By Jun-ya Kato and Charles J. Sherr

Interleukin-3 (IL-3)-dependent mouse myeloid 32DC13 cells differentiate to neutrophils in response to granulocyte colony-stimulating factor (G-CSF). Introduction of the human c-fms gene, which encodes the receptor for CSF-1, into 32DC13 cells gave rise to variants that were able to proliferate in medium containing either murine IL-3 or human recombinant CSF-1, but were unable to differentiate to granulocytes in response to G-CSF. Unlike parental 32CD13 cells, CSF-1-responsive derivatives expressed non-specific esterase when grown in CSF-1, but did not exhibit many other morphologic, immunologic, or functional properties of mononuclear phagocyte differentiation, or express murine CSF-1 receptors. Accelerated turnover of the human CSF-1 receptor was observed in response to CSF-1 and phorbol esters, but not after stimulation with IL-3 or bacterial lipopolysaccharide. Although both CSF-1 and IL-3 induced tyrosine phosphorylation of heterologous substrates in the dually responsive cells, differences in the patterns of substrate phosphorylation were observed in response to the two hematopoietins. We conclude that expression of the human CSF-1 receptor in 32DC13 cells not only induces CSF-1 responsiveness, but alters its phenotype in a way that prohibits granulocyte differentiation.

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Materials and Methods

Cell lines and culture conditions. IL-3-dependent 32DC13 cells and CSF-1-dependent BAC1.2F5 macrophages were generously provided by Dr Giovanni Rovera (Wistar Institute, Philadelphia, PA) and Dr E. Richard Stanley (Albert Einstein College of Medicine, Bronx, NY), respectively. The cells were maintained in Iscove’s medium containing 20% fetal calf serum (HyClone, Logan, UT), glutamine, antibiotics, and requisite growth factors (either 20% Wehi-3B cell conditioned medium as a source of IL-3 or 25% L-cell conditioned medium as a source of murine CSF-1). The human glioblastoma cell line, U87 MG, also provided by Dr Giovanni Rovera, was grown in complete Iscove’s medium without other growth factors and was used as a source of G-CSF.

A BamHI fragment containing the intact human c-fms cDNA was inserted in both the sense and antisense orientation into an inducible expression vector containing four tandem upstream copies of a metal response element, the β-globin TATA box, the BamHI cloning site, and a simian virus 40 polyadenylation signal downstream of the inserted gene. The c-fms plasmids were each electroporated into 32DC13 cells with a second plasmid (pSV2neo) that confers resistance to the antibiotic G418 (Geneticin, Sigma Chemicals, St Louis, MO). Electroporated cells were seeded at 3 x 10^6 cells per well into 24-well multiplate culture dishes, and selected sets of genes that independently contribute to each of these activities. In support of this hypothesis, the effects of CSF-1 on cell proliferation and survival can be dissociated, since submitogenic doses of CSF-1 can maintain the viability of nonproliferating bone marrow-derived macrophages that become growth arrested in the early G1 phase of the cell cycle. An unresolved issue concerns the extent to which the CSF-1R tyrosine kinase modulates the activity of genes that determine different stages of mononuclear phagocyte differentiation. To approach this problem in a model system, we introduced c-fms cDNA encoding human CSF-1R into mouse 32DC13 cells, which self-renew in IL-3 but terminally differentiate to neutrophils in response to G-CSF. Populations of CSF-1R-bearing cells were obtained that proliferated in either IL-3 or CSF-1, expressed monocyte esterases, and failed to differentiate in response to G-CSF. Our results suggest that CSF-1R both positively and negatively regulates the expression of gene products affecting differentiation within the monocyte and granulocyte lineages.

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Submitted November 17, 1989; accepted January 11, 1990.

Supported by the Howard Hughes Medical Institute, and by Cancer Center CORE Grant (P01-CA17675) to St Jude Children’s Research Hospital.

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Blood, Vol 75, No 9 (May 1), 1990: pp 1780-1787
for 3 weeks in complete medium containing IL-3 and 800 μg/mL G418. The medium was then supplemented with heavy metals (1 x 10⁻¹⁴ mol/L ZnSO₄ and 5 x 10⁻¹⁰ mol/L CdSO₄), and selection was continued in the presence of IL-3 and G418 for 2 more weeks.

Equal aliquots of cells from G418-resistant cultures electroporated with the c-fms gene in sense orientation were pooled, and cells expressing human CSF-1R were selected by fluorescence-activated cell sorting using a monoclonal antibody (MoAb) to the human receptor. The sorted CSF-1R-positive population (designated 32D[R⁺]) was maintained in medium containing IL-3. In parallel, cells from each independent culture were grown in complete medium supplemented with 2,000 U/mL (0.9 nmol/L) of human recombinant CSF-1. Seven cultures yielding CSF-1-responsive cells were obtained, three of which (designated 32D[R⁺]C1.1, C1.2, and C1.3) were characterized in detail.

Other analytical methods. Cells in suspension culture were concentrated on slides by cytocentrifugation, fixed in cold 9.25% formalin, 45% acetone in sodium phosphate buffer, pH 6.6, and stained for 1 hour at room temperature in an esterase reaction mixture (pH 6.3) containing 50 μg/mL α-naphthyl butyrate as a substrate. In some reactions, 1.25 mg/mL sodium fluoride was added to the reaction mixture to inhibit nonspecific esterase activity. Procedures for metabolic labeling with [³⁵S]methionine, cell lysis, immunoprecipitation, polyacrylamide gel electrophoresis, and immunoblotting with antisera to phosphotyrosine are described in detail in the references cited.

Preparation of rabbit antiserum to CSF-1R. An NcoI fragment (nucleotides 2271 to 3280) representing the 3' end of the human c-fms gene, was inserted into a pBR322-based expression plasmid, pSJH57, after conversion of its unique ClaI and BamHI cloning sites into NcoI and XhoI sites, respectively. The resultant construct contained the λ pμ promoter, and coded for a thermoinducible λ Cμ-c-fms fusion protein of 37.5 Kd containing the carboxyterminal 340 amino acids of human CSF-1R. The plasmid was inserted into Escherichia coli strain N4830, which contains a thermolabile repressor.

RESULTS

Transduction of human CSF-1R into IL-3-responsive 32DC13 cells. The mouse myeloid cell line, 32DC13, is dependent on IL-3 for proliferation and survival, but terminally differentiates into mature granulocytes when IL-3 is removed from the culture medium and is replaced by G-CSF. To determine whether 32DC13 cells could be reprogrammed to proliferate in CSF-1 and whether stimulation by CSF-1 would affect their pattern of differentiation,
IL-3 for survival and proliferation, but died in the absence of either factor. When these CSF-1-responsive cell lines were analyzed by FACS for expression of human CSF-1R, they expressed significantly higher levels of the receptor than pooled 32D[R+] cells grown in IL-3 (Fig 1). These levels of CSF-1R are equivalent to those detected on murine BAC1.2F5 macrophages (about 1 x 10^5 CSF-1 binding sites/cell). Although the experimental protocol prohibited an estimation of their frequency, the results suggested that only those cells that expressed high levels of human CSF-1R selectively grew out in response to CSF-1. Cells from the one factor-independent culture also exhibited very high levels of CSF-1R expression (data not shown), consistent with previous results suggesting that overexpression of CSF-1R in immature myeloid cells may contribute to a factor-independent phenotype.18

Growth and differentiation of CSF-1-responsive cells. Subsequent experiments were performed with the three CSF-1-responsive cell lines and with single cell subclones of each. Only similar data were obtained with each of these cell lines, only representative data with 32D[R+]Cl.1 cells are shown below. Figure 2 shows growth curves under different conditions for parental 32DC13 cells (A) and for a CSF-1-responsive clone (B). The parental cells proliferated only in the presence of IL-3. When transferred to medium containing human recombinant CSF-1, their viability declined rapidly, and no CSF-1-responsive or factor-independent variants arose from cultures containing more than 5 x 10^6 cells. In contrast, 32D[R+]Cl.1 cells grew equally well in response to murine IL-3, human CSF-1, or in medium containing both growth factors, but died in the absence of either. Although human CSF-1 can bind with high affinity to both human and mouse CSF-1R, murine CSF-1 does not bind to human CSF-1R and induce the growth of cells bearing the human receptor.22,23 As expected, murine CSF-1 did not support the growth or survival of 32D[R+]Cl.1 cells, implying that they did not express murine CSF-1R (see below) and that their growth in response to human CSF-1 was mediated by the transduced c-fms gene product.

When washed and transferred to medium containing G-CSF, 32DC13 cells eventually stopped growing (Fig 2A) and differentiated to granulocytes. Figure 3 contrasts the morphology of the parental cells maintained in IL-3 (A) with those cultured for 10 days in G-CSF (B) and illustrates the conversion of immature myeloid cells to mature granulocytes containing banded or segmented nuclei.14 In contrast, the survival of CSF-1-responsive 32D[R+]Cl.1 cells was not supported by G-CSF (Fig 2B). Although these cells could be maintained for a somewhat longer period of time in medium containing G-CSF than in unsupplemented medium, no viable cells remained after 10 days of G-CSF treatment. Moreover, no morphologic evidence of granulocytic differentiation was observed in G-CSF-treated 32D[R+]Cl.1, C1.2, or C1.3 cultures maintained during this period, nor did G-CSF inhibit their growth in response to either IL-3 or CSF-1 (data not shown).

Because the CSF-1-responsive cell lines were unable to differentiate to granulocytes in response to G-CSF, we considered the possibility that CSF-1 induced an alternative genetic program leading toward monocytic maturation. Therefore, the cells were assayed for production of nonspecific esterases as markers of monocytic differentiation. The term nonspecific esterase is reserved for enzymes capable of hydrolyzing simple esters of N-free alcohols and organic acids. For those enzymes that use α-naphthyl butyrate as a substrate, the reaction products are generally confined to monocytes.22 Parental 32DC13 cells lacked this activity (Fig 3A), as did those that had been induced to differentiate to granulocytes (Fig 3B). CSF-1R-positive 32D[R+] cells that had never been cultured in CSF-1 contained occasional nonspecific esterase-positive cells (Fig 3C), whereas 32D[R+]Cl.1 cells maintained in human CSF-1 exhibited high levels of enzymatic activity (Fig 3D). When the latter cells were washed, resuspended, and cultured for 2 more weeks in medium containing IL-3, the cells remained highly esterase-positive (Fig 3E), suggesting that induction of this activity by CSF-1 was not readily reversible. The enzyme was completely inhibited by addition of sodium fluoride to the reaction buffer (Fig 3F), providing further evidence that it represented a monocytic isoform.22

Although the CSF-1-responsive cell lines were esterase-positive, they lacked many other features of monocytic differentiation. The cells remained nonadherent, were nonphagocytic, could not present antigens to their respective T-cell hybridomas, were unable to support an antibody-dependent cytotoxic response to sheep erythrocytes, and expressed only low levels of the MAC-1 antigen20 equivalent to those detected on parental 32DC13 cells (negative data...
REPROGRAMMING THE CSF-1 RESPONSE

not shown). Treatment of these cells with other potential inducing agents including GM-CSF, LPS, TPA, PGE₂, LTB₄, 1,25-dihydroxyvitamin D₃, or 5-azacytidine did not induce monocytic maturation.

32D[R+]C1.1 cells do not express murine CSF-1R. The most typical marker of maturation in the mononuclear phagocyte lineage is expression of CSF-1R itself. The inability of murine CSF-1 to support the proliferation of 32D[R+]C1.1, C1.2, and C1.3 cells suggested that they did not express murine CSF-1R. To confirm these results, 32DC13, 32D[R+], and 32D[R+]C1.1 cells were metabolically labeled with [³⁵S]methionine, and human CSF-1R was immunoprecipitated from detergent lysates using an MoAb that does not crossreact with murine CSF-1R. The cleared lysates were then reacted with a polyvalent rabbit antiserum raised to a human c-fms polypeptide that crossreacts with the murine receptor. As a positive control, lysates of metabolically labeled murine BAC1.2F5 macrophages, which express high levels of the mouse CSF-1 receptor, were analyzed in parallel. All immunoprecipitates were denatured and separated on polyacrylamide gels containing sodium dodecyl sulfate, and receptors were detected by autoradiography of the dried slab gels. Under these labeling conditions, both the immature intracellular form (about 130 Kd) and mature cell surface form (about 165 Kd) of CSF-1R are detected; the two forms differ solely in their composition of asparagine-linked oligosaccharide chains.

Figure 4 (lanes 1 and 5) shows that parental 32DC13 cells did not express CSF-1R. As expected from FACS analysis (Fig 1), 32D[R+] cells expressed significantly lower levels of human CSF-1R (lane 2) than 32D[R+]C1.1 cells (lane 3), whereas both cell lines failed to express murine CSF-1R (lanes 6 and 7). The MoAb to human CSF-1R does not react with the murine receptor expressed in BAC1.2F5 cells (lane 4), but the latter species was readily detected with the polyvalent rabbit antiserum (lane 8). Again, by these criteria, the levels of human CSF-1R synthesis in 32D[R+]C1.1 cells were similar to those observed for the murine receptor in BAC1.2F5 macrophages. However, the molecular mass of human CSF-1R in 32DC13 cell derivatives was greater than that of the murine receptor in BAC1.2F5 cells, probably reflecting cell-specific differences in the processing of oligosaccharide chains. Similar results were previously observed for v-fms gene products expressed in murine myeloid and macrophage cell lines, respectively.

Human CSF-1R is not transmodulated by IL-3. After ligand binding, CSF-1R is rapidly internalized and degraded in lysosomes (receptor downmodulation). Activators of protein kinase C, such as the phorbol ester TPA, also induce accelerated receptor turnover by inducing a protease that cleaves CSF-1R near its transmembrane segment; this process (receptor transmodulation) results in the release of the receptor ligand-binding domain from the cell and concomitant internalization of the 50-Kd tyrosine kinase domain that is degraded intracellularly. Treatment of macrophages with bacterial lipopolysaccharide induces transmodulation of CSF-1R through the same mechanism. Because IL-3 has been reported to induce a loss of CSF-1 binding sites from mouse bone marrow cells, we attempted to determine whether it would similarly induce CSF-1R turnover in 32D[R+]C1.1 cells that expressed both classes of receptors. 32D[R+]C1.1 and BAC1.2F5 cells labeled with
Similarly, murine and human CSF-1 receptors were then immunoprecipitated and resolved on denaturing gels. Figure 5 shows that the mature cell surface form of human CSF-1R (lane 1) was degraded after treatment of 32D[R+]C1.1 cells with CSF-1 (lane 2) or TPA (lane 3), but was not transmodulated by IL-3 (lane 4). Similarly, murine CSF-1R expressed in BAC1.2F5 cells (lane 6) showed accelerated turnover in response to CSF-1 (lane 7) and TPA (lane 8), but was unaffected by IL-3 (lane 9). LPS transmodulated CSF-1R expressed in mouse macrophages (lane 10), but unexpectedly did not induce receptor turnover in 32D[R+]C1.1 myeloid cells (lane 5). The explanation for its differential effect in the two cell types remains unclear, although the simplest interpretation is that immature myeloid cells lack a receptor for LPS.

Tyrosine phosphorylation induced by CSF-1 and IL-3. Whereas CSF-1R functions as a protein tyrosine kinase, the structure of the IL-3 receptor and its mechanism of signal transduction remain unclear. Isfort et al. reported that murine IL-3 binds to a 140-Kd protein that becomes phosphorylated on tyrosine, suggesting that IL-3, like CSF-1, might mediate its effects through the activation of a protein tyrosine kinase. To determine whether CSF-1 and IL-3 would induce the phosphorylation of similar substrates in dually responsive cells, 32DC13, 32D[R+], and 32D[R+]C1.1 cells were incubated in medium lacking growth factors for 18 hours, and then were restimulated for 10 minutes with high concentrations of IL-3 or CSF-1. Cell lysates were separated on denaturing gels, transferred to nitrocellulose, and probed with an antibody to phosphorylurin. Important for the interpretation of these experiments, immunoblotting of total cell lysates with anti-phosphotyrosine is a relatively insensitive technique that only detects the presence of major phosphorytrosine-containing polypeptides.

After IL-3 stimulation, two major phosphotyrosine-containing bands were detected in each cell line (Fig 6, lanes 2, 4, and 7). A protein of 140 Kd corresponded in mass to the previously reported IL-3 binding protein, a second polypeptide of 95 Kd showed an even greater signal intensity. In contrast, CSF-1 stimulation induced tyrosine phosphorylation of CSF-1R (about 165 Kd) as well as the appearance of 110-, 95-, and 85-Kd phosphoproteins (lane 8). We also observed a phosphoprotein of the same molecular weight as CSF-1R in starved 32D[R+]C1.1 cells (lane 6); this background level of receptor tyrosine phosphorylation was even higher in the factor-independent variant (data not shown), suggesting that high levels of receptor expression are accom-
REPROGRAMMING THE CSF-1 RESPONSE

Similarly, introduction of the c-erbB proto-oncogene encoding the epidermal growth factor (EGF) receptor into 32DC13 or FDC-P1 cells rendered them responsive to EGF.\(^{39,41}\)

Although transduction of the CSF-1 and EGF receptor genes into mouse myeloid cell lines could, in some cases, induce ligand-dependent shifts toward more mature phenotypes,\(^{39,40}\) the cells did not terminally differentiate. Moreover, insertion of constitutive oncogene coded tyrosine kinases, such as v-erbB,\(^{42-44}\) v- abl,\(^{42,44}\) v-src,\(^{42,44}\) or v-fms,\(^{42}\) into these same cell lines abrogated their IL-3 dependence but did not induce differentiation and, in the case of 32DC13 cells, inhibited granulocyte maturation in response to G-CSF.\(^{44}\)

The growth of 32D[R+]C1.1, C1.2, and C1.3 cells in CSF-1 induced nonspecific esterase activity and rendered the cells unable to differentiate in response to G-CSF. Induction of esterase activity was not readily reversible, since recultivation of the cells for 10 days in IL-3 did not lead to a detectable diminution of enzymatic activity. One interpretation is that CSF-1 induced partial differentiation toward monocytes, leading concomitantly to a loss of G-CSF responsiveness. However, the cells did not express other functional or phenotypic markers of monocytic differentiation, including murine CSF-1R itself, nor could they be induced to mature within the monocye lineage by GM-CSF, LPS, PGE\(_2\), LTBB4, TPA, vitamin D3, or 5-azacytidine. Thus, although 32DC13 cells remain competent to differentiate to granulocytes, they may be inherently unable to complete the alternative differentiation program characteristic of normal mononuclear phagocytes.

Normal IL-3–responsive bone marrow progenitors are committed to differentiate, and although more mature myeloid elements arising from them can be isolated, they cannot be maintained in culture.\(^9\) In contrast, the process of establishing factor-dependent cell lines may select for the constitutive expression of transcriptional regulatory factors that inhibit receptor-mediated signals for differentiation. Mouse leukemic cells containing retrovirally activated \(c-myc\) and \(Evi-1\) genes represent examples of immortalized lines that remain IL-3–dependent but are unable to differentiate. The latter proto-oncogenes encode nuclear DNA-binding proteins that are likely to regulate gene expression. \(Evi-1\) is not normally expressed in hematopoietic cells,\(^{47}\) but its activation in myeloid cells interrupts their differentiation program without abrogating their growth factor dependence. We would predict that introduction of \(c-myc\) into such cells might render them CSF-1 responsive without inducing complete monocytic maturation. An additional caveat in interpreting our experiments is that human CSF-1R may not function appropriately in mouse myeloid cells; eg, FDC-P1 cells that expressed human CSF-1R showed no evidence of monocytic differentiation,\(^{18}\) whereas the murine \(c-myc\) gene induced monocytic features when the cells were propagated in CSF-1.\(^ {39}\)

After introduction of the human \(c-myc\) gene with \(pSV2 neo\), the levels of human CSF-1R expressed in a mixed population of receptor-positive 32D[R+] cells were relatively low, and only 7 of 21 individual G418-resistant cultures ultimately gave rise to cells that grew in CSF-1. In contrast, 32D[R+]C1.1, C1.2, and C1.3 cells, selected for growth in

**Fig 6.** Identification of phosphotyrosine-containing proteins in IL-3 or CSF-1 stimulated cells. 32DC13 cells (lanes 1 and 2), 32D[R+] cells (lanes 3 through 5), and 32D[R+]C1.1 cells (lanes 6 through 8) were cultured in factor-free medium for 18 hours and either left unstimulated (lanes 1, 3, and 6) or incubated for 10 minutes with 250 U/mL murine IL-3 (lanes 2, 4, and 7) or with 2 nmol/L human recombinant CSF-1 (lanes 5 and 8) beforeysis. Proteins were separated on denaturing polyacrylamide gels, transferred to nitrocellulose, and blotted with an antibody to phosphotyrosine followed by \(^{125}\)I-labeled \(Staphylococcus aureus\) protein A.\(^{24}\) The positions of known molecular weight standards are indicated in kilodaltons in the left margin. Tyrosine phosphorylated substrates discussed in the text are denoted by arrowheads in each lane.

panied by an increased basal level of receptor kinase activity, even in the absence of its ligand. Preincubation of the blots with phosphotyrosine, but not phosphoserine or phosphothreonine, completely inhibited the ability of the antibody to detect either CSF-1R or the 110-, 95-, and 85-kd phosphoproteins (data not shown). Together, the results suggest that IL-3 induces a different pattern of protein tyrosine phosphorylation than does the CSF-1R kinase, although one of the substrates (95 kD) may be phosphorylated in response to treatment by either factor.

**DISCUSSION**

IL-3–dependent mouse 32DC13 cells do not express detectable CSF-1 receptors and cannot survive in medium containing murine CSF-1. Insertion of the human \(c-myc\) gene into these cells enabled them to proliferate in human recombinant CSF-1 without affecting their ability to respond to murine IL-3. Thus, transduction of a macrophage-specific growth factor receptor into immature myeloid cells is sufficient to sensitize them to a growth factor that normally acts on more mature cells of the mononuclear phagocyte lineage. The ability of receptors of the protein tyrosine kinase gene family to reprogram growth factor responsiveness when expressed outside their normal cellular context was not unexpected. Introduction of the human \(c-myc\) gene into mouse NIH-3T3 fibroblasts dependent for growth on platelet-derived growth factor and insulin\(^{17,18}\) or into IL-3–dependent FDC-P1 mouse myeloid cells\(^{18,39}\) enabled them to proliferate in CSF-1.

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medium containing human recombinant CSF-1, expressed significantly more CSF-1R than cells passaged in IL-3, as judged both by FACS analysis and by biosynthetic labeling studies. Thus, only those transformants expressing high levels of human CSF-1R appeared to have had a proliferative advantage when the cells were shifted to medium containing CSF-1. In FDC-P1 cells, high levels of human CSF-1R expression were also associated with the emergence of factor-independent variants, which depended on CSF-1R-mediated signals for cell growth. In contrast, 32D[R+]C1.1, C1.2, and C1.3 cells remained factor-dependent throughout months of continuous passage in culture, as did single-cell subclones derived from them, and only one factor-independent cell line was obtained. Based on the elevated basal levels of receptor tyrosine phosphorylation observed in these cells, we presume that CSF-1R kinase activity can provide certain variants with a selective growth advantage, even in the absence of ligand.

Because 32D[R+]C1.1 cells remained dually responsive to IL-3 and CSF-1, we were able to examine whether IL-3 treatment transmodulated the CSF-1R receptor by inducing its accelerated degradation. We considered this possibility because IL-3 treatment at 37°C can induce the rapid loss of CSF-1 binding sites from normal mouse bone marrow cells by an as yet ill-defined mechanism. Although phospholipid and other inducers of protein kinase C can transmodulate CSF-1R by activating a protease that cleaves the receptor near its membrane-spanning segment, IL-3 treatment had no effect on CSF-1R turnover in 32D[R+]C1.1 cells. Reciprocally, insertion of the constitutively active v-fms oncogene into FDC-P1 cells, although able to confer factor-independence, did not affect the number or affinity of IL-3 binding sites expressed at the cell surface. TPA transmodulated CSF-1R in both 32D[R+]C1.1 and BAC1.2F5 cells, but LPS induced CSF-1R turnover only in BAC1.2F5 macrophages. This implies that LPS does not mediate its effects by directly activating protein kinase C, but rather, its active moiety (lipid A) may bind to another receptor that is differentially expressed in these two cell types.

Although the mechanisms of signal transduction by the IL-3 receptor remain unclear, IL-3 has been shown to bind to a 140-Kd cell surface glycoprotein and to stimulate its phosphorylation on tyrosine residues. After stimulation of 32D[R+]C1.1 cells with IL-3, immunoblotting analyses performed with antibodies to phosphotyrosine showed the presence of a 140-Kd substrate as well as a second major tyrosine phosphorylated species of 95 Kd. One or both of these proteins might well represent components of the IL-3 receptor or, alternatively, substrates of an IL-3 receptor-associated tyrosine kinase. CSF-1 treatment of the same cells induced the appearance of different tyrosine phosphorylated substrates, including human CSF-1R itself. However, like IL-3, CSF-1 induced tyrosine phosphorylation of a 95-Kd protein. Apart from their molecular masses, we have no further biochemical evidence that the 95-Kd phosphoproteins observed in response to IL-3 and CSF-1 represent the identical polypeptide. Therefore, although direct transmodulation of CSF-1R by IL-3 was not observed, our results do not preclude "crosstalk" between the two receptor signaling pathways.

ACKNOWLEDGMENT

We thank Drs Giovanni Rovera and Richard Stanley for murine cell lines; Dr Steven Clark for human recombinant CSF-1; Dr Richard A. Ashmun for performing flow cytometry; and Dr Martine Roussel and Virgil Holder for preparing the c-fms expression vector and antisera to its product.

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Human colony-stimulating factor 1 (CSF-1) receptor confers CSF-1 responsiveness to interleukin-3-dependent 32DC13 mouse myeloid cells and abrogates differentiation in response to granulocyte CSF

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