A new human myeloid cell line has been established recently from the bone marrow cells of a patient with chronic myelogenous leukemia in blast crisis. The active proliferation and survival of the cells in RPMI 1640 medium containing fetal calf serum are clearly dependent on the presence of either natural or recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF). Despite permanent culturing in rhGM-CSF (100 U/ml), the cells do not differentiate and bear the myelomonocytic surface markers CD34, CD13, CD36, as well as HLA-DR, but not CD3, CD7, CD10, CD11b, CD14, CD20, or CD42b. The predominant karyotype, apart from tetraploidy in several cells, is 45, XX,-9,-17,-19,-22, 7p-,-9q+ (der t[9;22]), der (13)q, with three additional marker chromosomes, from which one was observed in the patient’s leukemic cells. On BglII-digested DNA, Southern blot analysis with bcr 5’ as the probe detected two additional hybridizing restriction fragments of 8.6 and 11.0 kilobase pairs.

**CASE REPORT**

A 50-year-old female patient (G.M.) was referred to our clinic from another hospital due to a blast crisis of a CML, which had been diagnosed in February 1986.

**MATERIALS AND METHODS**

**Bone marrow cells.** With the patient’s prior informed consent, marrow cells were obtained by means of iliac crest puncture. Mononuclear cells were separated by density-gradient centrifugation on Ficoll (Nycomed AS, Oslo, Norway) and cultured in RPMI 1640 medium with 10% fetal calf serum (FCS) and growth factors. Part of the cells were preserved by freezing in liquid nitrogen with 10% dimethylsulfoxide (DMSO) and RPMI 1640 medium containing 40% FCS, and these were used later for karyotype analysis.

**Cytokines.** Recombinant human G-CSF (rhG-CSF), rhGM-CSF, rhIL-3, rhIL-4, and rhIL-6 were provided by L. Souza (AMGen, Thousand Oaks, CA), whereas rhIL-1α and rhIL-2 were obtained from Boehringer-Mannheim (FRG).

**Antibodies.** Rabbit anti-GM-CSF antiserum was also a gift from L. Souza (AMGen). Monoclonal antibodies (MoAbs) against CD34 (HPCA-1), CD7 (Leu 9), CD10 (CALLA), CD16 (Leu 11b), CD20 (Leu 16), HLA-DR, and fluorescein-conjugated goat anti-mouse were purchased from Becton-Dickinson (Mountain View, CA); CD36 (OKM 5), CD11b (OKM 1), CD13 (OKM 13), CD14 (OKM 14) and CD3 (OKT 3) from Ortho-Diagnostic System (Neckargemünd, FRG); and CD42b (AN 51) from Dakopatts (Hamburg, FRG).
Proliferation assay. After washing the cells three times in phosphate-buffered saline (PBS), the cells (1 × 10^6 cells/well) were incubated for 72 hours in flat-bottom 96-well microtiter plates in a final volume of 200 μL RPMI 1640 medium containing 10% FCS and various cytokines. \(^{1}\)H-thymidine (Amersham, Braunschweig, FRG) was added at 1 μCi (37 kBq) per well for 4 hours before the end of the culture. The cells were then harvested on fiberglass filters and measured by liquid scintillation counting. All assays were performed in triplicate and data are expressed as the mean counts per minute of \(^{1}\)H-thymidine incorporated.

Immunophenotyping. Cell surface antigens were detected by standard indirect immunofluorescence assay. In brief, the cells were incubated at 4°C for 30 minutes with mouse MoAbs, washed three times by centrifugation with PBS, followed by adding FITC-conjugated goat anti-mouse antibody for 30 minutes at 4°C. Stained cells were then evaluated with a UV-microscope. The cells were considered positive for the antigen in question when the fluorescence intensity on intact cells was clearly visible in comparison with the cells treated only with FITC-conjugated goat anti-mouse antibody.

Chromosome analysis. Karyotyping of GM/CSF cells and cryopreserved bone marrow cells was performed by standard techniques, including Giemsa-trypsin banding.

Southern blot analysis. High molecular weight DNA from cells was digested with BglII, electrophoresed on a 0.8% agarose gel, and blotted onto a nylon membrane (Genescreen Plus; NEN, duPont de Nemours, Bad Homburg, FRG). The bcr-5 probe (provided by C.R. Bartram, Ulm, FRG) was labeled by nick-translation and hybridized according to standard protocols.

Staining for microscopy. May-Grünwald and cytochemical stainings for PAS, myeloperoxidase, and α-naphthyl acetate esterase were performed in our routine hematology laboratory.

RESULTS

Establishment and cellular characteristics. Initially, bone marrow mononuclear cells were cultured in the presence of rhG-CSF, rhGM-CSF, and IL-3 (1,000 U/mL each) with the intention of studying the differentiation capacity of the blast cells. As usual, the culture medium used was RPMI 1640, supplemented with 10% FCS, 0.2 mmol/L L-glutamine, and antibiotics. Within the first 2 weeks there were no differences to be detected, either in proliferation rate or in maturation of the cells in the presence of these three hematopoietic growth factors. However, after several passages an active proliferation was sustained only by the cells exposed to rhGM-CSF. In subsequent steps, three stable subclones were established by using limiting dilution tech-
nique. These subclones differ in their morphology and generation time, but they still retain their requirement for external supply of rhGM-CSF. The dependency can be circumvented if the cells are cultured by carefully reducing GM-CSF for 2 or more weeks. As a consequence, cells that have once become independent do not require GM-CSF for their survival and proliferation, whereas they sustain to respond to GM-CSF with increased proliferation (data not shown). To avoid overly complex data, only the clone termed as GM/SO is discussed in this report.

Although there is a considerable variation in cell size, the typical morphology of GM/SO cells in May-Grünwald staining is a large, round, or slightly irregular nucleus, three to six prominent nucleoli, and deep blue cytoplasm with a perinuclear pale zone (Fig 1A). There is no granula present and the cytochemical staining for PAS, myeloperoxidase, and α-naphytol acetate esterase are negative. Moreover, the cells growing in 100 U/mL rhGM-CSF (unless otherwise stimulated) retain their morphology and display no overt differentiation in liquid culture. However, a rapid change

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**Fig 2.** Proliferative response of GM/SO cells to rhGM-CSF, rhG-CSF, rhIL-1α, rhIL-2, rhIL-3, rhIL-4, or rhIL-6 either alone at concentrations between 1 and 1,000 U/mL or in combination with 1 U/mL rhGM-CSF; measurement after 72 hours. The neutralizing antibody was tested in 1:40 final dilution, and the curves represent the mean values of four separate experiments.

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**Fig 3.** Stemline karyotype of line GM/SO: 45, XX, −9, −17, −19, −22, 7p−, 9q+ der t(9;22) (q34;q11), der (13q), plus three marker chromosomes (M1, M2, and M3) that could not be exactly classified.
into a monocyte-macrophage morphology and increased adherence to plastic surface can be induced by the addition of phorbol myristate acetate (PMA) (Sigma, St Louis, MO) at concentrations between 1.00 ng/mL and 1 ng/mL (Fig 1B).

Immunophenotype analysis showed that the GM/SO cells clearly bear surface antigens of myelomonocytic cells CD34 (greater than 75%), CD13 (greater than 96%), and CD36 (greater than 92%), but not the other typical granulocyte-macrophage antigens such as CD11b, CD14, or CD16. While HLA-DR (greater than 98%) is strongly expressed on the cells, lymphocytic markers such as CD3, CD7, CD10, CD20, and thrombocytic marker CD42b are also definitely absent.

As shown in Fig 2, the proliferative effect of GM-CSF on these cells is dose-dependent, and the saturating dose seems to be by 100 U/mL. However, by using a polyclonal rabbit antiserum against GM-CSF at neutralizing titers, the proliferation could be completely abolished in these assays. This inhibition apparently is not due to any toxic effect in antiserum, since the neutralizing effect could in turn be entirely suppressed by excess of rhGM-CSF in culture medium. In addition, we simultaneously tested rhG-CSF and rhIL-3, as well as rhIL-1α, rhIL-2, rhIL-4, and rhIL-6 to rule out any possible effect on this cell line. As the given examples demonstrate, there was no detectable proliferative effect of these cytokines at concentrations between 1 and 1,000 U/mL, either alone or in combination with 1 U/mL rhGM-CSF.

**Cytogenetic and DNA analysis.** To find out whether GM/SO cells possess the typical characteristics of a CML cell, eg. Ph' chromosome or bcr-abl rearrangement, we analyzed the karyotype with a G-banding technique and examined the DNA hybridization with a bcr-5' probe on Southern blots. The stemline karyotype was determined as 45, XX, -9, -17, -19, -22, 7p-, 9q+ (der §[9;22] [q34;q11]), der (13q), and three additional marker chromosomes (Fig 3). Since various numerical chromosomal anomalies were observed in the bone marrow blast cells, at least one of these markers (M1), together with the 9q+ chromosome, seems to be characteristic for the patient's leukemic transformation because it was also present in all metaphases of the bone marrow cells freshly frozen during the blast crisis. In the course of establishment, an increasing rate of tetraploid karyotypes was found in subsequent analyses. Southern blot analysis of BgIII digested DNA with bcr-5' as hybridization probe showed two BgIII restriction fragments of 8.6 and 11.0 kilobase pair (kb) size, giving clear evidence for a rearrangement typical for CML (Fig 4, lane A). Additionally, the signal of the 8.6 kbp fragment, if compared to the 5.0 kbp, is many times stronger, and in this case indicates an amplification process. The occurrence of two aberrant fragments was observed in several blotting and hybridization experiments; thus, an artifact due to partial digestions is excluded.

**DISCUSSION**

The cell lines that were established in recent decades have become an essential part of our contemporary biomedical research. Their contributions to discovering various new diagnostic and therapeutic agents can actually be exemplified with the development of MoAbs, purification of cytokines, bioassays, and so forth. In this context, the factor-dependent cell lines, being an ideal subject for studying the physiologic relevance of the factor in question, are undoubtedly of particular interest.

In this report we describe a new human myeloid cell line GM/SO, of which survival and proliferation in vitro is primarily dependent on the presence of hGM-CSF, either natural or recombinant. Apart from excluding probable effects of rhIL-1α, rhIL-2, rhIL-3, rhIL-4, rhIL-6, and rhGM-CSF on GM/SO by testing them directly, we provided evidence by using a neutralizing antibody that the factor responsible for the detected proliferation is restricted to GM-CSF. In contrast to the reported synergistic effect of rhG-CSF, IL-6, IL-22, and IL-3 on leukemic blasts or normal CD34+ cells, respectively, we failed to observe such a synergism on GM/SO cells. rhM-CSF and IL-5 have not yet been assayed.

In addition, we confirmed through immunophenotyping
and differentiation induction that GM/SO cells possess exclusively myelogenous, but no lymphoid, features. This is indeed the major difference in comparison with the other cell lines reported to be dependent on the presence of external GM-CSF. Another characteristic to be noted is the additional second signal with bcr-5' probe in Southern blot analysis of DNA from GM/SO cells. This finding is actually consistent with the observation made by Bartram et al. They could also detect an additional novel 5' bcr-fragment in blast cells of a patient with CML, and they suggest that one of the two Ph1 had to be secondarily rearranged at the 5' bcr site.

In summary, the data presented herein suggest that the GM/SO cell line possesses a functionally intact receptor for hGM-CSF as well as some characteristics of its normal counterpart. Apart from the use for bioassay, it is likely that prospective studies on this cell line may contribute to the understanding of the physiologic consequence of the interaction between GM-CSF and myelogenous cells.

REFERENCES


