Establishment and Characterization of a New Granulocyte-Macrophage Colony-Stimulating Factor–Dependent and Interleukin-3–Dependent Human Acute Myeloid Leukemia Cell Line (GF-D8)

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A novel factor-dependent human myeloid leukemia cell line (GF-D8) was established from the peripheral blood of an 82-year-old man suffering from acute myeloblastic leukemia (AML). By morphology, cytochemical staining, and analysis of surface antigens, GF-D8 cells are myeloblasts of immature progenitor origin. The consensus karyotype is 45, XY, −5, 7q-, inv(7) (q31.2q36), 8q+, +8q+, 11q+, 12p−, −15, −17, + marker. The long-term survival and proliferation of GF-D8 cells is dependent on the presence of either granulocyte-macrophage colony-stimulating factor (GM-CSF) or interleukin-3 (IL-3). Weak colony growth was observed after exposure of GF-D8 cells to stem cell factor (SCF) but not after exposure to granulocyte-CSF (G-CSF), macrophage-CSF (M-CSF), IL-1β, IL-2, IL-5, or tumor necrosis factor-α (TNF-α). GM-CSF– and IL-3–induced proliferation is dose dependent, with significant growth observed at concentrations as low as 0.1 ng/mL, but the combination of both factors has no synergistic effect. A significant proliferation is induced by GM-CSF and IL-3 even in serum-deprived cultures, although with a slightly decreased efficiency. GF-D8 cells were shown to express specific messenger RNAs for the α chains of the GM-CSF and IL-3 receptors as well as for the β chain, common to both receptors. Interestingly, despite the absence of biologic response to G-CSF, specific transcripts for the G-CSF receptor gene were similarly identified by reverse polymerase chain reaction analysis. GF-D8 cells represent a useful tool for studying chromosome abnormalities of human AML as well as the regulation of myeloid proliferation and differentiation in vitro.

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T HE IN VITRO growth of acute myeloblastic leukemia (AML) cells usually requires the addition of colony-stimulating factors (CSFs) and interleukins to the culture media[1] and only in a few cases of AML is the requirement of exogenous growth factors overcome by the ability of the same blasts to secrete the molecules they need to proliferate.[2] These observations suggest that growth factors are necessary for the expansion of the leukemic clones in vitro and probably also in vivo. In the past few years, the study of leukemogenesis took great advantage of the availability of several continuous cell lines that have been invaluable tools for studying leukemic as well as normal hematopoiesis.[3] However, unlike fresh leukemic cells, most AML cell lines are growth factor independent, probably as a consequence of additional genetic abnormalities acquired in vitro allowing their unrestricted proliferation even in the absence of growth factor supply. Recently, some new myeloid cell lines have been established that retain the original growth factor dependency for continuous growth and these cell lines have been of remarkable importance in cloning the genes of hematopoietic growth factors as well as in defining their mechanism of action.[4,5]

In this report, we describe the characteristics of a new granulocyte-macrophage CSF (GM-CSF)– and interleukin-3 (IL-3)–dependent human myeloid cell line (GF-D8) established in our laboratory from the peripheral blood of an 82-year-old man with acute myeloid leukemia. This cell line carries multiple chromosomal aberrations, including the inversion and interstitial deletion of chromosome 7. Because of all these findings, this cell line might contribute to the improvement of our knowledge of myeloid leukemogenesis and differentiation.

MATERIALS AND METHODS

Case history and cell culture. On April 4, 1989, an 82-year-old man was referred to our clinic from another hospital with a 3-month history of progressive fatigue, diffuse bone pain, and fever with a diagnosis of AML based on the following clinical features: white blood cell (WBC) count, 183×10^9/L (with 1% neutrophils, 3% lymphocytes, and 96% blasts); hemoglobin (Hb), 7.6 g/dL; and platelets (Plt), 76 $\times 10^9$/L. The patient did not receive any specific treatment before being admitted to our hospital. The diagnosis of AML was confirmed by morphology (M1 according to the French-American-British [FAB] classification), cytochemistry (the whole leukemic population was α-naphthyl-acetate positive, but only 20% of blasts were Sudan black positive), and immunophenotyping (CD13, CD33+, with the T- and B-cell antigens being negative). After having confirmed the diagnosis of AML (M1 according to the FAB classification) by morphology, cytochemistry, and immunophenotyping of bone marrow cells, with the patient's prior informed consent and with the approval from the Institutional Review Board for these studies, leukemic blasts were obtained from peripheral blood samples and purified by density-gradient centrifugation on Ficoll-Hypaque. The cells were seeded in Falcon plastic tissue culture flasks (Falcon, Becton Dickinson, Oxnard, CA) at 10^6/mL in 10 mL of RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 20% fetal calf serum (FCS; Hyclone, Sterile System Inc, Logan, UT) and 50 ng/mL of human recombinant GM-CSF (Genzyme, Inc, Boston, MA; specific activity 5×10^7 proliferation units/mg of protein) and were incubated at 37°C with a humidified atmosphere of 5% CO_2 in an incubator. At the beginning, cells were fed once weekly by partial replacement of spent medium with the fresh medium and GM-CSF.

Immunophenotype analysis. The surface immunophenotype of GF-D8 cells was assessed by indirect immunofluorescence using a

FACScan analyzer (Becton Dickinson, Mountain View, CA) and the following panel of monoclonal antibodies (MoAbs): anti-HLA-Dr, Leu HPCA1-CD34, Leu1-CD5, Leu9-CD7, Leu14-CD22, Leu IL-2R–CD25, and LeuM5-CD11c (Becton Dickinson); T11-CD2, T3-CD3, T4-CD4, and T8-CD8 (kindly provided by Dr S. Schlossmann, Dana Farber Cancer Institute, Boston, MA); J5-CD10, B4-CD19, B1-CD20, B2-CD21, and Plt1-CD41 (Coulter Immunology, Hialeah, FL); antiglycophorin (clone 39a3), Mo1-CD11b, My4-CD14, My7-CD13, My9-CD33, PM81-CD15, LAM-1, and clone 3C11C8 (reacting with human γ-interferon, used as negative control) were generously provided by Dr J. D. Griffin (Dana Farber Cancer Institute). MoAb to ICAM1-CD54 was a gift from Dr D. Haskard (Guy's Hospital, London, UK). MoAb to VLA 4-CD29-49d was a gift from Dr F. Sanchez (University of Madrid, Madrid, Spain). The secondary reagent was an affinity-purified fluorescein isothiocyanate (FITC)-labeled goat antimouse Ig antiserum (Technogenetics, S. Mauro Torinese, Italy). Nuclear terminal deoxynucleotidyl transferase (TdT) enzyme was assessed by fixing cytocentrifuged cell preparations in cold methanol for 30 minutes and staining by a rabbit anti-TdT antibody, followed by FITC-F(ab)$_2$ goat antirabbit IgG (both from Technogenetics).

Morphologic, cytochemical, and electron microscopic studies. Cytocentrifuged smears were stained with May-Grünwald-Giemsa (MGG) and various other stainings including: α-naphthyl-butirate esterase (α-NBE) and α-naphthylacetate esterase (α-NAE) with or without inhibition with sodium fluoride (NAaF), myeloperoxidase (MPO), leukocyte alkaline phosphatase (LAP), acid phosphatase (AcP), and Sudan Black (SB). For electron microscopy, cells were fixed for 30 minutes at 4°C with 1.25% glutaraldehyde in 0.1 mol/L phosphate buffer and cacodylate buffer, washed and postfixed in osmium tetroxide, embebbed in Epon resin, stained with uranyl acetate and lead citrate, and examined with a Zeiss 109 electron microscope.

Cytogenetic analysis. Chromosomes were analyzed using standard techniques and Giemsa Trypsin G (GTG) banding.

Clonal growth in semisolid culture. GF-D8 cells at a concentration of 2×10^3/mL were cultured in Iscove's Modified Dulbecco's Minimal Essential Medium (IMDM; GIBCO) supplemented with 20% FCS and 0.33% final concentration of agar (Noble Agar; DIFCO, Detroit, MI) by modification of a standard technique.[6] To evaluate the effect of growth factors on the plating efficiency of the cell line, various concentrations of GM-CSF, IL-3, stem cell factor (SCF), or medium alone were added and the culture mixture (1 mL) plated in triplicate in 35-mm Petri dishes (Falcon). After 11 days of incubation at 37°C in a fully humidified atmosphere of 5% CO_2 in air, GM colonies (>50 cells per aggregate) and clusters (<50 cells per aggregate) were scored using a dissecting microscope (40× magnification).

Proliferation studies. For proliferation studies, GF-D8 cells were cultured for different periods of time in 96-well round-bottom microtiter plates in the presence or absence of different concentrations of recombinant cytokines at 2.5×10^5/mL in RPMI 1640 medium, supplemented with 2 mmol/L L-glutamine and 20% FCS. Cells were pulsed with 0.5 mCi of ^{3}H-thymidine (specific activity, 25 Ci/mmol; Amersham, Buckinghamshire, UK) 12 hours before harvesting.

The following human recombinant cytokines were evaluated for their ability to promote GF-D8 proliferation: GM-CSF and macrophage-CSF (M-CSF; Dr S. Clark, Genetics Institute, Cambridge, MA); IL-3 (Dr D. Krumwiech, Behrinwerke, Marburg, Germany); granulocyte-CSF (G-CSF; Dr L. Souza, Amgen, Thousand Oaks, CA); SCF (Genzyme, Cambridge, MA); IL-1β (Dr D. Boraschi, Sclavo, Siena, Italy); IL-2 (Biogen, Geneva, Switzerland); IL-4 (Dr S. Gillis, Immunex, Seattle, WA); and tumor necrosis factor-α (TNF-α; Dr G. Adolf, Ernst Beerhinger Institute, Vienna, Austria).

Molecular studies. Southern and Northern blot analyses were performed as previously described.[7,8] Densitometric analysis was performed using a GS-300 Transmittance-Reflectance scanning densitometer (Hoefer Scientific Instruments, San Francisco, CA). The reverse polymerase chain reaction (PCR) technique was applied to demonstrate specific transcripts for the G-CSF receptor. To examine which messenger RNA (mRNA) of the G-CSF receptor were expressed by GF-D8 cells, experiments were performed according to the method described by Fukunaga et al.[9] Based on the published sequence, two oligonucleotide primers were derived from positions 2086 to 2105 and 2322 to 2303. A cDNA was synthesized directly in the amplification microtube by using 1 μg of total denatured RNA in a 20 μL reaction mixture containing 10 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 5 mmol/L $MgCl_2$, 1 mmol/L dNTPs, 50 U reverse transcriptase (Perkin Elmer-Cetus, Norwalk, CT), 20 U RNase inhibitor (Perkin Elmer-Cetus), and 15 pmol of an antisense primer. Each sample was then diluted with 80 μL of PCR mixture containing a final concentration of 2 mmol/L $MgCl_2$, 50 mmol/L KCl, 10 mmol/L Tris-HCl, pH 8.3, 2.5 U of Taq DNA polymerase (Perkin Elmer-Cetus), and 15 pmol of specific primers. PCR was performed on an automated heat block (PCR system 9600; Perkin Elmer-Cetus). After an initial denaturation at 95°C for 1 minute, denaturation was performed at 95°C for 10 seconds; annealing and extension were performed at 60°C for 15 seconds, followed by one more cycle of final extension at 60°C for 6 minutes. Finally, 30 μL of PCR mixture was run on a 3% Nusieve agarose gel stained with ethidium bromide and visualized under a UV lamp. In selected experiments, 10 μL of the PCR products fractionated by electrophoresis through a 1.5% agarose gel was transferred to nylon membranes (Genescreen Plus; New England Nuclear, Boston, MA), prehybridized, hybridized, and washed according to manufacturer's instructions.

DNA probes. The cDNAs for GM-CSF, G-CSF, M-CSF, and IL-3 inserted in the *Xho* site of the pXMT 2 vector[5] and for IL-1β were generously supplied by Dr S. Clark (Genetics Institute). The cDNA for the α chain of human GM-CSF receptor (clone pGMR138) was kindly provided by Dr N. A. Nicola (The Walter and Eliza Hall Institute of Medical Research, Victoria, Australia).[10] A cDNA for the α chain of the IL-3 receptor was cloned in our laboratory by PCR using primers derived from positions 359 to 381 and 981 to 1003 of the published sequence.[11] The cDNA for the β chain of the GM-CSF receptor (clone pkH97) was generously provided by Dr A. Miyajima (DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA)[12]; the cDNA for G-CSF receptor (clone D7) was kindly given by Dr S. Gillis (Immunex).[13] To determine an internal standard of total RNA content, membranes were rehybridized with a mouse α-actin cDNA obtained from the American Type Culture Collection (ATCC; Rockville, MD). A genomic probe for the second exon of human c-*myc* (plasmid MC413 RC) was obtained from Dr P.G. Pelicci (University of Perugia, Perugia, Italy).[14] The organization of the T-cell receptor (TCR) β and δ genes was studied with an *Eco*RI cDNA fragment hybridizing to both alleles (c$β_1$ and c$β_2$) of the constant region of the β chain[15] and with MH6, a 5.0-kb *Eco*RI fragment that lies between Jδ1 and Jδ2.[8] TCR probes were provided by Dr T. Mak (Ontario Cancer Institute, Toronto, Canada). Ig gene arrangement was investigated using a *Bam*HI-*Hind*III probe from the joining region of the IgH locus (J$_H$), kindly provided by Dr P.G. Pelicci. A 3.4-kb *Bam*HI fragment of Epstein-Barr virus (EBV) DNA (FF41)[16] was obtained through the courtesy of Dr M. Minden (Ontario Cancer Institute). Probes were labeled to a specific activity of 10^9 cpm/μg by using hexanucleotide primers and ^{32}P-dCTP.[17]

Radioreceptor binding experiments. Cells were washed twice in phosphate-buffered saline (PBS), once in binding buffer (PBS, 0.1% bovine serum albumin [BSA], and 0.02% sodium azide), and resus-
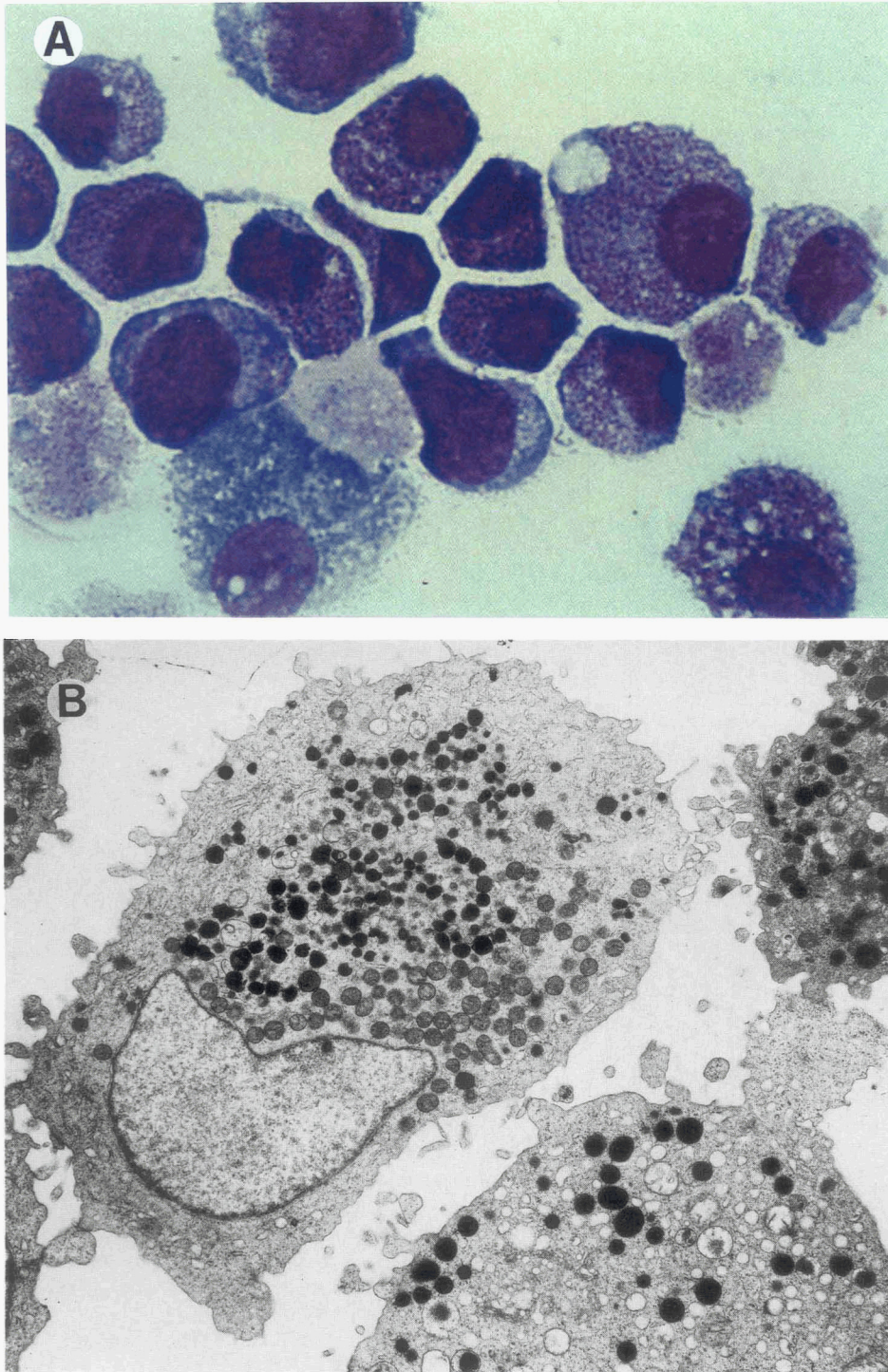
Fig 1. (A) Cell morphology of the GF-D8 cell line. A cytocentrifuged preparation stained with MGG is shown. Note the presence of multiple cytoplasmic granules. (B) Electron photomicrograph of GF-D8 cultured cells showing an eccentric nucleus and cytoplasm containing numerous mitochondria, primary granules, and empty vesicles (original magnification × 3,000).

pended in the binding buffer. Cells (1 to 2 × 10^6) were seeded in round-bottomed 96-well plastic plates containing various amounts of labeled CSFs with or without a 200-fold molar excess of unlabeled cytokines, with a final volume of 100 μL. After 1 hour of incubation at room temperature, separation of bound from free labeled CSFs was performed by centrifugation on a cushion of 20% saccharose-1% BSA. Specific binding was defined as the difference between the amount of radioactivity bound in the absence of unlabeled CSFs and that in the presence of excess unlabeled cytokines. For Scatchard analysis, parameters were generated by the ligand program.[18] ^{125}I–GM-CSF (1,200 Ci/mmol) and ^{125}I–IL-3 (520 Ci/mmol) were from Amersham. Human recombinant G-CSF was iodinated using the Iodo-gen reagent (Pierce, Rockford, IL) according to the manufacturer's instructions.
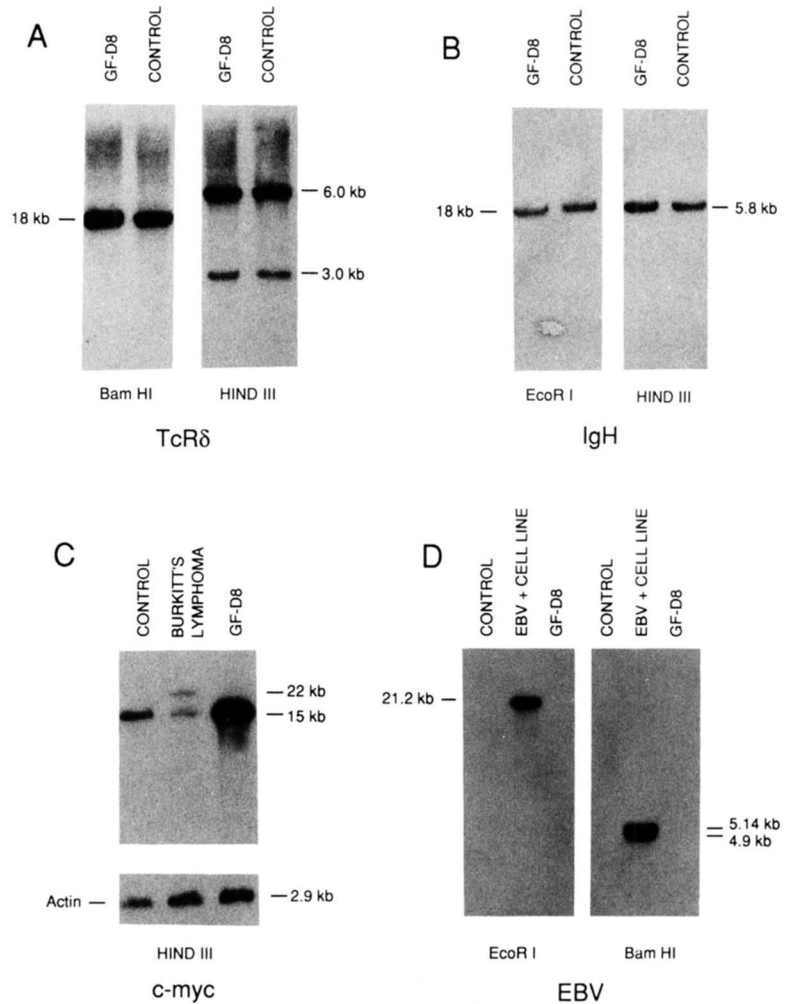
Fig 2. Southern blot analysis of GF-D8 cells showing the germline configuration of the δ chain of the T-cell receptor gene (A) as well as of the Ig heavy chain (B). The amplification of the c-myc gene and the lack of EBV DNA integration in GF-D8 cells are presented in (C) and (D), respectively.

RESULTS

Establishment of the GF-D8 cell line. After 4 weeks of initial culture, proliferation was noted. When growth became stable, the cells were cloned by a limiting dilution method and the GF-D8 clone was obtained. This clone as well as the parent cell line grow in single-cell suspension without forming cell clumps and with a doubling time of 48 to 72 hours in the presence of GM-CSF.

Morphology, cytochemistry, and immunophenotype. The morphologic appearance of GF-D8 cell line is consistently that of immature poorly differentiated myeloid blasts. The cells are usually round in shape with a basophilic cytoplasm, full of azurophilic granules with few vacuoles and no Auer rods; the nucleus is large, round, or slightly irregular (Fig 1A). A low percentage of giant cells is present. Cells are strongly positive for α-NAE and become almost negative after NAaF treatment. Cells are also positive for SB and AcP, but are negative for α-NBE and LAP. Only a small amount of MPO-positive cells can be seen. Electron microscopy shows most of the cells have a single, large, central, or eccentric nucleus with a single nucleolus and a low to moderate chromatin condensation. In many cells the nucleus is irregularly convoluted or lobed. The great majority of cells contain numerous developed primary granules in the cytoplasm, associated with vacuoles filled with low density material, predominant in some cells. Further, dense haloed structures are intermingled with the primary granules in a significant number of cells (Fig 1B). The immunophenotype analysis of patient's leukemic cells at diagnosis and GF-D8 cells was superimposable, showing a strong positivity only for myeloid-specific antigens (CD13 and CD33). All the erythroid-, platelet-, and lymphoid-associated antigens were always found to be negative. Among the adhesion molecules analyzed, GF-D8 showed some positive staining for only CD11a and CD11b.

Cytogenetic studies. At diagnosis, the patient's leukemic cells had the following karyotype: 44 XY, 5q−, 7q−, 8q+, 11q+, −15, −17. After 30 months of culture, the GF-D8 cell line still retained a near diploid chromosomes number. The consensus karyotype found in GF-D8 cells is: 45 XY, −5, del(7) (q32qter), inv(7) (q31.2q36), 8q+, +8q+, 11q+, 12p−, −15, −17+ marker chromosome.

DNA analysis. To demonstrate the lack of lymphoid commitment, the configuration of TCR-δ and IgH loci was
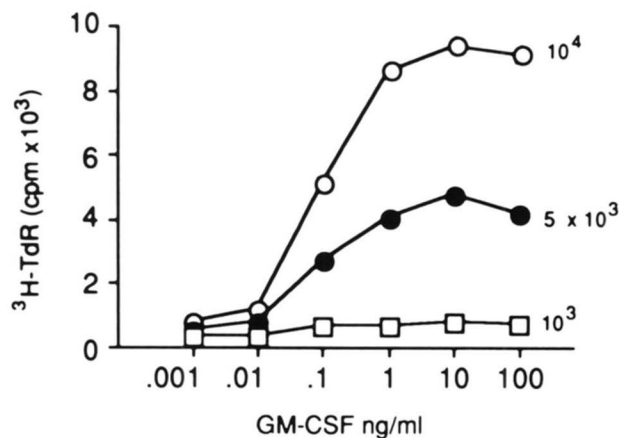
Fig 3. A representative experiment showing the proliferative response of different numbers of GF-D8 cells to GM-CSF. One thousand cells (□), 5×10^3 cells (●), or 10^4 cells (○) were cultured for 72 hours in the presence of the indicated concentration of GM-CSF. ^{3}H-TdR was added during the last 12 hours of cultures. Results are expressed as mean cpm of triplicate cultures. SD was always ≤ 2%.

Fig 4. Detection of human G-CSF receptor mRNA by PCR analysis. Total RNA (1 μg) from GF-D8 cells, the monoblastic leukemia cell line U937, and human placenta was amplified by the PCR method. The amplified products (10% of the reaction mixture) were analyzed on a 1.8% agarose gel. For size markers, *Msp* I-digested DNA from PBR322 was electrophoresed in parallel and sizes of the amplified DNA fragments are given in base pairs.

studied. As shown in Fig 2A, GF-D8 had conserved either for TCR-δ and IgH both alleles in the germline configuration. For the karyotypic abnormalities involving chromosome 8, the presence of c-*myc* amplification was investigated by Southern blot (Fig 2C). After normalization with the actin gene, scanning of the autoradiograms showed that the amount of c-*myc* DNA in GF-D8 cells was 4.3-fold more abundant than in control cells. Southern blot hybridization showed no bands specific for EBV (Fig 2D).

In vitro proliferation of GF-D8 cells is GM-CSF and IL-3 dependent. GF-D8 cell line was established in the presence of recombinant GM-CSF. In several experiments we tested the ability of GF-D8 cells to respond to several other recom-

Table 1. Plating Efficiency of GF-D8 Cells in Response to Hematopoietic Growth Factors

Stimulus	Colonies
Control*	5
5637-CM†	230
GM-CSF (ng/mL)	
0.01	73
0.1	89
1	193
10	210
IL-3 (ng/mL)	
0.01	85
0.1	165
1	144
10	164
SCF (ng/mL)	
0.1	36
1.0	35
10	35
100	20

Colonies (more than 50 cells) per 2×10^3 GF-D8 (mean of 3 cultures, SE of mean was less than 10% in each point).

* The control was IMDM containing 20% fetal bovine serum.

† 5637-CM was used at a final concentration (vol/vol) of 10%.
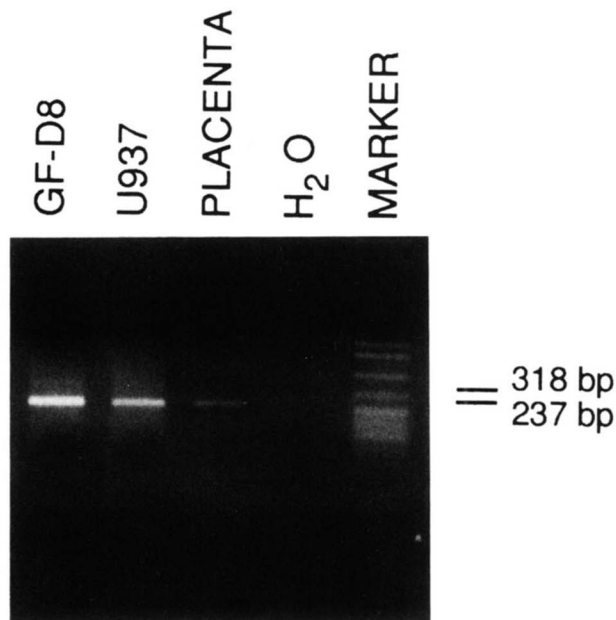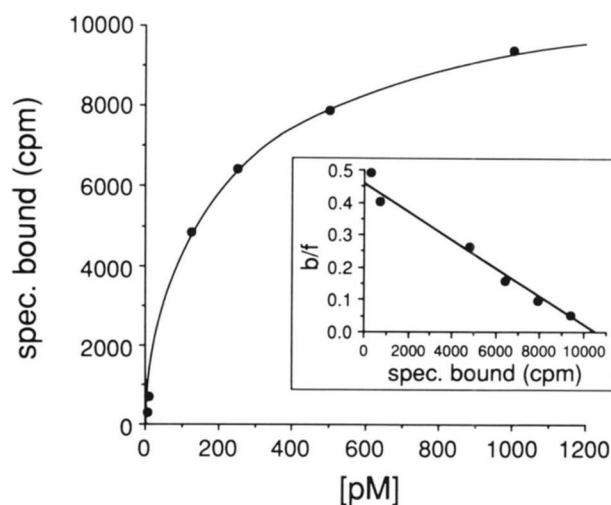

Fig 5. Equilibrium binding of ^{125}I–GM-CSF. GF-D8 cells ($\times10^6$) were incubated with ^{125}I–GM-CSF for 1 hour at room temperature. Data are on the specific bound cytokine in the presence of a 200-fold molar excess of unlabeled cytokine. Insert shows the Scatchard representation of specific binding.
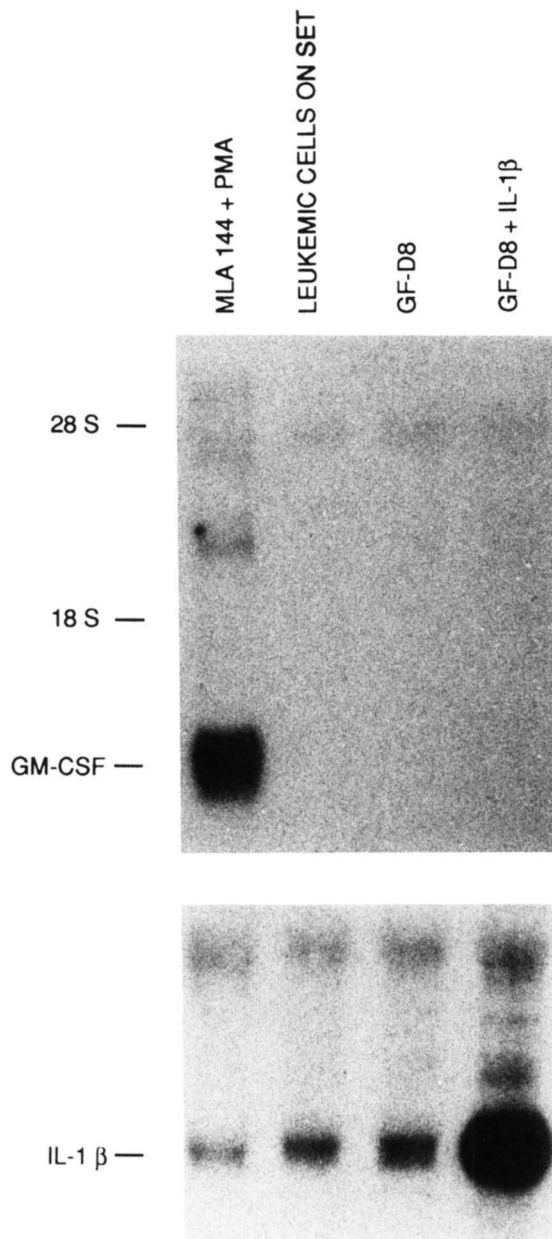
Fig 6. Expression of GM-CSF and IL-1β mRNA in the patient's leukemic cells at diagnosis and in GF-D8 cells in basal conditions and upon stimulation with IL-1β (100 U/mL final concentration). RNA from the gibbon lymphoma cell line UCD-MLA-144 stimulated with PMA (20 ng/mL) was used as the positive control.

proliferation even in the absence of serum, although with a slightly decreased efficiency (data not shown). Various chemical agents have been reported to be highly potent for inducing cell differentiation in myeloid leukemic cell lines. Specifically, it has been shown that phorbol myristate acetate (PMA) promotes monocyte-macrophage differentiation. In the presence of PMA (1 ng/mL) and after 48 hours of exposure, GF-D8 cells rapidly undergo monocyte-macrophage differentiation, as assessed by morphology and plastic adherence (data not shown).

Clonal growth in semisolid medium. The ability of various concentrations of GM-CSF, IL-3, and SCF to simulate the colony formation of the GF-D8 cell line is shown in Table 1. In the absence of exogenous growth factors, GF-D8 cells are unable to form colonies in agar. However, a clear clonogenic ability is evident in the presence of recombinant GM-CSF and IL-3 in a dose-dependent feature. The dose-response curve of the cells to increasing concentrations of IL-3 shows the maximal effect for 0.1 ng/mL because an increasing concentration does not improve the clonogenicity. Similarly, 5637-conditioned medium, which is a known source of hematopoietic growth factors, induced a significant colony growth. SCF induced a weak, although significant, colony growth, but without a dose-dependent response.

Expression of GM-CSF, IL-3, and G-CSF receptors in GF-D8 cells. Using Northern blot analysis we could demonstrate that GF-D8 cells express specific mRNAs for both the α chains of the GM-CSF and IL-3 receptors. Similarly, GF-D8 showed the 5.3-kb transcript for the β chain common to both the GM-CSF and IL-3 receptors (data not shown). Analysis of G-CSF receptor transcripts was performed by PCR analysis, as indicated by Fukunaga et al.[9] With a set of primers able to amplify the corresponding splicing of the class I and III human G-CSF receptor gene, we detected in GF-D8 cells the expected 237- and 318-bp amplified fragments (Fig 4). We then examined by radioiodinated cytokine binding experiments the expression of cytokines on the cell surface. GF-D8 cells were found to express specific binding sites for GM-CSF, G-CSF, and IL-3. Figure 5 shows a representative experiment (of three performed with similar results) in which the binding of ^{125}I–GM-CSF has been examined. Scatchard analysis showed 1,675 ± 105 binding sites/cell with a kd of 244 ± 75 pmol/L. The analysis of ^{125}I–G-CSF binding (two experiments) demonstrated the presence of 267 ± 109 binding sites/cell with a kd of 1.9 nmol/L. When IL-3 has been evaluated, specific binding was detectable but not accurately quantitated due to low levels of receptor expression.

Constitutive gene expression and production of IL-1β by GF-D8 cells. In the majority of AML cases, secretion of IL-1β has been reported and proposed to play a role in leukemic cell proliferation. To evaluate whether autocrine secretion of IL-1β was present in GF-D8 cells, Northern blot analysis was performed. As shown in Fig 6, no evidence of GM-CSF gene transcription could be observed under any culture conditions. On the contrary, the patient's leukemic cells purified at diagnosis as well as GF-D8 cells express a significant IL-1β transcript further enhanced after exposure of GF-D8 cells to IL-1β itself. By a specific radioimmunoassay[19] (with a detection limit of 10 pg/mL, using rabbit poly-

binant growth factors, including IL-3, G-CSF, M-CSF, IL-1β, IL-4, IL-5, IL-6, and TNF-α. However, apart from IL-3, none of the above cytokines showed any significant proliferative effect on GF-D8 cells. As shown in Fig 3, GM-CSF–induced proliferation was dose dependent and a significant proliferative activity could be observed at concentrations as low as 0.1 ng/mL up to 10 ng/mL. There was no further induction in proliferation using higher doses of either GM-CSF or IL-3. GM-CSF, as well as IL-3, induced a significant

clonal antibody against human IL-1β; kindly provided by Dr Paolo Ghiara, Siena, Italy), a range of 50 to 100 pg/mL of IL-1β was detected in culture supernatants of GF-D8 cells in three different experiments. Because the active secretion of IL-1β has been described to play an important role in supporting the proliferation of AML cells, we tested the effect of IL-1ra[20] and polyclonal antibodies against IL-1β (kindly provided by Dr P. Ghezzi, Istituto M Negri, Milano, Italy) on GF-D8 cell growth in vitro. However, no significant inhibition of the GM-CSF–induced proliferation was found (data not shown).

DISCUSSION

Several human AML cell lines have been established in culture and they have proven to be a very important tool for the study of myeloid differentiation. However, only a few of them were shown to be growth factor dependent for their continuous growth in vitro.[4,21-24] This property has been of great value in the defining and cloning of several hemato-poietic growth factors[5] and in the defining of multiple aspects of the CSFs' biology. A new acute myeloid leukemia cell line with several peculiar characteristics was established in our laboratory. This cell line is strictly factor dependent for growth in liquid as well as in semisolid culture medium. We have studied the effect of several recombinant cytokines on the in vitro proliferation of GF-D8 cells and found that only GM-CSF and IL-3, alone or in combination, have colony-stimulating activity. The CSFs are not simply proliferative stimuli for responsive cells because, invariably, they promote irreversible differentiation with cellular maturation and stimulation of functional activity. As far as judging from morphology, cytochemistry, and immunophenotype, GF-D8 cells appear to have properties of early myeloid progenitor cells and none of these characteristics were changed in response to IL-3 and GM-CSF. On the contrary, incubation with PMA induced a rapid monocyte-macrophage terminal differentiation. The GM-CSF and IL-3 dependency for in vitro growth is similar to those reported earlier for other cell lines and is consistent with the observation that GF-D8 cells express receptors for both cytokines. As previously demonstrated for other factor-dependent lines, GF-D8 can be used as a useful laboratory tool in demonstrating GM-CSF or IL-3 biologic activity.[25] Indeed, in our experimental conditions, this cell line was demonstrated to respond and proliferate to as low as 0.1 ng/mL when the cells are plated in culture at the appropriate cell density. Of special interest is the observation that the cell proliferation can be observed in vitro even in the absence of serum, thus confirming the specific response to GM-CSF and IL-3. The molecular analysis of GM-CSF and IL-3 receptor genes allowed to demonstrate the presence of the specific transcripts for both. However, using PCR analysis, we could also demonstrate specific G-CSF receptor mRNAs, thus suggesting that the lack of biologic response to G-CSF does not reflect the absence of the receptor gene transcription. The selective response of GF-D8 cells to GM-CSF and IL-3 can be used to investigate specific molecular and biochemical pathways of myeloid cell differentiation and proliferation. We also showed evidence of active IL-1β gene transcription and production in GF-D8 cells. Several reports

have proposed IL-1β as a key molecule in regulating paracrine and autocrine pathways of AML proliferation. However, in this cell line, despite an active secretion of IL-1β, proliferation could not be observed spontaneously nor in response to exogenous IL-1β. However, it is possible that autocrine secretion of IL-1β can allow or facilitate the proliferative response of GF-D8 cells to GM-CSF and IL-3. The lack of significant response to G-CSF, despite the presence of G-CSF receptors on the cell surface, is of some interest, although it is not surprising because other human myeloid leukemia cell lines (ie, U937) present a similar feature.[26]

Cytogenetic analysis of GF-D8 cells showed an abnormal karyotype, and the original leukemic cells were also found to have an abnormal karyotype, although partially different than that of the established cell line. The loss and duplication of chromosomes (as far as chromosome 5 and 8 in GF-D8 cell line) appears to be a common feature of human cell lines carried in culture for a long time. The deletion of chromosome 7 (q32-qter) is a consistent abnormality found at the time of leukemia onset as well as in the GF-D8 cell line. Cytogenetic analysis of patients with myelodysplastic syndromes and AML shows many partial deletions of the long arm of chromosome 7.[27-30] In this context, GF-D8 could become a useful tool for the molecular identification of the deleted region.

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