Introduction of a Human Colony Stimulating Factor-1 Gene Into a Mouse Macrophage Cell Line Induces CSF-1 Independence but not Tumorigenicity

By Martine F. Roussel, Carl W. Rettenmier, and Charles J. Sherr

The SV40-immortalized mouse macrophage cell line, BAC1.2F5, is strictly dependent on CSF-1 for its survival and proliferation in culture. Introduction of a retroviral vector containing a 1.6 kilobase (kb) pair human CSF-1 cDNA into these cells abrogated their growth factor dependence but did not render the cells tumorigenic in nude mice. The infected macrophages contained multiple copies of the vector provirus, expressed both membrane-bound and secreted forms of CSF-1, and exhibited constitutive receptors expressed on hematopoietic cells of the mononuclear phagocyte lineage. The CSF-1 receptor is identical to the product of the c-fms proto-oncogene and is one of a family of growth factor receptors that exhibits tyrosine-specific protein kinase activity.

Autocrine synthesis of CSFs can induce the malignant transformation of established cell lines expressing their respective receptors. For example, the introduction of genes encoding the granulocyte-macrophage colony stimulating factor (GM-CSF) or multi-CSF (IL-3) into the myeloid cell line FDC-P1, or insertion of the interleukin 2 (IL-2) gene into a T cell line, abrogated their factor dependence and rendered the cells tumorigenic. Similarly, a CSF-1 dependent mouse macrophage cell line immortalized by a murine retrovirus encoding the c-myc oncogene gave rise to variants in which the CSF-1 gene was rearranged. The latter cells produced CSF-1, were factor independent for growth in culture, and induced tumors when inoculated into syngeneic animals. Coinfection of mouse NIH-3T3 cells with retroviral vectors containing the human c-fms and CSF-1 genes also led to cell transformation and tumorigenicity in nude mice, indicating that simultaneous expression of a hematopoietic growth factor and its receptor in fibroblasts from a heterologous species was sufficient to induce a fully transformed phenotype.

To determine if the constitutive production of CSF-1 by macrophages would be sufficient to induce cell transformation, we introduced a human CSF-1 gene into an SV40-immortalized murine macrophage cell line, BAC1.2F5. The latter cells are strictly dependent on exogenous CSF-1 for survival and proliferation in culture and respond to either the murine or human growth factor. Although the infected cells synthesized CSF-1 and grew autonomously in vitro, they failed to induce tumors in nude mice, indicating that, even in these immortalized cells, factor independence was not associated with a tumorigenic phenotype.

MATERIALS AND METHODS

Infection of BAC1.2F5 macrophages with a retrovirus encoding CSF-1. A 1.6 kb human CSF-1 cDNA clone was inserted at the BamHI cloning site of a murine retrovirus vector, pZIPneoSV(X)1, and the plasmid DNA was transfected into the ψ2 mouse packaging cell line. The latter cells contain a mutant Moloney leukemia provirus that is defective in its packaging sequences and cannot encapsidate its genomic RNA into virions. The ψ2 provirus provides retroviral replicative functions in trans, so that vector genomic RNA is efficiently packaged into viral particles.
produced by the transfected cells. These “helper free” viruses can infect other murine cells, leading to efficient integration of the vector provirus. Since the vector lacks genes necessary for virion production, the target cells do not release infectious particles. The construction of a vector containing the CSF-1 and neomycin-resistance (neo) genes was described previously. Transfected cells were selected in G418 (Geneticin; Sigma Chemicals, St Louis), and G418-resistant $\phi$2 cells expressing CSF-1 were selected by flow cytometry using an antiserum to the growth factor. All cell lines were cultured in RPMI 1640 medium containing 15% fetal calf serum; the medium was supplemented with nonessential amino acids, glutamine, sodium pyruvate, and antibiotics, each supplied as 50 to 100× concentrates by the manufacturer (GIBCO Laboratories, Grand Island, NY).

Supernatants from transfected $\phi$2 cells were titered for virus production by infection of NIH-3T3 cells expressing a previously inserted human c-fms gene. The latter cells undergo morphologic transformation when infected with viruses producing CSF-1, and the number of transformed foci is a linear function of the input virus dilution. Twenty-four hour culture supernatants from $\phi$2 cells selected for the expression of membrane-bound CSF-1 by flow cytometry produced 5 $\times$ 10^3 focus forming units (FFU) per milliliter. These viruses were used to infect SV40-immortalized, CSF-1 dependent mouse BAC1.2FS5 macrophages as previously described, and 24 hours after infection the cells were shifted to medium containing both G418 and CSF-1. After three weeks of selection, the neomycin-resistant cells were maintained in complete medium containing CSF-1 alone.

Several sources of CSF-1 were used in these experiments and included mouse L cell conditioned medium and medium from mouse C127 cells transfected with a bovine papilloma virus vector encoding human recombinant CSF-1. Both sources of CSF-1 are equally active in supporting the growth of BAC1.2FS5 macrophages and in eliciting the formation of macrophage colonies in semisolid medium from normal mouse bone marrow progenitor cells. Selection, cloning, and growth properties of infected cells. Cells expressing the highest levels of membrane-bound CSF-1 (5% of the total population) were sorted by fluorescence-activated flow cytometry, expanded in medium containing CSF-1, and plated in semisolid medium in the presence or absence of the growth factor. Factor-independent colonies derived from single cells were grown up and subjected to further analysis. Experiments documenting CSF-1 production and receptor down modulation were performed with a representative subclone, designated BAC1.CSF-1 clone 3, whereas three independent clones were tested for tumorigenicity in nude mice.

To determine the growth rates of macrophage cell lines, either 1 $\times$ 10^4 or 5 $\times$ 10^4 cells from each line were seeded in a series of 60-mm diameter plastic dishes and were cultured in 5 mL of complete medium in the presence or absence of 10,000 U/mL human recombinant CSF-1 for six days. The culture medium was changed at two-day intervals. BAC1.2FS5 in the presence (A) or absence () of CSF-1; BAC1.CSF-1 in the presence (Δ) or absence (Δ) of CSF-1; BAC1.CSF-1 clone 3 in the presence (■) or absence (○) of CSF-1.

RESULTS

Selection and growth properties of virus-infected CSF-1 independent macrophage cell lines. The complete 1.6 kb human CSF-1 cDNA was cloned into a Moloney murine leukemia virus vector, pZIPneoSVX(1), and transfected into the $\phi$2 packaging cell line to generate helper-free virus stocks. Transfected cells expressing the neomycin resistance gene were selected in medium containing G418, and the titer of viruses encoding CSF-1 was determined by infection of NIH-3T3 cells harboring a previously introduced human c-fms gene encoding the CSF-1 receptor. High titer helper-free viruses were used to infect CSF-1 dependent BAC1.2FS5 macrophages, and 24 hours after infection, the cells were shifted into medium containing both G418 and CSF-1. When tested 3 weeks later for their ability to form colonies in semisolid medium, approximately 5% of the virus-infected cells (designated BAC1.CSF-1) formed colonies in the presence of CSF-1, whereas a low percentage (0.14%) formed colonies in the absence of the growth factor (Table 1). Since the product of the 1.6 kb human CSF-1 cDNA is expressed as a membrane-bound glycoprotein at the cell surface, G418-resistant cells could be subsequently selected for CSF-1 expression by fluorescence-activated flow cytometry using an antiserum to CSF-1. After two sequential rounds of cell sorting during which the recovered macrophage population was expanded and maintained in medium containing CSF-1, the mean fluorescence of the recovered cell population was only twofold higher than that of the uninfected, control BAC1.2FS5 macrophages. However, a significantly higher
The frequencies of colony formation represent CSF-1 0.3% noble agar in medium containing lacking 10,000 U/mL human exogenous CSF-1.

clones grew at a similar rate in the presence or absence of $10^6$ x

uncloned G4l8-resistant population contained a mixture of the first three days after plating, after which their overall absence, their doubling time was significantly prolonged for

approximately fivefold greater than that of the sorted median, and at efficiencies equivalent to that of BAC1.CSF-1 cells and at efficiencies equivalent to that of BAC1.CSF-1 cells grown in the presence of CSF-1 (Table 1).

The doubling times of the factor-independent cells in the absence of CSF-1 remained similar even when cultures were initiated with fivefold fewer cells. Based on their growth rates, the cloned cells did not have a proliferative advantage over uninfected BAC1.2F5 macrophages grown in the presence of the growth factor.

Both uncloned BAC1.CSF-1 cells and the three factor-independent subclones were examined for their ability to induce tumors in nude mice (Table 1). Animals were injected both intraperitoneally and subcutaneously with $5 \times 10^6$ cells at each site. As a positive control, nude mice were also injected with v-fms-transformed BAC1.2F5 cells shown previously to be both CSF-1 independent for growth and tumorigenic. After 6 months of observation, neither the BAC1.CSF-1 cells nor their CSF-1 independent subclones induced tumors, indicating that factor independence did not correlate with tumorigenicity. Because other established hematopoietic cell lines can be transformed by autocrine mechanisms, the following experiments were undertaken to verify that BAC1.CSF-1 cells contained the vector provirus, synthesized and correctly processed the human growth factor, and exhibited evidence of constitutive stimulation of their CSF-1 receptors.

Analysis of viral integration. To confirm that the factor independent cells contained an integrated CSF-1 provirus, cellular DNA was extracted, digested with restriction endonucleases, and analyzed by Southern blotting using a CSF-1 specific probe (Fig 2). A schematic map of the vector provirus showing relevant sites of restriction is shown at the bottom of Fig 2. Using XhoI, which recognizes an internal proviral DNA fragment containing the entire CSF-1 gene, a diagnostic 2 kb fragment was detected in DNA from both the uncloned (lane 2) and cloned (lane 3) factor independent cells. No equivalent DNA fragment was detected in DNA from noninfected BAC1.2F5 cells (lane 1). The two high molecular weight bands observed in lane 3 correspond to the endogenous mouse CSF-1 gene detected with the same probe (see legend to Fig 2).

The number of proviruses integrated into the DNA of the cloned infected cells was determined by digestion with EcoRI, an enzyme that recognizes a cleavage site within the CSF-1 gene. Using the CSF-1 probe, each integrated provirus should generate one hybridizing EcoRI fragment containing the 5' portion of the provirus joined to flanking mouse cellular sequences; in addition, an internal 2.9 kb fragment consisting only of proviral DNA should be detected. Because proviruses can integrate at many sites in cellular DNA, the length of the 5' EcoRI fragment depends on adventitious sites of EcoRI cleavage in host cellular DNA, so that each integration event should generate a unique hybridizing band. In cloned cell lines, each cell should contain the same proviral insertion(s), and the number of 5' proviral DNA fragments should therefore correspond to the number of integrated proviruses. As shown in lane 5, the CSF-1 probe hybridized to three 5' fragments in addition to the internal 2.9 kb band (arrowheads), indicating that three copies of the vector proviruses were integrated in the cellular DNA of BAC1.CSF-1 clone 3 macrophages.

Table 1. Properties of BAC1.CSF-1 Cell Lines

<table>
<thead>
<tr>
<th>Cell Line*</th>
<th>Colony Formation in Agar (%)†</th>
<th>Tumors in Nude Mice‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAC1.2F5</td>
<td>3.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BAC1.v-fms</td>
<td>6.9</td>
<td>6.0</td>
</tr>
<tr>
<td>BAC1.CSF-1 (unsorted)</td>
<td>5.0</td>
<td>14.0</td>
</tr>
<tr>
<td>BAC1.CSF-1 (sorted)</td>
<td>4.0</td>
<td>0.7</td>
</tr>
<tr>
<td>BAC1.CSF-1 c1.l</td>
<td>3.7</td>
<td>3.9</td>
</tr>
<tr>
<td>BAC1.CSF-1 c1.l2</td>
<td>2.8</td>
<td>3.2</td>
</tr>
<tr>
<td>BAC1.CSF-1 c1.l3</td>
<td>4.0</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Abbreviation: NT, not tested.

* v-fms transformed BAC1.2F5 cells (BAC1.v-fms) were derived previously and characterized in detail elsewhere.

† 1.5 $\times$ $10^6$ cells were seeded in 60-mm diameter culture dishes in 0.3% noble agar in medium containing or lacking 10,000 U/ml human recombinant CSF-1. Macroscopic colonies of >100 cells were enumerated after 2 weeks. The frequencies of colony formation represent averages of quadruplicate determinations.

‡ Nude mice were injected subcutaneously and intraperitoneally with $5 \times 10^6$ cells at each site and observed for a period of 6 months. Animals inoculated with BAC1.v-fms cells developed highly malignant histiosarcomas within 5 weeks.

The number of proviruses integrated into the DNA of the cloned infected cells was determined by digestion with EcoRI, an enzyme that recognizes a cleavage site within the CSF-1 gene. Using the CSF-1 probe, each integrated provirus should generate one hybridizing EcoRI fragment containing the 5' portion of the provirus joined to flanking mouse cellular sequences; in addition, an internal 2.9 kb fragment consisting only of proviral DNA should be detected. Because proviruses can integrate at many sites in cellular DNA, the length of the 5' EcoRI fragment depends on adventitious sites of EcoRI cleavage in host cellular DNA, so that each integration event should generate a unique hybridizing band. In cloned cell lines, each cell should contain the same proviral insertion(s), and the number of 5' proviral DNA fragments should therefore correspond to the number of integrated proviruses. As shown in lane 5, the CSF-1 probe hybridized to three 5' fragments in addition to the internal 2.9 kb band (arrowheads), indicating that three copies of the vector proviruses were integrated in the cellular DNA of BAC1.CSF-1 clone 3 macrophages. The two additional...
bands (lane 5) correspond to endogenous mouse CSF-1 sequences that were also detected in uninfected BAC1.2F5 cells (lane 4).

**CSF-1 synthesis by factor independent BAC1.CSF-1 clone 3 macrophages.** Biologically active CSF-1 released into the culture medium by factor independent macrophages was tested for its ability to induce macrophage colonies from murine bone marrow cell progenitors plated in semisolid medium. Medium (5 mL/60 mm plate) conditioned for five days by confluent cultures of BAC1.CSF-1 clone 3 cells showed only low levels of CSF-1 activity (200 U/mL) as compared with mouse L cell conditioned medium (2,000 U/mL) used as a positive control in the bioassay. Measurement of the CSF-1 concentration by radioimmunoassay independently confirmed these findings. The low levels of extracellular CSF-1 may be partially due to the fact that the 1.6 kb CSF-1 cDNA encodes a membrane-bound form of the growth factor that is inefficiently cleaved from the cell surface. However, we cannot exclude the possibility that CSF-1 released into the medium can bind to receptors on the same cells and be subsequently internalized and degraded. Parental BAC1.2F5 cells did not release detectable CSF-1 activity.

Biogenesis of CSF-1 was also measured by metabolic radiolabeling procedures (Fig 3A). BAC1.CSF-1 clone 3 cells were labeled with [35S]methionine for 60 minutes, and immunoreactive molecules were precipitated from detergent lysates with an antiserum to CSF-1 and analyzed by electrophoresis on denaturing polyacrylamide gels. In the absence of reducing agents, a 64 kd species diagnostic of human CSF-1 encoded by the 1.6 kb cDNA3 was specifically precipitated with antiserum to CSF-1 (lanes 2) but was neither precipitated with a control antiserum (lane 1) nor detected in uninfected BAC1.2F5 cells (lanes 3 and 4). To confirm that the 64 kd species corresponded to the homodimeric cell surface form of the growth factor, viable cells were labeled with 125I using lactoperoxidase, lysed with detergent, and the labeled plasma membrane form of CSF-1 was precipitated with antiserum and similarly analyzed (Fig 3B...
and C). In the absence of reducing agents, the 64 kd species was specifically precipitated only from infected, factor independent cells (Fig 3B) and yielded subunits of ca. 32 kd on reduction (Fig 3C). The slightly reduced molecular weight of the membrane-bound CSF-1 homodimer produced by infected macrophages (64 kd) as compared with the 68 kd form previously detected in mouse NIH-3T3 cells infected with the same vector is most likely due to differences in the processing of asparagine-linked oligosaccharide chains by the two cell types.

**Human CSF-1 stimulates mouse CSF-1 receptor kinase activity.** Because we transferred the human CSF-1 gene into a mouse cell line, we wanted to verify that the mouse CSF-1 receptor kinase could be stimulated by the human growth factor. BAC1.2F5 cells were grown overnight in the absence of CSF-1 receptors at the cell surface. Membranes were then prepared and incubated with purified human recombinant CSF-1 or stage I purified mouse CSF-1 in the presence of divalent cations and [γ-32P]-ATP. The membranes were then analyzed by electrophoresis either directly (lanes 1 through 3) or after immunoprecipitation (lanes 4 through 6) with antiserum to the CSF-1 receptor gene product. The mobilities of the mature cell surface form of CSF-1 and the phosphorylated membrane proteins on denaturing polyacrylamide gels. The mouse CSF-1 receptor kinase can be stimulated by human CSF-1, consistent with the ability of the human growth factor to support the proliferation and survival of normal mouse macrophages.

**Factor independent BAC1.CSF-1 clone 3 cells exhibit constitutively accelerated turnover of the CSF-1 receptor.** The murine CSF-1 receptor expressed on the plasma membrane of BAC1.2F5 macrophages is rapidly internalized and degraded after exposure of the cells to mouse CSF-1. In BAC1.CSF-1 clone 3 cells producing the human growth factor, human CSF-1 should similarly bind to and stimulate the mouse CSF-1 receptor, inducing receptor mediated endocytosis with subsequent degradation of ligand-receptor complexes. To test this possibility, we studied the rate of synthesis and turnover of the mouse CSF-1 receptor in both factor dependent BAC1.2F5 and factor independent BAC1.CSF-1 clone 3 macrophages. BAC1.2F5 (Fig 5, panel A) and BAC1.CSF-1 clone 3 cells (Fig 5, panel B) were metabolically labeled for 15 minutes with [35S]methionine. The labeling medium was removed and the cells were transferred to “chase” medium containing a 100-fold excess of unlabelled methionine either lacking or containing human recombinant CSF-1. Detergent lysates were prepared at various time intervals after the labeling period, and the murine CSF-1 receptor was immunoprecipitated and analyzed electrophoretically on denaturing polyacrylamide gels. The mouse c-fms gene encodes an immature 130 kd glycoprotein (gpl30) that undergoes modification of its N-linked oligosaccharide chains during trans-
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...port to the cell surface, yielding mature receptor molecules of 165 kd (gp165).25 In control BAC1.2F5 cells chased in the absence of CSF-1 (Fig 5, panel A, left), gp130 was converted almost completely to gp165 within one hour, and the mature cell surface form of the receptor turned over with a half life of approximately three hours. When the cells were chased in the presence of exogenously added CSF-1 (Fig 5, panel A, right), the time of conversion of gp130 to gp165 was similar, whereas the half life of the mature receptor was considerably shortened with only trace amounts of gp165 remaining two hours after synthesis. By contrast, the factor independent cells (Fig 5, panel B) exhibited a greatly accelerated turnover of gp165 even in the absence of added CSF-1. Since rapid down modulation and accelerated turnover of the CSF-1 receptor is mediated by its ligand,3,6 the results imply that a substantial proportion of mature receptor molecules are persistently stimulated in these cells.

DISCUSSION

We have used a retroviral vector containing a selectable neomycin-resistance gene to introduce the human CSF-1 gene into a CSF-1 dependent, SV40-immortalized macrophage cell line. Although the parental cells are strictly dependent on CSF-1 for their proliferation and survival in culture, cells expressing relatively high levels of the acquired CSF-1 gene grew autonomously. Because CSF-1 independent variants could not be recovered from uninfected BAC1.2F5 cells grown in the absence of the growth factor, insertion of the vector must have induced the factor-independent phenotype. The characteristic molecular weight of the CSF-1 homodimer and its expression as a membrane-bound form at the cell surface confirmed that the growth factor was encoded by the human 1.6 kb cDNA.

The factor-independent subclones exhibited a growth rate similar to parental BAC1.2F5 cells grown in the presence of CSF-1 and therefore did not have a proliferative advantage when maintained in medium containing the growth factor. The selection of these factor-independent cells was achieved by fluorescence-activated cell sorting with an antiserum to CSF-1 and by subsequent cloning of the enriched CSF-1-positive population in semisolid medium lacking the growth factor. The frequency of factor-independent cells was increased approximately fivefold after selection by cell sorting and an additional fivefold by cloning the cells in semisolid medium. Although most of the original G418-resistant population remained factor-dependent and expressed only low levels of CSF-1, the factor-independent subclones expressed significantly higher levels of the growth factor. BAC1.CSF-1 clone 3 cells contained multiple proviral insertions, consistent with the possibility that integration of several copies of the vector in a low percentage of the original G418-resistant population was responsible for their increased levels of CSF-1 expression and factor independence. In experiments in which an analogous vector containing the v-fms oncogene was inserted into the IL-3-dependent mouse myeloid cell line, FDC-P1, the abrogation of factor independence also occurred through a similar selective process.25

The factor-independent clones grew exponentially in the absence of exogenous CSF-1, suggesting that they did not need to condition their medium to proliferate. Consistent with this interpretation, the addition of exogenous CSF-1 to the medium did not accelerate their rate of growth. Nonetheless, the cells exhibited persistent down modulation of their CSF-1 receptors, suggesting either that cleavage of the membrane-bound form of the endogenously synthesized growth factor is rapidly followed by binding to receptors at the cell surface or that ligand-receptor interactions can occur within the secretory compartments of the cells. Under these conditions, the steady state level of cell surface CSF-1 molecules exceeds that of unoccupied receptors on the plasma membrane so that interference with cell growth using neutralizing antibodies may not be possible.3

To our knowledge, our results represent the first instance in which abrogation of CSF dependence by an autocrine mechanism in an established hematopoietic cell line did not result in a tumorigenic phenotype. Expression of the mouse GM-CSF cDNA via a retroviral vector in the FDC-P1 myeloid cell line induced both factor independence and tumorigenicity.10 Similarly, rearrangement and expression of the endogenous murine CSF-1 gene has been implicated as a second transforming event leading to tumorigenicity in a mye-immortalized macrophage line.13 In conceptually related experiments, the introduction of the IL-3 gene into hematopoietic progenitors derived from mouse fetal liver led to both cell proliferation and differentiation, as judged by the formation of factor-independent clones identical in their cell composition and finite self-renewal capacity to those derived from normal progenitors grown in the presence of the exogenous growth factor.11 Mast cell lines derived from such colonies remained nontumorigenic when injected into nude mice. By contrast, retroviral-mediated insertion of the IL-3 gene into established myeloid cell lines led to their autonomous growth and tumorigenicity. Thus, the property of IL-3 independence in itself was insufficient to convert normal cells to malignant ones, but immortalized cell lines became frankly neoplastic, presumably because they had undergone other genetic alterations during their establishment in culture. However, in our system BAC1.2F5 cells were not rendered tumorigenic in nude mice after introduction of a human CSF-1 cDNA, even though they were previously immortalized by SV40.14 The latter property differentiates these cells from mouse NIH-3T3 fibroblasts, which undergo morphologic transformation and become tumorigenic following cointroduction of retroviruses containing the human c-fms and CSF-1 cDNAs.15

The disparity in these results from those obtained in other systems is underscored by the fact that introduction of the v-fms oncogene into BAC1.2F5 macrophages induced both factor independence and tumorigenicity.20 Several mechanisms might account for the differential response of these cells to v-fms versus autocrine CSF-1 expression. One possibility is that persistent stimulation of the murine CSF-1 receptor by an endogenous ligand does not lead to levels of substrate phosphorylation equivalent to those obtained in v-fms transformed cells. Truncation of the v-fms gene product at its extreme carboxyterminus has removed a critical tyrosine residue whose autophosphorylation may negatively regulate the receptor kinase activity.15,26 Thus, the constitu-
tive v-fms-coded kinase may be upregulated as compared with that of the normal CSF-1 receptor. Moreover, the CSF-1 receptor undergoes down modulation and is rapidly degraded either in response to its ligand or phospholipid esters, whereas the turnover of the v-fms-coded glycoprotein is unaffected by either compound. If physiologic substrates for the receptor kinase reside at the plasma membrane, the inability to accelerate the turnover of the v-fms product might also contribute more persistent signals for cell growth.

An alternative possibility is that the v-fms gene product phosphorylates substrates that are not recognized by the normal CSF-1 receptor. Since v-fms can transform fibroblasts and IL-3–dependent myeloid cell lines that do not express CSF-1 receptors, and can induce fibrosarcomas and hematopoietic neoplasms involving nonmonocytic cells in animals, the oncogene product may be more promiscuous than the normal receptor in substrate recognition. Thus, both quantitative and qualitative differences in receptor signaling mechanisms could account for the observed results.

The bone marrow progenitors of mononuclear phagocytes respond to CSF-1 by proliferation and differentiation, whereas mature monocytes and macrophages require CSF-1 primarily for their survival. These pleiotropic actions of the growth factor are mediated through its interaction with a single class of high affinity CSF-1 receptors whose number per cell increases tenfold as the cells differentiate and mature. If the differential response to receptor signals is modulated by the emergence of diverse physiologic substrates for the receptor kinase as a part of the differentiation program, the most mature cells might be relatively refractory to the growth promoting activities of CSF-1. Indeed, monocytes and macrophages produce CSF-1 in response to a variety of physiologic inducers, including γ-interferon and GM-CSF, suggesting that autocrine or paracrine mechanisms might normally function to modulate the activities of monocytes and macrophages during an inflammatory response. Activated macrophages also release a variety of inflammatory modulators including platelet-derived growth factor (PDGF), tumor necrosis factor (TNF or cachectin), interleukin 1 (IL-1) (reviewed in reference 35), and transforming growth factor beta (TGF-β), at least some of which may inhibit cell proliferation. Possibly, the release of other cytokines by the factor-independent cells affects their tumorigenicity. Although autocrine mechanisms may contribute to leukemogenesis, possibly as part of a multistep process involving other genetic events, our results suggest that additional regulatory mechanisms can limit the tumorigenic potential of immortalized, autocrine-stimulated hematopoietic cells.

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