Biological Properties and Growth in SCID Mice of a New Myelogenous Leukemia Cell Line (KBM-5) Derived from Chronic Myelogenous Leukemia Cells in the Blastic Phase

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ABSTRACT

The establishment and the biological properties of a new leukemic cell line (KBM-5) derived from a patient in the blastic phase of chronic myelogenous leukemia are described. The cells exhibited multiple copies of the Philadelphia chromosome, and a high level of p210BCR-ABL kinase activity was detected with rabbit anti-Ab1 and anti-BCr (exon 3) peptide antisera. Use of specific primers and polymerase chain reaction followed by Southern blotting revealed that KBM-5 cells carried a bcr3-ABLII splice junction. While a normal BCR message was detected, no normal ABL message was found. The cells were phenotypically myeloid with monocytic differentiation. The high cloning efficiency in semisolid media was independent of the presence of exogenous colony-stimulating factors. In vitro exposure to inducers of differentiation, such as retinoic acid, dimethyl sulfoxide, or hemin, failed to influence the growth rate of the cells and their level of differentiation. KBM-5 cells are highly resistant to the antiproliferative action of recombinant α- and γ-interferons. Although sensitive to recombinant tumor necrosis factor α, they were completely resistant to natural killer cell action. KBM-5 cells constitutively expressed mRNA for tumor necrosis factor α but not for γ-interferon, other interleukins, or hematopoietic growth factors. The KBM-5 cells that were transplanted into SCID mice manifested metastatic potential and tissue invasiveness similar to the way leukemic cells in humans do. This new KBM-5 cell line represents a helpful model for examining in vitro and in vivo modulation of the growth and properties of leukemic cells by using biological and chemotherapeutic agents.

INTRODUCTION

In CML, the long-term prognosis is poor despite recent advances in biological therapy and chemotherapy (1–3). Although a fraction of patients can be cured with allogeneic bone marrow transplantation (4), in the majority of patients, the development of a blast phase heralds a terminal stage of the disease for which there is no effective treatment (5). The cellular processes that cause such a transformation are unknown. Once developed, the blastic phase of CML is highly resistant to therapy, including chemotherapy combinations and allogeneic bone marrow transplantation (4–6).

To develop new treatments, an understanding of the events of blastic transformation is necessary, which is best gained through the study of in vivo and in vitro preclinical models. Studying leukemic cell lines can help us to obtain this information. By manipulating these cell lines, we can study the mechanisms that regulate the proliferation and differentiation of leukemic cells as well as evaluate the response of the cells to chemotherapeutic and biological agents. Myeloid leukemia cell lines are relatively difficult to establish, and their availability is limited. It is difficult to generalize the validity of observations made from one particular cell line, because it may represent a selected subclone of leukemic cells that are able to continue growth in vitro. Most existing lines are also poorly characterized. Thus, there is a need to establish cell lines that have defined biological properties to (a) study the leukemic process and (b) test the efficacy of various treatment approaches.

The establishment of the new leukemic cell line (KBM-5) can be valuable for investigating transformation events, the production of autocrine and paracrine growth factors, the significance of additional chromosomal abnormalities, and the role of cellular oncogenes in leukemic transformation. Only a limited number of Ph+ cell lines have been established (7–17). Here we describe a new leukemic cell line, KBM-5, established from the peripheral blood of a patient in the blastic phase of CML. Because these cells were found to have a metastatic potential in SCID mice, their grafting in such animals creates a new human/animal model of the disease that could be used to compare in vitro with in vivo results and that could provide unique opportunities for preclinical in vivo studies on chemotherapeutic and biological agents potentially active in the blastic phase of CML.

MATERIALS AND METHODS

Case Report of the Patient from Whom the KBM-5 Cell Line Was Derived.

A 67-year-old female with a 2-year history of Ph+ CML and a recent rapid increase in peripheral WBC presented with a hemoglobin of 6.0 g/dl, a platelet count of 188 × 10^3/μl, and a WBC of 74 × 10^3/μl, 50% of which were blasts. The morphological and cytochemical characterization of the blasts was compatible with the myeloid blastic phase of CML. The patient was treated with 9 doses of 1-β-D-arabinofuranosylcytosine (3 g/m² for 2 h every 12 h). Although this treatment reduced circulating blast cells, on day 15 of therapy, the peripheral blast count rose again. At this time WBC were obtained for cultures from which the KBM-5 cell line was derived.

Culture of Leukemic Cells and Establishment of the Cell Line.

Mononuclear cells were separated by Hypaque-Ficoll density gradient sedimentation (1.077 g/cm³), 10 million cells were suspended in IMDM (GIBCO, New York, NY) with 25% FCS (GIBCO) and placed in 25-cm² plastic flasks (Corning Glass Works, Comin, NY). The cultures were maintained in closed boxes at 37°C in an atmosphere of 5% CO₂, 12% O₂, and 83% N₂. After a lag period of 3 weeks, the cells resumed proliferation and were split and reseeded, initially at a cell density of 5 × 10⁶/ml. After the tenth passage, the cell line was maintained by biweekly passages in IMDM supplemented with 15% FCS at an initial density of 3 × 10⁶/ml, but without the addition of conditioning factors. For clonogenic assays, the cells were cloned in IMDM supplemented with 15% FCS and 0.3% agar (Bacto Difco Agar) and plated in 35-mm Petri dishes at 1 × 10⁴ cells/ml/dish. After incubation for 7–8 days in a fully humidified atmosphere of 5% CO₂, 12% O₂, and 83% N₂, clones of more than 50 cells were counted using an inverted microscope.

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Amplification was carried out in a Thermocycler (Perkin-Elmer Cetus) for 30 cycles. The temperature profile, identical for all cytokine primers, was as follows: 1 min at 94°C for denaturation; 1 min at 58°C for annealing; 1 min at 72°C for primer extension. PCR products were separated on ethidium bromide-stained agarose gel (1.6% agarose; Pharmacia) and the size compared with molecular size standards (φX174 RF DNA/HaeIII; Gibco). The OKT3-stimulated mononuclear blood cells expressed all cytokines and served as a positive control for each set of primers to determine whether the PCR product was the correct size (36).

Amplification of Human DNA-specific Sequences (HLA-DQα) by PCR. DNA was prepared from fresh or frozen tissues (1–2 mg) or cells (1–2 × 10⁶) (37) after removal of RBC by lysis buffer. A portion (5 μl) of the DNA extract was used for amplification of DNA in the Thermocycler in a total volume of 50 μl with 1.25 units of Taq DNA polymerase under the following reaction conditions: annealing for 1 min at 55°C; extension for 1.5 min at 72°C; and denaturation for 1 min at 95°C. Previously published primers for α chain of HLA-DQα1 were used (38). Specific positive and negative controls were used for internal consistency in every PCR assay. The amplified product (usually one-fifth of the 50-μl volume) was separated by electrophoresis in 1.6% agarose gel and stained with ethidium bromide. The positive results were detected as a 225-base pair band.

Grafting of KBM-5 Cells into SCID Mice. Female C.B. 17 SCID mice were obtained from Bommice, Ltd., Denmark. They were kept in sterile conditions at the SCID animal facility of the Department of Immunology, Karolinska Institute, Stockholm, Sweden, without antibiotic prophylaxis. They were in good health with NK activity close to zero, indicating pathogen-free conditions. There were no electrophoretically detectable immunoglobulin levels in their plasma.

KBM-5 cells (1 × 10⁷) from Mycoplasma-free, exponentially growing cultures were injected into animals using s.c. (2 animals), retroorbital (2 animals), i.p. (2 animals), or i.v. (2 animals) routes. The animals were not conditioned prior to or after grafting. Animals were observed twice daily for signs of disease and killed when they became terminally ill, at which time the blood was also obtained. Postmortem examination detected the presence of macroscopic tumors and splenomegaly and samples of tissues were collected so that the histology and presence of human DNA in the tissues could be determined.

RESULTS

Establishment of the Cell Line. The search for optimal culture conditions using various supplementations of IMDM did not result in an advantageous growth in any of the conditions tested. Between weeks 3 and 4 in culture, the cells resumed growth and could be split to grow at a high density (5–7 × 10⁶/ml). After the tenth passage, the cells were diluted to an initial density of 3 × 10⁵/ml. In medium supplemented by 15% FCS, the cells grew with a doubling time between 24 and 60 h, depending on the passage, to a saturation density of 1.5–2.0 × 10⁶/ml. The cells grew in suspension as single, round, nonadherent cells. Characterization of exponentially growing, acridine orange-stained KBM-5 cells with flow cytometry revealed a cell population with a DNA index of 1.0 and a high RNA content (data not shown). Different passages of the cell line, designated KBM-5, were frozen at regular intervals. The cell line was kept free of Mycoplasma.

Morphology and Cytochemical Profile of Fresh Leukemic Cells and KBM-5 Cells. On Wright-Giemsa-stained smears, the patient's leukemic cells were immature blasts with morphological characteristics of acute myelomonocytic leukemia according to the French-American-British classification (39). Granules were not observed in the original cells or KBM-5 cells. The original cells were positive for naphthol-ASD chloroacetate esterase (100%), MPO (20%), acid phosphatase (100%), and α-naphthol butyrate (30%) but negative for periodic acid-Schiff. The cytochemical profile of KBM-5 cells was similar, with the exception of a lower MPO positivity (3%), and compatible with the myelomonocytic lineage. On Wright-Giemsa-stained smears (Fig. 1A), the KBM-5 cells exhibited some anisocytosis and variation in the nucleocytoplasmic ratio. The nuclei were pleomorphic and had fine chromatin and rather small nucleoli. The relatively scarce cytoplasm was intensely basophilic and contained free eosinophilic granules and many vacuoles. The ultrastructural morphology of KBM-5 cells (passage 13) was blast-like and had an invasive nucleus and some marginate chromatin. The cytoplasm contained few mitochondria, some granules, and a moderate amount of endoplasmic reticula (Fig. 1B). The KBM-5 cells were terminal deoxynucleotide transferase negative, and they did not express Epstein-Barr virus nuclear antigen protein. PCR amplification of the BamW region of EBV did not reveal the presence of EBV-DNA integrated in the KBM-5 genome.

Phenotypic Characterization. The patient's leukemic cells from which the KBM-5 cell line was established demonstrated a phenotype consistent with monocytic lineage based on My4 and OKM1 expression (40), although the absence of Ia reactivity was unusual (Table 1). Phenotypic changes occurred with the establishment of the cell line and persisted with a trend towards increased My7, My9, and OKT4 expression and loss of My4 and OKM1 expression. KBM-5 cells were negative for surface immunoglobulin. The cells did not form rosettes with sheep erythrocytes. Approximately 25–30% of the KBM-5 cells formed EAC rosettes, which indicated the presence of complement receptors.

Isoenzyme Analysis. Eight loci proved to be informative in that genetic variants were present in either the control cells (HL-60 and HeLa) or the KBM-5 cell line. The phenotypes (which directly reflect the genotypes, because alleles at isozyme loci are codominant) for the material studied at each of the informative loci are shown in Table 2. The coidentity of KBM-5 and the original tumor was documented. All passages of KBM-5 were the cell line and derivatives of the original tumor (KBM-5-0). The presence of the rare PGM1.7 allele clearly...
marked the KBM-5 cells and distinguished them from HL-60, K-562, and two other new myeloid leukemia cell lines, KBM-7 and KBM-3, recently established in our laboratory (14, 41).

Cytogenetic Analysis. In each case, cytogenetic analysis of a Giemsa-banded karyotype was performed on 50 metaphases. The analysis of KBM-5 metaphases (Fig. 2A) showed a broad range in the number of chromosomes, with 46 metaphases having between 69 and 87 chromosomes and 4 having between 107 and 170 chromosomes. The cells in the triploid range exhibited similar numerical and structural alterations: +6, +7, +8, +8, +8, −9, −11, +q, −13, 14q−, +15, 17p+, +20, +22q−, +22q−, +frag, +DMs. The presence of common chromosomal changes indicates multiclonality and clonal evolution. The Ph1 chromosome t(9;22) and 17p+, +6, +8, −9, and a few minute chromosomes have also been identified in metaphases from a bone marrow sample obtained from the patient (Fig. 2B) at the initiation of the cell cultures. Minute chromosomes observed at a low frequency in the direct marrow preparation were present in multiple copies in all metaphases of the cell line. The KBM-5 cells carried multiple copies of the Philadelphia chromosome and also chromosomes 7, 8, 15, and 17 (Fig. 2A).

![Fig. 2. A. Giemsa-banded karyotype of a metaphase spread from KBM-5 (passage 12). Arrows, numerical (e.g., chromosomes 6, 7, 8, 11, 15, 20), as well as structural (e.g., chromosomes 9q+, 14q−, 17p+, 22q−) abnormalities. Note presence of numerous double minute (DM) chromosomes. The presence of Ph1 chromosome, the 17q+, +6, +8, and −9 are markers shared between original tumor and KBM-5 cells. B. Giemsa-banded karyotype of a metaphase from patient bone marrow. Multiple copies of Ph1 chromosome and numerous chromosomal abnormalities (arrows) are shown.](cancerres.aacrjournals.org)
Clonogenic Growth and Response to Colony-stimulating Activity. KBM-5 cells grow as compact colonies in agar- or methylcellulose-containing media. The cloning efficiency, initially 10–15%, increased after 20–30 passages to 20–30%. When cloned at low density, the cells tended to grow better in the presence of autologous serum, the cells tended to grow better in the presence of autologous serum.

Cells is not affected by recombinant G-CSF, granulocyte-macrophage CSF, interleukin 3.4 Addition of human recombinant stem cell factor increased less than 10% after induction with DMSO, RA, and TPA. When exposed to induced maturation toward erythropoiesis as evaluated by benzidine phagocytosis polystyrene beads, and their phagocytic activity in response to induced maturation toward erythropoiesis as evaluated by benzidine phagocytosis was 20–30%. When cloned at low density, the cells tended to grow better in the presence of autologous serum.

Cloning efficacy of the colonies (Table 3). The cell growth and differentiation of KBM-5 cells is not affected by recombinant G-CSF, granulocyte-macrophage CSF, interleukin 3.4 Addition of human recombinant stem cell factor (kindly provided by Immunix Research Laboratory, Washington, DC) at concentrations between 25 and 200 ng/ml failed to influence growth, while 400 ng/ml proved inhibitory (40% inhibition of growth).

Response to Inducers of Differentiation. Noninduced cells only occasionally reduced NBT and the number of NBT-reducing cells increased only marginally after stimulation with TPA. When exposed to either RA or DMSO in concentrations optimal to stimulate growth of granulocyte-macrophage colony-forming cells nor media conditioned by several other myeloid leukemia cell lines affected the number or size of the colonies (Table 3). The cell growth and differentiation of KBM-5 cells is not affected by recombinant G-CSF, granulocyte-macrophage CSF, interleukin 3.4 Addition of human recombinant stem cell factor (kindly provided by Immunix Research Laboratory, Washington, DC) at concentrations between 25 and 200 ng/ml failed to influence growth, while 400 ng/ml proved inhibitory (40% inhibition of the growth).

Sensitivity to NK-mediated Cell Lysis. Chromium-labeled KBM-5 cells were exposed to both noninduced and α-interferon-activated NK cells from normal donors (target:effector cell ratio, 1:5–1:50). K562 cells were studied as a positive control. KBM-5 cells proved to be completely resistant to any cytolytic action of NK cells in a 4-h chromium release assay (Table 4).

Immune Complex Kinase Assay for p210 Bcr-Abl. Assays were performed concomitantly with K-562 cells that are known to have amplified c-ABL (42). As expected, high levels of p210 Bcr-Abl phosphokinase activity were detected in K562 cells when rabbit anti-Ab as well as anti-Bcr (exon 3) peptide antisera were used (Fig. 3). The M, 190,000 protein in K-562 cells, detected in variable amounts by the two antisera, is thought to be a proteolytic fragment of p210 (42). In KBM-5 cells, p210 Bcr-Abl kinase activity was observed using both antisera (Fig. 3), albeit at lower levels than in K562 cells. It has been demonstrated previously (43) that the break on chromosome 22 may occur between bcr exons 3 and 4, presumably generating alternate BCR-ABL messages that either contain or lack exon 3. The significance of these molecular variations remains unclear. The detection of p210 Bcr-Abl by an anti-Bcr serum, which was made against a domain within exon 3, demonstrates that KBM-5 cells, like K-562 cells, express the variant which includes BCR exon 3.

Detection of BCR-ABL Splice Junction and BCR-ABL, ABL, and ABL Message. Use of the polymerase chain reaction followed by Southern blotting revealed that KBM-5 cells carried a BCR 3-ABL II splice junction. There was no BCR 2-ABL II splice junction or c1-a2 junction (the latter encodes the p190 Bcr-Abl). In addition, normal BCR message was detected by the polymerase chain reaction followed by Southern blotting. Because the primers used for the latter assay where derived from cDNA sequences distal to the junction where BCR is spliced to ABL, they would be expected to identify normal BCR message, but not the BCR-ABL message, since only the more proximal BCR sequences are found in the BCR-ABL mRNA. No normal ABL message was discerned, as determined by polymerase chain reaction followed by the hybridization protection assay.

Cytokine mRNA Expression. PCR and specifically designed primers were used to analyze the early and late passages (13 and 250, respectively). This approach detected constitutive expression of only TNFα in KBM-5 cells (with β-actin expression being consistently positive (Fig. 4). The assay failed to detect mRNA for hematopoietic growth factors, i.e., G-CSF; granulocyte-macrophage CSF; c-kit ligand; macrophage CSF; interleukins 3, 4, 6, and 10; and IFNγ. The efficacy of the PCR procedure and quality of the primers were monitored with OKT3-stimulated human mononuclear blood cells expressing mRNA for all factors/cytokines studied, with the exceptions of c-kit ligand, and G-CSF.

Growth of KBM-5 Cells in SCID Mice. Eight SCID mice were given injections of a dose of 1 x 10^7 cells, and all became terminally ill between 18 and 62 days after inoculation. The results are summarized in Table 5. Engraftment was achieved in all of the mice regardless of the mode of inoculation. In the two animals that were given s.c. injections, s.c. and regional lymph node tumors developed. In two other animals that were inoculated i.p., one developed intraabdominal tumors and ascites and another a s.c. tumor. After retroorbital inoculation, one animal developed a regional lymph node tumor and the second an abdominal tumor. The last two i.v.-injected animals developed both intraabdominal and submandibular tumors. All of the tumors were composed of blast cells. The majority of the tissues studied, e.g., blood, marrow spleen, liver, lungs, and brain tissues that were examined using primers for the α chain of HLA-DQA1 (38), demonstrated the presence of human DNA (Table 5). The presence of a specific leukemic marker, i.e., bcr/abl mRNA, was documented by amplification of bcr/ abl transcript from most tissues of SCID mice studied in 3 animals after i.v. (1 animal) and i.p. (2 animals) injection of KBM-5 cells. (Fig. 5; Table 6). The histology of selected tissues revealed the presence of foreign cells the blast cell morphology of which was compatible with that of the grafted KBM-5 leukemic cells. Fig. 6 shows splenic involvement and intraabdominal KBM-5 tumor.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>E/T ratio</th>
<th>MWBC</th>
<th>Recombinant IFNa/MWBC</th>
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<tr>
<td>K-562</td>
<td>10:1</td>
<td>6.2</td>
<td>22.0</td>
</tr>
<tr>
<td></td>
<td>40:1</td>
<td>19.8</td>
<td>59.8</td>
</tr>
<tr>
<td>K562</td>
<td>10:1</td>
<td>-2.0</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>40:1</td>
<td>2.5</td>
<td>-1.7</td>
</tr>
</tbody>
</table>

* MWBC, mononuclear (density ≤10,077 g/cm^3) WBC from normal blood.

a MWBC from normal blood activated by exposing 1 x 10^6 cells/ml to 500 units recombinant IFNa for 2 h.

4 M. Beran, unpublished data.
Fig. 3. Immune complex kinase assay in KBM-5 cell extracts. p210bcr'"'bl phosphokinase activity in KBM-5 cells was detected by immune complex kinase assay. Antisera used were anti-Abb antiserum (Lanes 1, 2, 5, 6, 9, 10) and anti-bcr3 antiserum (Lanes, 3, 4, 7, 8, 11, 12). In even lanes, the reactivity is blocked by addition of cognate peptide. Lanes 1–4, KBM-5 cells; Lanes 5–8, K562 cells; Lanes 9–12, HL-60 cells (Ph'i-negative leukemic cell line). Kd, molecular weight in thousands.

Fig. 4. Cytokine expression (mRNA) in KBM-5 cells as measured by PCR. PCR products are electrophoretically separated on 3% agarose gels (70 V for 60 min). Lane 1, molecular size standards; Lane 2, KBM-5, b-actin; Lane 3, H2O control; Lane 4, TNFa; Lane 5, H2O control; Lanes 6 and 7, interleukin 1 and interleukin 1, H2O control, respectively; Lane 8, IFN'y. bp, base pairs.

Fig. 5. Amplification of bcr/abl transcript from the organs of SCID mice, 4 weeks after i.p. inoculation with 1 X 10^7 KBM-5 cells. Lane 1, molecular markers; Lane 2, KBM-5 cells, positive control; Lane 3, KBM-7 cells (14), positive control; Lane 4, blood; Lane 5, liver; Lane 6, negative control, normal SCID liver; Lane 7, CML, chronic phase, bone marrow. bp, base pairs.

growing adjacent to spleen (Fig. 6A) as well as involvement of the subarachnoidal space with KBM-5 cells (Fig 6B).

In vitro cultures of bone marrow and spleen cells from animals grafted with KBM-5 cells which have leukemic cells in these organs, documented by PCR/DQa, produced outgrowth of KBM-5 cells with phenotype identical to that of inoculated KBM-5 cells (CD33 97.8% and 98.8% positive KBM-5 and KBM-5/SCID, respectively; CD13 98.6% and 96.4% for KBM-5 and KBM-5/SCID, respectively).

**DISCUSSION**

The significance of chromosomal abnormalities other than t(9;22) evolving during the transformation of CML into the blastic phase is obscure (39, 40). Knowledge about the relationship of these chromosomal aberrations to the biology and phenotype of leukemic cells may contribute to the development of future treatments. The availability of immortalized cell lines derived from patients with blastic phase of CML might facilitate such studies. The studies with the first such established line, K-562 (derived from the pleural fluid of a patient in the lymphoid blastic phase of CML) (7), have revealed an immature phenotype of these cells compatible with transformation at the multipotent stem cell level. Another remarkable characteristic of K-562 cells was their sensitivity to lytic action of NK cells (7, 28). Questions arise, however, about the extent to which K-562 cells are representative of the blastic phase phenotype. To answer these questions, other cell lines must be obtained from patients in the blastic phase of CML.

Additional CML blastic phase-derived myeloid cell lines (8–16) as well as lymphoid cell lines (17) with various phenotypes from the CML blastic phase have been reported. A review of their principal characteristics indicates their phenotypic heterogeneity (8–17). Some lines carry multiple copies of the Ph chromosome whereas others have only one copy. Descriptions of other chromosomal markers have been reported in detail only for KBM-7 (14), TOM-1 (17), and the present KBM-5 cells. Isoenzyme analysis has proved that, like KBM-7 cells, KBM-5 cells originate from the donor-derived leukemic population. Furthermore, these cells display a chromosomal constitution similar to that in the original donor cells. Despite their blast appearance and large nucleus:cytoplasm ratio, many cells are strongly positive for butyrate which indicates differentiation along the monocytic pathway. Unlike K-562, KBM-5 cells are completely resistant to the action of both unstimulated and a-interferon-activated NK cells. They are also completely resistant to the antiproliferative action of recombinant a-

<table>
<thead>
<tr>
<th>Route of inoculation</th>
<th>No. of animals inoculated/no. engrafted</th>
<th>Survival (days)</th>
<th>Organs analyzed and involved</th>
<th>Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>s.c.</td>
<td>2/2</td>
<td>28, 30</td>
<td>Blood, liver, lungs, brain</td>
<td>s.c.: regional lymph nodes in both animals</td>
</tr>
<tr>
<td>i.p.</td>
<td>2/2</td>
<td>28, 35</td>
<td>Blood, liver, lungs, brain</td>
<td>Abdominal: ascites; s.c.</td>
</tr>
<tr>
<td>r.o.</td>
<td>2/2</td>
<td>21, 56</td>
<td>Lungs, brain, liver, blood</td>
<td>Submandibular nodes: abdominal</td>
</tr>
<tr>
<td>i.v.</td>
<td>2/2</td>
<td>56, 62</td>
<td>Blood, marrow, lungs, liver, brain</td>
<td>Submandibular nodes: abdominal</td>
</tr>
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</table>

*Human DNA sequences detected.

* r.o., retroorbital; involves i.v. and local implantation.
and γ-interferons; this resistance is accompanied by an absence of 2′-5′-oligoadenylate synthetase induction and an inability to enhance the production of this enzyme when challenged by interferons.5

Because the cell lines are constantly in danger of being cross-contaminated, their origin must be carefully documented. Enzyme markers trace the origin of the new KBM-5 cell line to the patient sample, and compatible cytogenetics traces the origin to the original tumor. As with other leukemic cell lines, the biological factors that allow the cells to survive in vitro are not known. It is noteworthy that molecular analysis of the original frozen sample and the KBM-5 cell line revealed an approximately 8-fold amplification of c-ABL, her, and C immunoglobulin genes in the KBM-5 cells but not in the fresh, uncultured cells from which the line was derived. These findings indicate a possible role of c-ABL and BCR amplification in the establishment of in vitro growth or the selective advantage of a minor preexisting clone in the establishment of a cell line. Interestingly, while the expression of c-ABL mRNA is consistently found in many fresh samples of blast cells from patients with CML in the blast phase that do not display c-ABL amplification (46), no ABL transcript was detected in KBM-5 cells in the present studies. This finding makes KBM-5 cells suitable for studies of the role of c-ABL gene expression for the biological properties of cells. A recently described Ph+ lymphoid cell line, TOM-1, failed to show c-ABL gene amplification, and although the BCR was rearranged, the cell line contained BCR gene transcripts of only normal length (17).

The KBM-5 cell line has interesting biological characteristics. Although this cell line shares with K-562 cells the property of being highly resistant to the inhibitory action of recombinant IFNγ and recombinant IFNα in vitro (47), the K-562 cells are very sensitive to NK cells and resistant to recombinant TNFα, whereas the KBM-5 cells are completely resistant to NK cell action and highly sensitive to the inhibitory action of recombinant TNFα (47). Finally, KBM-5 and K-562 lines share comparable in vitro dose responses to a variety of chemotherapeutic agents, including doxorubicin and amsacrine.4 Thus, we believe that a series of well-defined CML blastic phase-derived cell lines will offer new opportunities to study the biological and therapeutic significance of various phenotypic and genotypic phenomena in the blast phase of the CML.

The observation that KBM-5 cells engraft and disseminate in tissues of SCID mice in a mode comparable to the clinical situation is particularly relevant, because it opens the possibility of studying such phenomena in vivo. Only a limited knowledge exists about the growth of chronic myelogenous leukemia-derived cells in SCID mice. The engraftment in SCID mice of cell lines derived from blast phase CML was first reported for K-562 cells (48) and also for two other PH+ myeloid and lymphoid cell lines, EM-2 and ALL-1, respectively (48). Primary cells from 2 patients in blastic phase of CML were also reported to grow locally and invasively after implantation under kidney capsule and spread to bone marrow as demonstrated by flow cytometry (48). Attempt to engraft cells derived from patients with chronic phase CML were thus far unsuccessful. Transient local engraftment in the peritoneal cavity was noted in few animals after i.p. injection with cells from two of six patients. No human cells were detected in tissues of animals given injections of cells from patients with chronic phase of CML by any route (48).

We have recently studied the engraftment of acute myelogenous leukemia-derived cell lines (HL-60, K562-3) as well as their counterparts, which were rendered resistant to antileukemic drugs. All lines engrafted consistently and were found to invade numerous tissues, including the central nervous system. After passage through SCID mice, the phenotype of both sensitive and drug-resistant cells remained unchanged.6 Similar results were recently reported for other cell lines derived from patients with AML, i.e., HL-60 (48, 49) and KG-1 (48). The engraftment of primary myeloid leukemia cells has been less consistent although successful engraftment of primary blasts from AML was reported from four patients (48). Only in one case was the presence of human AML cells detected after i.v. injection of K562 cells into SCID mice. Mice were sacrificed when terminal illness was first noted. Engraftment in the peritoneal cavity was noted in few animals after i.p. injection with cells from two of six patients. No human cells were detected in tissues of animals given injections of cells from patients with chronic phase of CML by any route (48).

Table 6  Polymerase chain reaction-amplified BCR/ABL mRNA transcript in organs of SCID mice transplanted with KBM-5 human leukemic cells

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Inoculation route</th>
<th>Blood</th>
<th>Marrow</th>
<th>Spleen</th>
<th>Liver</th>
<th>Lung</th>
<th>Brain</th>
<th>Tumor</th>
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<tbody>
<tr>
<td>1</td>
<td>r.o.</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>i.p.</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
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*+, positive for BCR/ABL; ND, not done; NR, tumor not found; r.o., retroorbital. Mice sacrificed when terminally ill.

5 M. Beran et al., unpublished data.
6 M. Beran and P. Pisa, submitted for publication.
20. Reedman, B. M., and Klein, G. Cellular localization of an Epstein-Barr virus (EBV) useful for preclinical evaluation of antileukemic drugs and biological cates a factor-independent proliferation of KBM-5 cells both in vitro and in vivo. Engraftment of KBM-5 cells in mice without prior conditioning of recipients with irradiation or drugs makes this model useful for preclinical evaluation of antileukemic drugs and biological agents, currently ongoing in our laboratory.

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Biological Properties and Growth in SCID Mice of a New Myelogenous Leukemia Cell Line (KBM-5) Derived from Chronic Myelogenous Leukemia Cells in the Blastic Phase

Miloslav Beran, Pavel Pisa, Susan O'Brien, et al.


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