fms* and LTR-2/c-*fms*[S301, F969] vectors were engineered from the previously described LTR-2 vector (9) by using a full-length human c-*fms* cDNA clone (10) or a human c-*fms*[S301,F969] cDNA (containing mutations resulting in Leu-301 → Ser and Tyr-969 → Phe substitutions; ref. 6; a gift from Charles Sherr, St. Jude's Children's Research Hospital, Memphis, TN), respectively.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CSF, colony-stimulating factor; CSF-1, macrophage-CSF; G-CSF, granulocyte-CSF; GM-CSF, granulocyte/macrophage-CSF; IL, interleukin; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; $Fc_\gamma R$, Fc_γ receptor; MFI, mean fluorescence intensity; NBT, nitroblue tetrazolium; u, unit(s).

Gene transfer was achieved by a modified method of electroporation (11). Stable transfectants were selected in medium containing 80 mM mycophenolic acid, 0.2 mM sodium hypoxanthime, 0.4 mM aminopterin, and 16 mM thymidine.

Immunoblot Analysis and Radioreceptor, and Mitogenic Assays. Immunoblot analysis was performed on membrane protein as described (9), using an anti-c-Fms peptide serum directed against amino acids 791–806. Purified recombinant human CSF-1 (a gift of Steven Clark, Genetics Institute, Cambridge, MA) was radioiodinated by the chloramine-T method (specific activity, 3.4×10^5 cpm/ng). Saturation binding assays were performed with ^{125}I-labeled CSF-1 at 50 ng/ml, as described (9). For mitogenic assays, 3×10^5 cells per ml were plated in 24-well plates in RPMI-1640 medium containing 15% fetal bovine serum in the presence or absence of various concentrations of human CSF-1 (Genetics Institute) or murine IL-3 (Genzyme). The [^{3}H]thymidine incorporation assays were then performed as described (11). A unit (u) of human CSF-1 = 0.5 fmol and 1 u of IL-3 = 3.7 fmol.

Hematopoietic Differentiation and Chemotaxis Assay. α-Naphthyl acetate (12), chloroacetate esterase (12), myeloperoxidase (8), lysozyme (13), lactoferrin (8), and the nitroblue tetrazolium (NBT) reduction activity (14) were assayed. The ability of cells to phagocytose zymosan particles was determined by incubating 10^6 cells per ml with 10^9 zymosan particles for 1 hr at 37°C. Cells with at least 10 internalized particles were scored as positive. Fluorescence-activated cell sorter analysis was performed by forward and right-angle light scatter. A 4-decade logarithmic green fluorescence histogram of 1000-channel resolution was collected from 10,000 viable cells for each sample analyzed. The mean fluorescence intensity (MFI) of stained cells was determined to provide a measure of the relative density of antigen on the cell surface. Monoclonal antibodies used were anti-Mac-1 (M1/70), anti-Mac-2 (M3/38), anti-Mac-3, (M3/84), and anti-Ia (M5/114) (Hybritech); anti-Fc$_\gamma$ receptor (Fc$_\gamma$R) (2.4G2) (a gift from B. J. Mathieson, National Cancer Institute-Frederick Cancer Research Facility) (15); and anti-CFS-1 receptor (Oncogene Sciences, Manhasset, NY). Nonspecific Fc$_\gamma$R-mediated binding was controlled for by lack of staining of the cells with an irrelevant isotype-matched antibody. A 1:40 dilution of fluorescein isothiocyanate (FITC)-labeled goat anti-rat IgG (Kirkegaard & Perry Laboratories, Gaithersburg, MD) was used as the second antibody. The CSF-1-maintained 32D-c-*fms* cells were sterilely sorted for high Mac-1 or CSF-1 receptor levels by selecting 10% of the population expressing the highest MFI (FAST Systems, Gaithersburg, MD).

Cell migration of 32D cells was assayed by a modified Boyden-chamber technique, using Nuclepore filters (5-μm pore size), as described (16). The IgG fraction of rabbit polyclonal anti-human CSF-1 serum (50 μg) (Genzyme) was incubated with various concentrations of human CSF-1 at 37°C for 2 hr prior to chemotaxis analysis for neutralization studies.

RESULTS

Transfection and Expression of Human c-*fms* cDNAs in 32D Cells. Initial studies revealed that exposure of the IL-3-dependent 32D cell line to CSF-1 failed to support either its proliferation or survival (Table 1). Thus, we introduced expression vectors for human c-*fms* or its activated counterpart c-*fms*[S301,F969] into 32D cells by electroporation and selection in medium containing IL-3 and mycophenolic acid (13). When membrane proteins were analyzed by immunoblotting with anti-c-Fms serum (Fig. 1*A*), 165- and 130-kDa proteins were detected in the 32D-c-*fms* and 32D-c-*fms*-[S301,F969] transfectants but not in the control 32D cells. Similarly sized c-Fms proteins were observed in a murine macrophage cell line, P388D$_1$, which is known to express CSF-1 receptors (3). In quantitative saturation binding assays, both 32D-c-*fms* and 32D-c-*fms*[S301,F969] bound ^{125}I-labeled recombinant human CSF-1 10-fold more efficiently than P388D$_1$ cells (Fig. 1*B*). Conversely, 32D cells demonstrated no significant CSF-1 binding.

Table 1. Mitogenic response and colony-forming efficiency of 32D-c-*fms* transfectants

Cell line	Addition: Factor(s)	Addition: Conc., u/ml	SI*	Cloning efficiency†
32D	IL-3	300	280	30.0
	CSF-1	3000	1	NT
		300	1	<0.1
		30	1	NT
	None	—	1	<0.1
32D-c-*fms*	IL-3	300	310	32.4
	Both	300	330	29.5
	CSF-1	300	320	20.7
		100	310	NT
		30	220	NT
		10	100	NT
		3	20	NT
		1	1	NT
	None	—	1	<0.1
32D-c-*fms*-[S301,F969]	IL-3	300	290	32.8
	CSF-1	300	320	23.5
	None	—	120	3.6

*Stimulation index: [^{3}H]thymidine incorporation (cpm) with treatment/[^{3}H]thymidine incorporation (cpm) of 32D without added factors. Data are the means of duplicate samples.

†Colony-forming efficiency was established by plating cells at 10-fold serial dilutions in growth medium with 0.45% plaque agarose. Visible colonies were scored at 14 days after plating and data are the means of duplicate plates. NT, not tested.

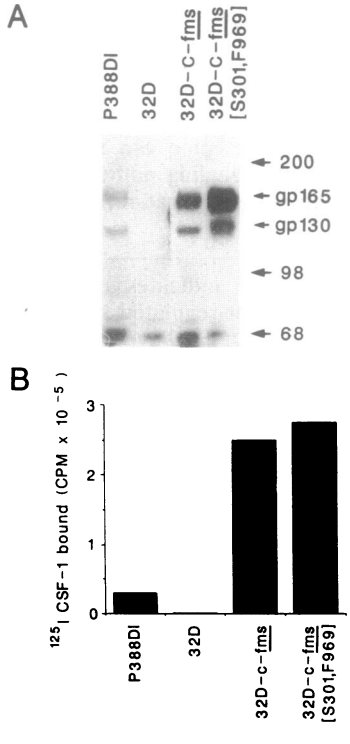

FIG. 1. Expression of c-Fms proteins in 32D cells transfected by electroporation with LTR-2/c-*fms* or LTR-2/c-*fms*[S301,F969]. (*A*) Extracts immunoblotted with a rabbit anti-c-Fms peptide serum. Sizes are shown in kilodaltons; gp, glycoprotein. (*B*) Saturation binding of ^{125}I-labeled CSF-1 to 32D-c-*fms* transfectants in comparison to 32D and P388D$_1$ cells. Data are the means of triplicate points.

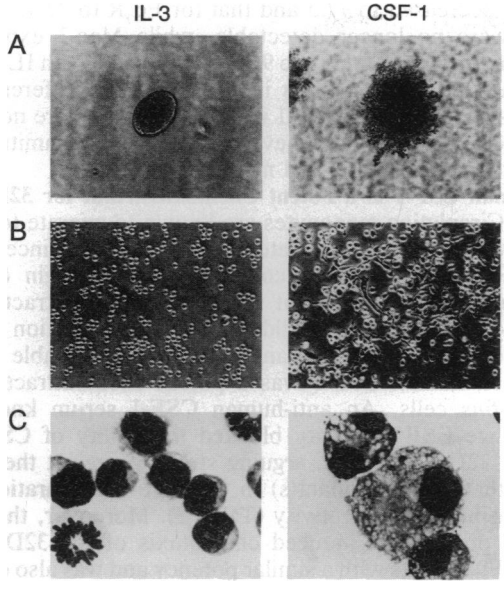

FIG. 2. Comparison of morphological features of 32D-c-*fms* cells in medium with IL-3 or CSF-1. (*A*) Semisolid medium. (×24.) (*B*) Liquid medium. (×60.) (*C*) Wright–Giesma-stained preparations of cells. (×600.).

Activation of Mitogenic Signaling Pathways in 32D-c-*fms* Transfectants. The ability of the human c-Fms protein to transduce a mitogenic signal in 32D-c-*fms* cells was analyzed under growth-limiting conditions (Table 1). The 32D-c-*fms* cells failed to incorporate [^{3}H]thymidine in the absence of added cytokines but responded dramatically to addition of either human CSF-1 or murine IL-3. The 32D-c-*fms*[S301, F969] transfectant also responded mitogenically to CSF-1. However, 32D-c-*fms*[S301,F969] cells incorporated [^{3}H]-thymidine even in the absence of added growth factor. These results were paralleled by those obtained in a semisolid-medium assay (Table 1). Under these conditions, 32D cells produced colonies only in the presence of IL-3, whereas 32D-c-*fms* and 32D-c-*fms*[S301,F969] cells formed colonies in the presence of either CSF-1 or IL-3. Moreover, 32D-c-*fms*[S301,F969] cells, but not 32D-c-*fms* cells, were able to form colonies at a low efficiency in medium containing only fetal bovine serum.

It has been possible to propagate 32D-c-*fms* cells in CSF-1-containing growth medium for several months. These cells remained factor-dependent even after long-term growth with CSF-1 and were nontumorigenic in 10 nude mice tested during a 3-month period (10^6 cells per mouse). By contrast, cell lines established from the 32D-c-*fms*[S301,F969] transfectant were factor-independent and formed tumors in 10 out of 10 nude mice within 6 weeks. These results demonstrate that the c-Fms protein couples with mitogenic pathways inherently present in 32D cells. In addition, its constitutively activated counterpart c-Fms[S301,F969] abrogates growth factor dependence and induces the transformed phenotype.

CSF-1 Induces Reversible Monocytic Differentiation of 32D-c-*fms* Cells. We next investigated whether CSF-1 could induce differentiation of 32D-c-*fms* cells. Colonies induced by IL-3 were compact and contained rounded cells, whereas colonies that grew in response to CSF-1 were diffuse and contained cells with spindle-shaped protrusions (Fig. 2*A*). Morphological alterations were also noticeable in liquid culture: 32D-c-*fms* cells grown in IL-3 medium were round and nonadherent, but they became adherent with elongated filopodia after CSF-1 exposure for 48 hr (Fig. 2*B*). The 32D-c-*fms* cells maintained in IL-3 medium displayed an immature phenotype by Wright–Giemsa stain, while CSF-1-treated cells showed signs of morphological maturation such as decreased nucleus/cytoplasm ratio and membrane ruffling (Fig. 2*C*). No morphological markers of granulocytic differentiation were detected.

Histochemical analysis confirmed that CSF-1 triggered a monocytic differentiation pattern (Table 2). Whereas 32D-c-*fms* cells grown with IL-3 were negative for markers of monocytic differentiation such as lysozyme and nonspecific esterase (12, 13), exposure to CSF-1 induced a rapid appearance of both these markers within 48 hr. However, CSF-1-treated 32D-c-*fms* did not efficiently phagocytose zymosan particles or reduce NBT, functional markers of mature macrophages (14). Histochemical markers of granulocytic differentiation, including myeloperoxidase, lactoferrin, and chloroacetate esterase (8, 12), remained low or undetectable after CSF-1 treatment. The factor-independent 32D-c-*fms*[S301, F969] cells showed a similar differentiation phenotype even when propagated without exogenous CSF-1 or IL-3 (Table 2), indicating that sustained activation of the c-Fms protein was invariably associated with conversion of the 32D cells to a monocytic phenotype.

Fluorescence-activated cell sorter analysis revealed that CSF-1 treatment increased the expression of certain cell surface antigens known to be expressed on monocytes. Upon CSF-1 stimulation of 32D-c-*fms* cells, the MFI increased from 83.7 to 181.7 and from 78.0 to 123.4 for Mac-1 and $Fc_\gamma R$, respectively. However, induction of Mac-2, Mac-3, and Ia, surface antigens expressed on highly differentiated macrophages (17, 18), was only weakly triggered by CSF-1 treatment (data not shown). CSF-1 induction of these latter markers was, however, prominent in 32D-c-*fms* cells selected for a higher level of CSF-1 receptors by fluorescence-activated cell sorting. In this latter population, CSF-1 induced a pronounced increase in Mac-1 and $Fc_\gamma R$ (Fig. 3 *A* and *D*), which was accompanied by a significant increase in the

Table 2. Effect of CSF-1 on differentiation of 32D-c-*fms* transfectants

	32D-c-*fms*							
			CSF-1 to IL-3†				32D-c-*fms*-	
Marker*	IL-3	CSF-1	D1	D3	D5	D7	[S301,F969]	P388D$_1$
Lysozyme	0.2	6.3	6.0	2.5	0.6	0.2	4.8	6.8
Nonspecific esterase	0	100	80	35	<5	0	100	100
Phagocytosis	0	0	0	0	0	0	0	100
NBT reduction	0	0	0	0	0	0	0	100
Myeloperoxidase	<5	<5	<5	<5	<5	<5	<5	100
Lactoferrin	0	0	0	0	0	0	0	0
Chloroacetate esterase	0	0	0	0	0	0	0	0

*Histochemical values indicate the percentage of positive cells for all markers analyzed except lysozyme, which is indicated as μg per 10^6 cells per 24 hr.

†Time-course experiments were performed on 32D-c-*fms* cells sorted for high Mac-1 expression, and values are designated at various days (D) after shifting from CSF-1 to IL-3.

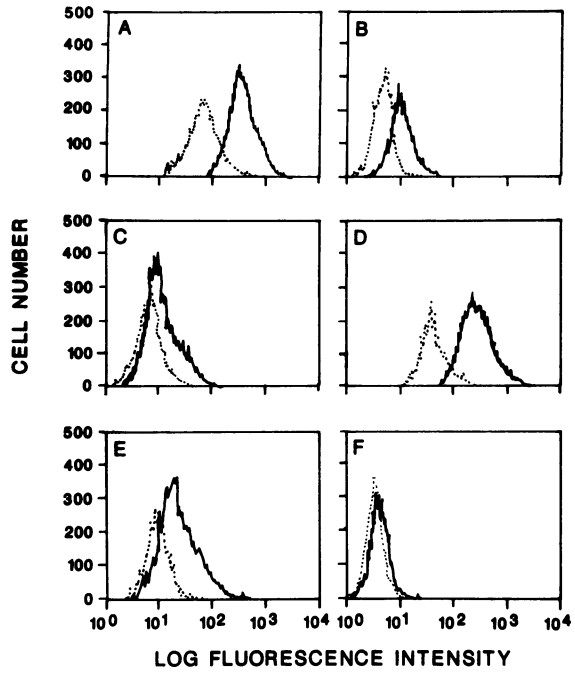

FIG. 3. Induction of monocytic cell surface antigen expression in 32D-c-*fms* cells by CSF-1. IL-3-propagated (···) or CSF-1 treated (—) cells were tested for expression of specific cell surface antigens by flow cytometry: Mac-1 (*A*); Mac-2 (*B*); Mac-3 (*C*); $Fc_\gamma R$ (*D*); Ia (*E*); control fluorescence with fluorescein-conjugated goat anti-rat serum (*F*).

percent of cells expressing cell surface antigens of more mature monocytes (Fig. 3 *B*, *C*, and *E*). However, the cells were still unable to mediate phagocytosis or reduce NBT. These results indicate that CSF-1 initiates a monocytic differentiation program in 32D-c-*fms* cells but is unable to direct terminal differentiation to macrophages.

To determine whether the monocytic phenotype induced by CSF-1 was reversible, 32D-c-*fms* cells propagated with CSF-1 for several months were selected for the highest levels of Mac-1 antigen by fluorescence-activated cell sorting (Mac-1^+ cells) and then subcultured in either IL-3- or CSF-1-containing medium. Mac-1^+ cells maintained with CSF-1 retained their monocytic phenotype, displaying high levels of Mac-1 and $Fc_\gamma R$ (MFI, 234.6 and 205.9, respectively). Moreover, Mac-2 was expressed on 18.2%, Mac-3 on 36%, and Ia on 62.3% of the population (data not shown). By contrast, Mac-1^+ cells shifted to IL-3 began to lose monocytic characteristics within 1–3 days and had completely reverted to an immature phenotype within 1 week (Table 2). The MFI for Mac-1 decreased to 77.5 and that for $Fc_\gamma R$ to 72.4. Mac-2^+ cells were no longer detectable, while Mac-3 expression decreased to 7.1% and Ia to 9.6% after 7 days with IL-3 (data not shown). These results indicate that the differentiation signals generated by CSF-1 in 32D-c-*fms* cells are not sufficient to induce events that evoke irreversible commitment to a fully mature or activated macrophage.

Table 3. Chemotactic activity of CSF-1 for 32D-c-*fms* transfectants

Addition		Chemotactic activity*			
Factor(s)	Conc., u/ml	32D	32D-c-*fms*	32D-c-*fms*-[S301,F969]	$P388D_1$
CSF-1	300	21 ± 3	312 ± 20	353 ± 16	163 ± 12
	100	18 ± 7	182 ± 24	197 ± 12	88 ± 6
CSF-1 + anti-CSF-1	300	NT	38 ± 9	46 ± 4	NT
	100	NT	26 ± 6	22 ± 1	NT
IL-3	300	34 ± 4	26 ± 5	NT	12 ± 1
	100	28 ± 8	22 ± 1	NT	9 ± 2
CSF-1 + IL-3	300	NT	325 ± 16	NT	NT
	100	NT	205 ± 25	NT	NT
None		22 ± 4	25 ± 3	18 ± 7	10 ± 3

*Average number of viable cells migrating to the lower chamber as counted by hemocytometer. Each value represents the mean ± SD of triplicate samples. NT, not tested.

Human CSF-1 Is a Potent Chemoattractant for 32D-c-*fms* Cells. Circulating monocytes are known to migrate to tissue sites where they differentiate to macrophages. Since CSF-1 is produced by cells of connective-tissue origin (2), we reasoned that CSF-1 might serve as a chemoattractant for 32D-c-*fms* cells. CSF-1 did not induce migration of the parental 32D cell line at any concentration (Table 3). By contrast, human CSF-1 was a potent chemoattractant for 32D-c-*fms* cells. An anti-human CSF-1 serum known to neutralize CSF-1 activity blocked the ability of CSF-1 to attract 32D-c-*fms* cells, arguing strongly against the possibility that a contaminant(s) in the CSF-1 preparation was responsible for this activity (Table 3). Moreover, the same CSF-1 preparation induced chemotaxis of the 32D-c-*fms*-[S301,F969] cells with a similar potency and was also chemotactic for the $P388D_1$ cell line. Interestingly, IL-3 was not chemotactic for 32D-c-*fms* cells at any concentration analyzed, nor did it block CSF-1-modulated chemotaxis (Table 3). These results establish that CSF-1 induces specific migration of 32D-c-*fms* cells, whereas IL-3 does not activate this signaling pathway.

DISCUSSION

The present studies establish that monocytic differentiation can be triggered by an external signal mediated by CSF-1 through activation of its receptor. Although maturation along the mononuclear phagocyte pathway could be demonstrated, we did not observe terminal differentiation of 32D-c-*fms* cells in response to CSF-1. In addition, CSF-1-induced monocytic differentiation was reversible, suggesting that the concentration of specific cytokines in a particular microenvironment may influence whether a monocyte continues along a differentiation pathway or returns to a self-renewing myeloid progenitor pool. Several cytokines, including tumor necrosis factor α, IL-1, IL-2, the interferons, and granulocyte/macrophage-CSF (GM-CSF), are thought to contribute to the development of mature macrophages (19–23). Therefore, these cells may provide a model system for determining how various external factors contribute to the progression of monocytic differentiation.

CSF-1 treatment induced only monocytic differentiation of 32D-c-*fms* cells. However, 32D-c-*fms* cells propagated with IL-3 and then switched to G-CSF still retained the capacity to terminally differentiate to neutrophilic granulocytes (data not shown). Thus, in the 32D system, it appears that activation of each receptor couples with a distinct myeloid differentiation pathway. A study published during preparation of our manuscript reported that murine CSF-1 triggered both monocytic and granulocytic differentiation in FDC-P1 cells expressing murine c-*fms* (24). Whether this reflects differences in the target cells utilized or divergence in the substrate specificity of murine and human CSF-1 receptors remains to be determined.

Selective recruitment of leukocytes to the extravascular sites from the peripheral blood is of critical importance in the inflammatory process, tissue injury, and tumor infiltration. It has been suggested that leukocyte migration to tissue sites may be directed, in part, by the local cellular release of specific cytokines. Two studies have shown that GM-CSF and CSF-1 are chemotactic for peripheral blood monocytes (25, 26), but others have demonstrated that GM-CSF inhibits granulocyte migration (27, 28) and that CSF-1 is not chemotactic for human monocytes (D. Liu and P. Ralph, personal

communication). Our analysis of 32D-c-*fms* cells showed that CSF-1 is a potent chemoattractant for cells expressing high levels of CSF-1 receptor. IL-3 was not chemotactic for 32D-c-*fms* cells, suggesting that only specific cytokines can induce migration of cells within a particular lineage. Thus, it appears that certain hematopoietic CSFs can act in a cell-specific manner to direct cell migration.

The IL-3 dependence of 32D can be abrogated by at least three retroviral oncogenes of the tyrosine kinase family, v-*abl*, v-*src*, and v-*erbB* (11, 29, 30). In each case, oncogene-induced factor independence was associated with conversion to the malignant phenotype. A recent study demonstrated that c-*fms* overexpression in FDC-P1 cells led to IL-3 independence at a low frequency (31). These factor-independent clones were tumorigenic in nude mice, whereas FDC-P1-c-*fms* clones that remained factor-dependent were nontumorigenic. In the present study, we found that overexpression of human c-*fms* in the myeloid progenitor 32D line did not release them from IL-3 dependence, reduce their growth factor requirements, or render them tumorigenic. In other studies, overexpression of receptors for epidermal growth factor (EGF) or platelet-derived growth factor (PDGF) also did not abrogate growth factor dependence of IL-3-dependent cell lines (11, 16, 32). Thus, overexpression of these normal growth factor receptors in 32D cells was insufficient to convert the cells to factor independence, or levels of overexpression required to see such an effect were not possible to achieve. We were able to establish that expression of point-mutated c-*fms*[S301,F969] abrogated IL-3 dependence of 32D cells, and these cells readily formed tumors in nude mice. Further, the monocytic phenotype of IL-3-independent 32D-c-*fms*[S301,F969] cells resembled that of CSF-1-induced 32D-c-*fms* cells, implying that partial monocytic maturation did not interfere with progression of these cells to a malignant state. These findings suggest that the c-*fms* gene product may contribute to the progression of myeloid leukemia either by its constitutive activation through an autocrine or paracrine mechanism or by genetic alterations in c-*fms* itself.

Recent evidence indicates that activation of the intrinsic tyrosine kinase activity of EGF and PDGF receptors leads to rapid tyrosine phosphorylation of several substrates including c-Raf, phospholipase C, a phosphatidylinositol kinase, and the GTPase-activating protein for c-Ras (33–38). Each of these proteins has been directly or indirectly implicated in mitogenic signal transduction, and evidence indicates that many interact directly with the PDGF receptor kinase, arguing that they are primary substrates of receptor phosphorylation. Since CSF-1 triggers mitogenesis, monocytic differentiation, and chemotaxis pathways in 32D cells, it will be of interest to determine whether the CSF-1 receptor interacts with any of these substrates and whether other tyrosine kinase containing receptors direct similar or different functions in these cells. By this approach it may be possible to identify tyrosine-phosphorylated substrates involved in regulating these specific pathways.

We thank Donald Bottaro, Purvin Anklesaria, and Mohammad Heidaran for helpful discussions. We thank Charles Knicley for excellent technical assistance and Paul Arnstein for tumorigenicity testing.

1. Metcalf, D. (1989) *Nature (London)* **339**, 27–30.
2. Stanley, E. R., Guilbert, L. J., Tushinski, R. J. & Bartelmez, S. H. (1983) *J. Cell. Biochem.* **21**, 151–159.
3. Guilbert, J. & Stanley, E. R. (1986) *J. Biol. Chem.* **261**, 4024–4032.
4. Byrne, P. V., Guilbert, L. J. & Stanley, E. R. (1981) *J. Cell Biol.* **91**, 848–853.
5. Sherr, C. J., Rettenmier, C. W., Sacca, R., Roussel, M. F., Look, A. T. & Stanley, E. R. (1985) *Cell* **41**, 665–676.
6. Roussel, M. F., Downing, J. R., Rettenmier, C. W. & Sherr, C. J. (1988) *Cell* **55**, 979–988.
7. Greenberger, J. S., Sakakeeny, M. A., Humphries, R. J., Eaves, C. J. & Eckner, R. J. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2931–2935.
8. Valtieri, M., Tweardy, D. J., Caracciolo, D., Johnson, K., Mavilio, F., Altmann, S., Santoli, D. & Rovera, G. (1987) *J. Immunol.* **138**, 3829–3835.
9. Di Fiore, P. P., Pierce, J. H., Fleming, T. P., Hazan, R., Ullrich, A., King, C. R., Schlessinger, J. & Aaronson, S. A. (1987) *Cell* **51**, 1063–1070.
10. Wang, L., Geihi, D. K., Choudhury, G. G., Mirter, A., Martinez, L., Weber, D. & Sachaguchi, A. (1989) *Biotechniques* **7**, 1–7.
11. Pierce, J. H., Ruggiero, M., Fleming, T. P., Di Fiore, P. P., Greenberger, J. S., Varticovski, L., Schlessinger, J., Rovera, G. & Aaronson, S. A. (1988) *Science* **239**, 628–631.
12. Yam, L. T., Li, C. Y. & Crosby, W. H. (1971) *Am. J. Clin. Pathol.* **55**, 283–294.
13. Osserman, E. F. & Lawlor, D. F. (1966) *J. Exp. Med.* **124**, 921–928.
14. Geissler, K., Harrington, M., Srivastava, C., Leamhuis, T., Tricot, G. & Broxmeyer, H. (1989) *J. Immunol.* **143**, 140–146.
15. Unkeless, J. (1979) *J. Exp. Med.* **150**, 580–596.
16. Matsui, T., Pierce, J. H., Fleming, T. P., Greenberger, J. S., LaRochelle, W. J., Ruggiero, M. & Aaronson, S. A. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 8314–8318.
17. Springer, T., Galfre, G., Secher, D. S. & Milstein, C. (1979) *Eur. J. Immunol.* **9**, 301–306.
18. Springer, T. A. & Ho, M.-K. (1982) in *Progress in Cancer Research and Therapy* (Raven, New York), Vol. 21, pp. 35–46.
19. Warren, M. K. & Vogel, S. N. (1985) *J. Immunol.* **134**, 982–989.
20. Carracciolo, D., Shirsat, N., Wong, G. G., Longe, B., Clark, S. & Rovera, G. (1987) *J. Exp. Med.* **166**, 1851–1860.
21. Sariban, E., Mitchell, T., Griffin, J. & Kufe, D. (1987) *J. Immunol.* **138**, 1954–1961.
22. Horiguchi, J., Warren, M. K. & Kufe, D. (1987) *Blood* **69**, 1259–1264.
23. Malkovsky, M., Loveland, B., North, M., Ashorson, G., Gao, L., Ward, P. & Fiers, W. (1987) *Nature (London)* **325**, 262–265.
24. Rohrschneider, L. & Metcalf, D. (1989) *Mol. Cell. Biol.* **9**, 5081–5092.
25. Wang, J.-M., Colella, S., Allavena, P. & Mantovani, A. (1987) *Immunology* **60**, 439–444.
26. Wang, J.-M., Griffin, J. D., Rambaldi, A., Chen, Z. G. & Mantovani, A. (1988) *J. Immunol.* **141**, 575–579.
27. Weisbart, R. H., Billing, R. & Golde, D. W. (1979) *J. Lab. Clin. Med.* **93**, 622–629.
28. Gasson, J. C., Weisbart, R. H., Kaufman, S. E., Clark, S. C., Hewick, R. M., Wong, G. G. & Golde, D. W. (1984) *Science* **226**, 1339–1344.
29. Rovera, G., Valtieri, F., Mavilio, F. & Reddy, P. (1987) *Oncogene* **1**, 29–35.
30. Watson, J. D., Eszes, M., Overell, R., Conlon, P., Widmer, M. & Gillis, S. (1987) *J. Immunol.* **139**, 123–129.
31. Kato, J.-Y., Roussel, M. F., Ashmun, R. A. & Sherr, C. J. (1989) *Mol. Cell. Biol.* **9**, 4069–4073.
32. von Rüden, T. & Wagner, E. F. (1988) *EMBO J.* **7**, 2749–2756.
33. Kaplan, D. R., Whitman, M., Schaffhausen, B., Pallas, D. C., White, M., Cantley, L. & Roberts, T. M. (1987) *Cell* **50**, 1021–1029.
34. Morrison, D. K., Kaplan, D. R., Escobedo, J. A., Rapp, U. R., Roberts, T. M. & Williams, L. T. (1989) *Cell* **58**, 649–657.
35. Margolis, B., Rhee, S. G., Felder, S., Mervic, M., Lyall, R., Levitzki, A., Ullrich, A., Zilberstein, A. & Schlessinger, J. (1989) *Cell* **57**, 1101–1107.
36. Meisenhelder, J., Suh, P.-G., Rhee, S. G. & Hunter, T. (1989) *Cell* **57**, 1109–1122.
37. Wahl, M. I., Olashaw, N. E., Nishibe, S., Rhee, S. G., Pledger, W. J. & Carpenter, G. (1989) *Mol. Cell. Biol.* **9**, 2934–2943.
38. Molloy, C. J., Bottaro, D. P., Fleming, T. P., Marshall, M. S., Gibbs, J. B. & Aaronson, S. A. (1989) *Nature (London)* **342**, 711–714.