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The v-*fms* Oncogene Induces Factor-Independent Growth and Transformation of the Interleukin-3-Dependent Myeloid Cell Line FDC-P1

ESTHER F. WHEELER,[1] DAVID ASKEW,[2] STRAT MAY,[3] JAMES N. IHLE,[2] AND CHARLES J. SHERR[1]*

Department of Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, Tennessee 38105[1]; and Molecular Mechanisms of T Cell Leukemogenesis Section, Basic Research Program, Frederick Cancer Research Facility, Frederick, Maryland 21701[2]; The Johns Hopkins Oncology Center, Baltimore, Maryland 21205[3]

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The normal cellular counterpart of the v-*fms* oncogene product is a receptor for the mononuclear phagocyte colony-stimulating factor, CSF-1. An interleukin-3 (IL-3)-dependent mouse myeloid cell line, FDC-P1, was infected with a murine retrovirus vector containing v-*fms* linked to a gene encoding resistance to neomycin (*neo*). Infected cells selected for resistance to the aminoglycoside G418 contained few proviral DNA copies per haploid genome, expressed low levels of the v-*fms*-coded glycoprotein, remained IL-3 dependent for growth, and were nontumorigenic in nude mice. In contrast, infected cells selected for their ability to grow in the absence of IL-3 contained an increased number of proviral insertions, expressed high levels of the v-*fms*-coded glycoprotein, and were tumorigenic in nude mice. The IL-3-independent cells expressed IL-3 receptors of comparable number and affinity to those detected in uninfected FDC-P1 cells and did not produce a growth factor able to support replication of the parental cells. Thus, the synthesis of high levels of the v-*fms* gene product in FDC-P1 cells abrogated their requirement for IL-3 and rendered the cells tumorigenic by a nonautocrine mechanism. The data suggest that v-*fms* encodes a promiscuous tyrosine kinase able to transform cells of the myeloid lineage that do not normally express CSF-1 receptors.

The product of the v-*fms* oncogene of the Susan McDonough strain of feline sarcoma virus (SM-FeSV) is an integral cell surface glycoprotein with an intrinsic tyrosine-specific protein kinase activity (2, 3, 5, 20, 29, 40, 44, 45). Although SM-FeSV transforms fibroblasts in cultures and produces fibrosarcomas in cats (33, 47), the virus has not been demonstrated to produce hematopoietic malignancies. The fact that the v-*fms* oncogene product is related to the receptor for the macrophage-specific colony-stimulating factor, CSF-1 (49), raised the possibility that its transforming activity might reflect retroviral transduction of a CSF-1 receptor gene into mesenchymal cells that synthesize the growth factor. Although fibroblasts transformed by SM-FeSV express v-*fms*-coded CSF-1-binding sites at their cell surfaces, antibodies to the v-*fms* gene product that inhibit CSF-1 binding or neutralizing antibodies to CSF-1 itself do not affect the transformed phenotype (46). In membrane preparations, the tyrosine kinase activity of the CSF-1 receptor is stimulated by its ligand (49), whereas the enzyme activity of the v-*fms* oncogene product appears to function constitutively (46). Thus, structural alterations in the v-*fms* gene which occurred as a result of viral transduction appear to have altered the kinase activity of the v-*fms* gene product so that transformation is mediated by a CSF-1-independent mechanism (43). In support of this interpretation, helper-free SM-FeSV infection of a simian virus 40-immortalized, CSF-1-dependent mouse macrophage cell line rendered the cells factor independent and tumorigenic through a nonautocrine mechanism (54). Expression of the v-*fms* gene product in these transformed macrophages affected neither the expression of normal CSF-1 receptors nor their down-modulation in response to ligand or phorbol ester treatment. These results suggest that the growth-promoting signals induced by v-*fms* bypass the proximal CSF-1 receptor pathway.

We have further tested this concept by introducing the v-*fms* gene into a myeloid cell line, FDC-P1, which lacks CSF-1 receptors but expresses receptors for granulocyte-macrophage-specific colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3) (11). FDC-P1 cells require either GM-CSF or IL-3 to proliferate in cultures and are nontumorigenic in nude mice. The results showed that FDC-P1 cells that express high levels of the v-*fms* gene product grow independently of IL-3 and are tumorigenic. Transformation occurred by a nonautocrine mechanism which did not affect the expression or affinity of the IL-3 receptor. Taken together, the results demonstrate that v-*fms* can transform both immature and mature cells of the myeloid lineage as well as fibroblasts.

MATERIALS AND METHODS

Virus infection and culture conditions. The *gag-fms* fusion gene of molecularly cloned SM-FeSV (12) was inserted into the unique *Bam*HI site of the murine retroviral shuttle vector pZIP*neo*SV(X)-1 (6). This construction links the v-*fms* gene to DNA sequences encoding neomycin resistance (*neo*); the polyprotein is translated from the full-genome-length RNA, whereas *neo* is synthesized from a spliced mRNA. The retroviral construct was transfected by the calcium phosphate method (17, 44) into the ψ2 packaging cell line (30). The ψ2 cell line contains an integrated Moloney murine leukemia provirus that provides the *gag*, *pol*, and *env* gene products in *trans* that are necessary for packaging retroviral RNA into virions. Because the ψ2 provirus contains a mutated packaging signal, its own genome cannot be packaged into viral particles. Following transfection, ψ2 cells were cultured in medium containing 800 μg of G418 (Geneticin; GIBCO Laboratories, Grand Island, N.Y.) per

* Corresponding author.

TABLE 1. Cloning efficiencies in agar of virus-infected FDC-P1 cells in the presence and absence of IL-3[a]

Cell line	Selection procedure		% Cloning efficiency in the presence (+) or absence (−) of IL-3	
	IL-3	G418	+	−[b]
FDC-P1	+	−	15.7	<0.01
FD(*neofms*)-1	+	+	8.9	<0.01
FD(*neofms*)-2	+	+	7.6	<0.01
FI(*neofms*)	−	−	11.2	7.1

[a] Infected FDC-P1 cell lines, selected as indicated in medium containing (+) or lacking (−) IL-3 and G418, were seeded in semisolid medium in the presence or absence of IL-3. Cells were plated in duplicate at dilutions of 10^3, 10^4, and 10^5 cells per plate, and continuously growing colonies of >100 cells were counted 3 weeks later. The efficiencies of colony formation represent averages from two individual experiments and were independent of the cell density. FI(*neofms*) cells were derived from FD(*neofms*)-1 cells by sequential reselection as described in the text.

[b] No factor-independent variants were obtained from the FD(*neofms*) cell lines after plating 10^5 cells. Because the efficiency of colony formation of FI(*neofms*) cells was ca. 10%, we estimate that the frequency of factor-independent variants in the FD(*neofms*) population was less than 1 in 10^4 cells.

ml, and cell lines producing helper-free transforming pseudotype virus at titers in excess of 10^6 focus-forming units per ml were obtained (55).

A murine myeloid cell line, FDC-P1 (11), was infected by cocultivation for 24 h with adherent ψ2 producer cells grown to 80% confluence. The infection was performed in the presence of 10 μg of Polybrene (Sigma Chemicals, St. Louis, Mo.) per ml in RPMI 1640 medium (GIBCO) supplemented with 4 U of high-pressure liquid chromatography-purified IL-3 (22) per ml and 10% fetal calf serum. After cocultivation, nonadherent FDC-P1 cells were collected, and G418-resistant cells were selected in complete medium containing 20 U of IL-3 and 800 μg of G418 per ml. Some of these G418-resistant cells were then further selected for IL-3 independence by being cultured in medium lacking both IL-3 and G418.

Infected FDC-P1 cells were tested for their ability to form colonies in semisolid medium by suspension of serial dilutions of cells in 0.3% Noble agar containing complete Iscove medium (GIBCO) in the presence or absence of 20 U of IL-3 per ml. G418 was not included in the cultures. For each experiment, the cells were plated in duplicate in 60-mm culure dishes at dilutions containing 10^3, 10^4, or 10^5 cells per plate. Continuously growing colonies of greater than 100 cells were scored 3 weeks after plating. The efficiencies of colony formation shown in Table 1 represent averages from two separate experiments and were independent of the cell density.

Biological assays. Possible growth factors produced by infected FDC-P1 cells were assayed by culturing parental FDC-P1 cells in RPMI 1640 medium supplemented with conditioned medium from the factor-independent, virus-infected cell cultures. Conditioned medium was obtained by culturing the infected cells for 48 h in complete RPMI medium lacking IL-3. After the incubation period, the medium was removed, filtered, and titrated for growth-promoting activity against the parental FDC-P1 cells. Serial dilutions (0.05 ml) of the conditioned medium were prepared in 96-well microtiter plates. The FDC-P1 cells were washed free of IL-3, and 5×10^4 cells in 0.05 ml were added to each well. Following an overnight incubation, 1 μCi of [^{3}H]thymidine (2 Ci/mmol; New England Nuclear Corp., Boston, Mass.) was added per well, and the cells were incubated for an additional 6 h. Samples were harvested with an automated cell harvester unit (Bellco Glass, Inc., Vineland, N.J.), plated onto filter paper strips, and counted by liquid scintillation for acid-insoluble [^{3}H]thymidine incorporation.

The BAC1.2F5 murine macrophage cell line was used to assay conditioned medium from virus-infected FDC-P1 cells for CSF-1 activity. BAC1.2F5 cells require CSF-1 for growth and survival in culture and die within 5 days in the absence of the factor (48; C. Morgan, J. W. Pollard, and R. E. Stanley, J. Cell. Physiol., in press). The macrophages were seeded at a density of 10^4 cells per well in 24-well microtiter plates. Filtered medium from virus-infected FDC-P1 cells was used to supplement 3 volumes of complete Dulbecco modified Eagle medium containing 15% fetal calf serum and glutamine. After 5 days of incubation of BAC1.2F5 cells in 25% conditioned medium, the medium was removed, and the cells were stained with Giemsa stain and scored by comparison with cultures maintained in 25% murine-L-cell-conditioned medium, used as an exogenous source of CSF-1. In the absence of CSF-1, no BAC1.2F5 colonies were observed.

[^{125}I]IL-3 equilibrium binding to FDC-P1 cells was used to quantitate receptor density and affinity for ligand as previously described (32).

Protein analysis. Cell surface expression of v-*fms*-coded epitopes was analyzed by flow cytometry with a monoclonal antibody (2) as previously described (44). Quantitation of cell surface expression of the myeloid differentiation markers Mac-1, Mac-2, and thy-1 was performed by analogous procedures. The v-*fms*-coded glycoprotein was metabolically labeled by incubating 3×10^7 cells in 2.5 ml of methionine-free medium containing 0.2 mCi of L-[^{35}S]methionine per ml for 45 min at 37°C; 2 ml of complete RPMI 1640 medium was added, and incubation was continued for an additional 45 min. Cells were rinsed in ice cold phosphate-buffered saline and lysed in RIPA buffer (50 mM Tris hydrochloride, [pH 7.4], 150 mM NaCl, 20 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.5% sodium dodecyl sulfate [SDS]) containing 2% aprotinin and 1% phenylmethylsulfonyl fluoride as protease inhibitors. Immunoprecipitation with antiserum to recombinant v-*fms*-coded polypeptide expressed in bacteria (15, 49) was performed as previously described (3). Washed immune complexes were denatured and analyzed by electrophoresis on polyacrylamide gels containing SDS (3). Under these labeling conditions, the proteolytically processed *gag-fms* polyprotein precursor (gP180$^{gag\text{-}fms}$) was not detected in the myeloid cell lines, and only the v-*fms*-coded glycoproteins gp120$^{v\text{-}fms}$ and gp140$^{v\text{-}fms}$ were observed.

DNA purification and analysis. DNA was prepared (19) and digested with restriction endonucleases under the conditions described by the manufacturers. The resulting digests were fractionated by electrophoresis through 0.8% agarose gels and transferred (51) to Hybond nylon filters (Amersham Corp., Arlington Heights, Ill.). Filters were hybridized for 48 h with a nick-translated (41) v-*fms* probe at 42°C in the presence of 50% formamide–0.75 M NaCl–0.075 M sodium citrate–0.2% Denhardt solution (51) 0.1% SDS–50 μg of sheared calf thymus DNA per ml–10 mM Tris hydrochloride (pH 7.8). The probe consisted of a ca. 2.4-kilobase-pair (kbp) *Cla*I-*Bgl*II fragment of SM-FeSV DNA that includes about 65% of the v-*fms* gene (12). Following hybridization, the filters were washed three times for 20 min at room temperature in 0.3 M NaCl–0.03 M sodium citrate–0.1% SDS and four times for 15 min at 52°C in 0.015 M NaCl–0.0015 M

sodium citrate–0.1% SDS. The filters were rinsed in distilled water and dried, and hybridization was quantitated by autoradiography on X-ray film in cassettes with Cronex Lightning-Plus intensifying screens (Du Pont Co., Wilmington, Del.) for 1 to 3 days.

RESULTS

Selection of virus-infected, IL-3-independent myeloid cell lines. FDC-P1 cells were infected with a helper-free retrovirus containing the v-*fms* oncogene linked to the *neo* gene (55) (see bottom of Fig. 3 for schematic map), and after 24 h, the infected cells were selected in medium containing G418 and IL-3. From two separate experiments, two G418-resistant cell lines [FD(*neofms*)] were derived and designated FD(*neofms*)-1 and FD(*neofms*)-2. Although both cell lines could proliferate in the presence of high concentrations of G418, the majority of the cells retained an absolute requirement for IL-3 for growth. From the FD(*neofms*)-1 line, IL-3-independent variants were selected by culturing the cells in the absence of the growth factor. The factor-independent cells, designated FI(*neofms*), retained resistance to G418.

To determine the frequency of the factor-independent variants in the original virus-infected cell lines, we plated FD(*neofms*) and FI(*neofms*) cells in agar in the presence or absence of IL-3. Each of the cell lines formed colonies in medium containing IL-3 at an efficiency ranging from 7 to 11%, whereas only FI(*neofms*) cells formed colonies in medium lacking IL-3 (Table 1). These results suggested that the frequency of factor-independent variants in the FD(*neofms*) cells was less than 1 per 10^4 cells (Table 1).

The different cell lines were also examined for their ability to induce tumors when injected into nude mice. The FI(*neofms*) cells induced tumors within 3 weeks. In contrast, mice injected with either of the FD(*neofms*) lines or with parental FDC-P1 cells did not develop tumors during a 3-month observation period. Therefore, factor independence correlated with tumorigenicity.

Expression of v-*fms* in FD(*neofms*) and FI(*neofms*) cell lines. In both fibroblasts (44) and macrophages (54), high levels of expression of the v-*fms*-coded glycoprotein at the cell surface are required for transformation. To determine whether the factor independence of the FI(*neofms*) cells was related to v-*fms* expression, we analyzed the various myeloid cell lines by flow cytometry for the presence of v-*fms*-coded epitopes on the plasma membrane. Both factor-dependent lines expressed low but detectable levels of v-*fms*-coded antigen (Fig. 1A and B). In contrast, the factor-independent variants expressed high levels of v-*fms*-coded epitopes (Fig. 1C), comparable to those observed on transformed fibroblasts (40, 44). The difference in the levels of expression between the factor-dependent and -independent cells was approximately fivefold. As expected, the parental FDC-P1 cells did not bind the v-*fms*-specific monoclonal antibody (Fig. 1D). Thus, relatively high levels of v-*fms* expression correlated with IL-3-independent growth.

The differences in the levels of cell surface expression of the v-*fms*-coded glycoprotein could be due to the rates of synthesis or processing of the v-*fms* gene products. To distinguish between these possibilities, we metabolically labeled cultures with [^{35}S]methionine and precipitated detergent lysates with a rabbit antiserum to a recombinant v-*fms*-coded polypeptide expressed in bacteria (15, 49). Washed immune complexes were then denatured and analyzed by SDS-polyacrylamide gel electrophoresis. Although the primary translation product of the *gag-fms* open reading frame is a 180-kilodalton glycoprotein, the polyprotein is cotranslationally cleaved to yield an immature form of the v-*fms*-coded glycoprotein, gp120$^{v\text{-}fms}$ (45, 55). The latter molecule is transported to the plasma membrane, undergoing concomitant modification of its asparagine (N)-linked oligosaccharide chains and giving rise to the mature cell surface form of the glycoprotein, gp140$^{v\text{-}fms}$. Because gp120$^{v\text{-}fms}$ is inefficiently converted to gp140$^{v\text{-}fms}$, the immature form of the glycoprotein is the major v-*fms*-coded species detected at steady state (3).

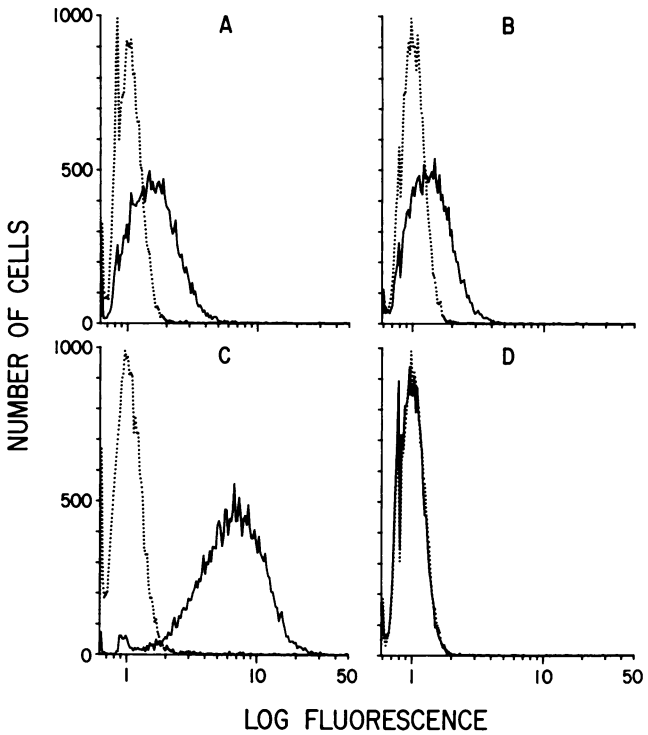

FIG. 1. Flow cytometric analysis of virus-infected FDC-P1 cells. The flow cytometric profile in each panel was recorded after binding of SM 2.6.3, a monoclonal antibody to a v-*fms* epitope, to the surfaces of viable cells (solid lines). Control fluorescence (dashed lines) was obtained by using cells similarly incubated with an irrelevant isotype-matched rat myeloma protein. (A and B) Profiles obtained with the two virus-infected factor-dependent cell lines, FD(*neofms*)-1 and FD(*neofms*)-2, respectively, selected in medium containing G418 and IL-3. (C) Fluorescence profile obtained with the factor-independent cell line FI(*neofms*) selected in medium lacking IL-3. (D) Profile obtained with parental FDC-P1 cells.

Figure 2 shows that both FD(*neofms*) cell lines synthesized relatively low levels of gp120$^{v\text{-}fms}$ (lanes 2 and 3), whereas FI(*neofms*) cells expressed about fivefold-higher levels of the glycoprotein (lane 4). In contrast to SM-FeSV-infected NIH 3T3 cells used as a control (lane 5), the mature form of the glycoprotein expressed in infected myeloid cells had an apparent molecular mass of about 160 kilodaltons, presumably due to host cell-specific differences in the processing of its N-linked oligosaccharide chains. Similar variations in the size of the mature v-*fms*-coded glycoprotein were previously observed in a murine macrophage cell line (54). Irrespective of these differences, the data indicate that the levels of expression of the v-*fms*-coded glycoprotein at the surfaces of FD(*neofms*) and FI(*neofms*) cells (Fig. 1) reflect their rates of synthesis.

High levels of v-*fms* expression in FI(*neofms*) cells results

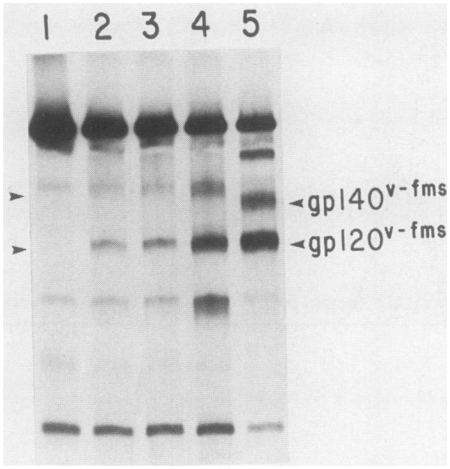

FIG. 2. Metabolic radiolabeling of the v-*fms* gene products gp120$^{v\text{-}fms}$ and gp140$^{v\text{-}fms}$ expressed in virus-infected FDC-P1 cells. Cells were metabolically labeled with [^{35}S]methionine, and viral proteins were immunoprecipitated from cell lysates, subjected to SDS-polyacrylamide gel electrophoresis, and detected by fluorography of the dried polyacrylamide slab gel. The arrowheads on the left indicate the mobilities of viral glycoproteins present in infected FDC-P1 cells; the mobilities of gp120$^{v\text{-}fms}$ and gp140$^{v\text{-}fms}$ synthesized in NIH 3T3 cells containing the same retroviral vector are indicated on the right. Lanes: 1, FDC-P1; 2, FD(*neofms*)-1; 3, FD(*neofms*)-2; 4, FI(*neofms*); 5, NIH 3T3 containing the pZIP*neo-gag-fms* vector.

from multiple proviral insertions. To determine the possible basis for the differences in the levels of synthesis of the v-*fms* gene product in the factor-dependent and -independent cell lines, we extracted cellular DNA and analyzed it for the presence of integrated proviruses by using a v-*fms*-specific probe. The results of Southern blotting analysis with DNAs from the three G418-resistant cell lines and parental FDC-P1 cells are shown in Fig. 3. A schematic map of the retroviral vector defining relevant sites of restriction is shown at the bottom of the figure.

Using *Bam*HI (Fig. 3A) and *Kpn*I (Fig. 3B), both of which recognize multiple cleavage sites within the vector, we detected proviral DNA fragments of the expected sizes in each of the infected cell lines (lanes 1 to 3); no hybridization was detected with the DNA of uninfected FDC-P1 cells under the same conditions (lane 4). In addition to the expected 5.0-kbp *Bam*HI and 3.7- and 2.2-kbp *Kpn*I fragments, some additional hybridizing bands were observed in the infected cells. The latter bands did not represent murine c-*fms* sequences, which would also be detected in uninfected FDC-P1 cells, but could have represented incompletely digested proviruses, possibly including some that had undergone rearrangement. Although similar quantities of DNA were run in each lane, the intensity of hybridization varied between the different cell lines. The DNA of both FD(*neofms*) cell lines (lanes 1 and 2) generated relatively weak hybridization signals, whereas the extent of hybridization seen with the DNA of FI(*neofms*) cells was at least fivefold greater (lanes 3). Thus, on the average, the factor-independent cells contained significantly more proviral DNA insertions per cell.

DNAs from the different cell lines were also examined after digestion with *Eco*RI, an enzyme which recognizes a unique cleavage site within the 3′ end of the proviral *neo* gene (Fig. 3C). With the v-*fms* probe, each proviral insertion should generate a unique hybridizing *Eco*RI fragment representing the 5′ end of the provirus joined to flanking mouse cellular sequences. The minimum size of such bands should correspond to the size of the 5′ *Eco*RI fragment of the linear provirus, or ca. 8 kbp. The probe hybridized to unresolved fragments greater than 8 kbp in length, suggesting that proviral insertions occurred at multiple sites in the cellular DNA (Fig. 3C). As expected from the data in Fig. 3A and B, the overall hybridization intensity of proviral DNA in FI(*neofms*) cells was greater than that in the FD(*neofms*) cell lines, confirming that the factor-independent cells had acquired an increased average number of proviruses. Thus, the increased level of expression of the v-*fms* gene in the latter cell population was due to the presence of multiple proviruses in cells that survived selection in medium lacking IL-3.

IL-3 independence is not due to an autocrine mechanism. The abrogation of factor dependence is transformed FI(*neofms*) cells could be due to the unscheduled expression of a relevant growth factor or to v-*fms*-induced growth-promoting signals that bypass the normal IL-3-dependent

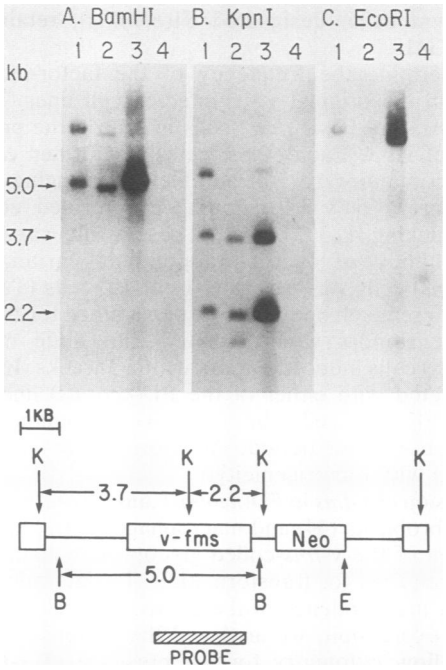

FIG. 3. Southern blot analysis of restricted high-molecular-weight DNAs from virus-infected FDC-P1 cells. DNAs from the infected FD(*neofms*) and FI(*neofms*) cell lines and control FDC-P1 cells were digested with *Bam*HI (A), *Kpn*I (B), or *Eco*RI (C). Similar quantities of DNA (25 μg) were run in each lane. The predicted restriction sites within the vector DNA and the origin of the probe DNA fragment are indicated on the map at the bottom of the figure (B = *Bam*HI, K = *Kpn*I, and E = *Eco*RI). The boxes at the ends of the proviral DNA map indicate the positions of the long terminal repeats. *Bam*HI generates a 5-kbp internal proviral DNA fragment that hybridizes to the v-*fms* probe, whereas *Kpn*I generates two internal fragments of 3.7 and 2.2 kbp. The mobilities of these fragments on the blot are indicated by arrows to the left of panel A. The sizes of the bands were calculated by comparison with *Hin*dIII λ phage DNA digests run in parallel. *Eco*RI recognizes a single restriction site in the provirus and generates v-*fms*-containing fragments representing the 5′ end of proviral DNA joined to host cellular sequences flanking the integration sites. The sizes of these 5′-junctional fragments are predicted to be >8 kbp. All panels were made from a single blot. Lanes: 1, FD(*neofms*)-1 DNA; 2, FD(*neofms*)-2 DNA; 3, FI(*neofms*) DNA; 4, parental FDC-P1 DNA. kb and KB, Kilobase pair.

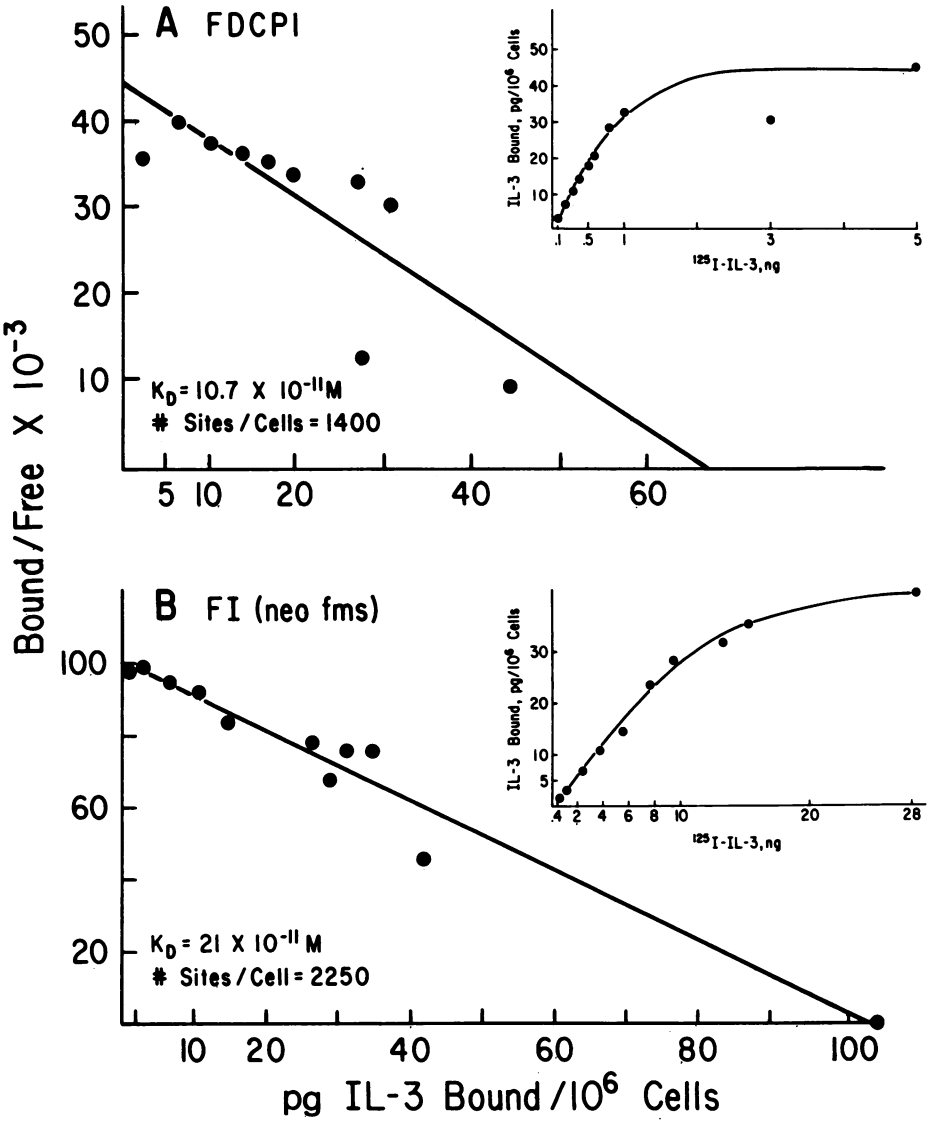

FIG. 4. Equilibrium binding of [^{125}I]IL-3 to parental FDC-P1 cells (A) and the factor-independent derivative FI(*neofms*) (B). Specific binding of [^{125}I]IL-3 to the cells was measured at 37°C with increasing concentrations of labeled factor. Data are plotted as the mean of triplicate determinations, and all values are within ±5% of the standard error of the mean.

pathway. To test if infected cells produced hematopoietic growth factors, we assayed conditioned medium from the FI(*neofms*) cultures for its ability to support the growth of parental FDC-P1 cells. No growth-stimulating activity was detected, implying that the cells did not produce growth factors with either IL-3 or GM-CSF activities. In addition, no CSF-1 activity was detected in analogous assays with CSF-1-dependent BAC1.2F5 macrophages as targets.

Expression of v-*fms* does not affect IL-3 receptor number or affinity. Previous studies have suggested that activation of one hematopoietic growth factor receptor may result in the down-regulation of receptor-binding sites for other hematopoietic growth factors (53). To determine whether the expression of the v-*fms*-coded analog of the CSF-1 receptor would affect the expression of IL-3 receptors on FI(*neofms*) cells, we performed equilibrium binding assays with IL-3. The factor-independent cells expressed a single class of high-affinity receptors for IL-3 (K_d, 21 × 10^{-11} M) comparable to those expressed by IL-3-dependent parental FDC-P1 cells (K_d, 10.7 × 10^{-11} M) (Fig. 4). Both cell lines also expressed similar numbers of receptors per cell (1,400 to 2,250). Thus, expression of the v-*fms* gene product abrogated the requirement for IL-3 without transmodulating IL-3 receptors.

Expression of differentiation-specific myeloid antigens in cells expressing v-*fms*. Because it was conceivable that high levels of expression of the v-*fms* gene product might alter the differentiation phenotype of FDC-P1 cells, the various cell lines were examined morphologically and assayed by flow cytometry for the presence of several other myeloid cell surface markers, including Mac-1, Mac-2, and an undesignated antigen detected by the monoclonal antibody RB6-8C5 (R. Coffman and J. Keller, personal communication). In general, there were no morphologic differences between the parental and virus-infected cells, regardless of their IL-3 requirement, and the expression of myeloid cell surface markers was unaltered. Parental FDC-P1 cells also expressed low levels of thy-1, an antigen expressed on lym-

phoid T cells and transiently on myeloid precursors at early stages of differentiation (23). The FI(*neofms*) cells did not express detectable thy-1, whereas both FD(*neofms*) cell lines expressed levels of thy-1 comparable to those expressed by parental cells. The significance of this finding is not understood but does not appear to correlate with differentiation, as assessed with the other cell surface markers.

DISCUSSION

To examine the ability of the v-*fms* oncogene to abrogate IL-3 dependence, we introduced a retroviral vector containing the v-*fms* oncogene linked to the dominant selectable marker, *neo*, into the myeloid cell line FDC-P1. By selecting infected cells in the presence of both IL-3 and G418, we recovered cells containing the vector provirus irrespective of their growth factor requirements. Although the vast majority of infected cells remained dependent on IL-3 for growth, reselection of these cells in medium lacking IL-3 gave rise to rare variants (<1 in 10^4 cells) which were both factor independent and tumorigenic. Because IL-3-independent variants could not be recovered from uninfected FDC-P1 cells grown in the absence of IL-3, insertion of v-*fms* must have contributed to the factor-independent phenotype. Selection for factor independence resulted in the isolation of cells that expressed approximately fivefold-higher levels of the v-*fms* oncogene product than did cells which remained dependent on IL-3 for growth. Although epigenetic suppression of v-*fms* could possibly have occurred during G418 selection (13, 14), the increased expression of v-*fms* in IL-3-independent cells was associated with an amplified number of integrated proviruses. Since the replication-defective virus was produced in ψ2 cells and since infected FDC-P1 cells did not, in turn, release infectious virus, it seems likely that only rare cells acquiring multiple proviral insertions immediately after infection had a selective advantage in the absence of IL-3.

The induction of factor independence by v-*fms* does not involve an autocrine mechanism, since the factor-independent cells did not synthesize growth factors able to stimulate either parental FDC-P1 cells or CSF-1-dependent macrophages. The v-*abl* oncogene also abrogates the growth factor requirements of FDC-P1 cells and concurrently potentiates tumorigenesis by an apparently similar mechanism (7). In addition, v-*abl* (31, 34, 38), like many other oncogenes encoding tyrosine kinases, can transform other hematopoietic cell lines (4, 24, 25, 37, 42). In chicken hematopoietic cells, several oncogenes of the *src* family as well as v-*mil* can abrogate requirements for exogenous growth factors by inducing growth factor synthesis (1, 16). Murine FDC-P1 cells can also be transformed by an autocrine mechanism after infection with retroviral constructs containing either GM-CSF (26) or IL-3 (A. Habel, G. F. Vande Woude, H. Campbell, N. Young, and T. Robbins, Lymphokine Res., in press; P. Wong and A. Nienhuis, personal communication) genes. To date, factor-independent FDC-P1 cells, whether induced by an autocrine mechanism, by genes encoding tyrosine kinases, or by the v-*myc* gene (39), have been found to be tumorigenic in nude mice. Although these data suggest that a stage of transformation of myeloid cells involves the abrogation of factor dependence, many myelogenous leukemias retain a requirement for hematopoietic growth factors in vitro (recently reviewed in reference 18).

The ability of some oncogenes encoding tyrosine-specific protein kinases to induce IL-3 independence might suggest that IL-3 itself promotes signal transmission through tyrosine phosphorylation. Cross-linking studies have demonstrated that the IL-3 receptor is biochemically distinct from known growth factor receptors of the tyrosine kinase gene family in being considerably smaller, with an apparent molecular mass of ca. 65 kilodaltons (32, 35, 36, 50). However, these results do not preclude the possibility that the ligand-binding polypeptide is a receptor subunit that can noncovalently associate with a membrane-bound kinase. FI(*neofms*) cells expressed binding sites for IL-3 on the cell surface at a number and an affinity comparable to those in parental FDC-P1 cells. Thus, v-*fms* abrogates the requirement for IL-3 without down-regulating IL-3-binding sites. Similar results were obtained with an SM-FeSV-infected, CSF-1-independent macrophage cell line that expressed normal numbers of CSF-1 receptors (54). In the latter case, the normal receptors underwent down-modulation in response to either CSF-1 or phorbol ester, again suggesting that v-*fms* nullified the requirement for CSF-1 without affecting the synthesis or turnover of its normal receptor.

The v-*fms* oncogene encodes a constitutive kinase activity that most likely results from critical mutations in the c-*fms* proto-oncogene. The most obvious structural difference between the c-*fms* proto-oncogene and v-*fms* oncogene products occurs at the extreme carboxyl-terminal ends of the proteins, where the terminal 40 amino acids of the c-*fms* product are replaced by 11 unrelated amino acids in the v-*fms*-coded glycoprotein (10). This unique region of the normal CSF-1 receptor contains a single tyrosine residue (Tyr-969) that was deleted from the v-*fms* gene product (20). A potential tyrosine phosphorylation site (Tyr-527) is located in an analogous position of $pp60^{c\text{-}src}$ and was deleted from its v-*src*-coded counterpart (52). In the c-*src* protein, Tyr-527 is phosphorylated in vivo (8, 21, 27) and may play a role in negatively regulating its kinase activity (8, 9). Although deletion of Tyr-527 activates c-*src* as a transforming gene, single point mutations in the body of c-*src* also generate products with transforming activity (21, 28). Tyr-969 in c-*fms* may regulate its kinase activity, but other activating mutations elsewhere in the gene appear to be required for transformation (43). The latter alterations appear to confer CSF-1-independent enzyme activity and allow v-*fms* to act as a promiscuous receptor kinase in transforming a variety of target cells, including cells like FDC-P1 which do not express CSF-1 receptors.

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