Spontaneous Epstein-Barr Virus Transformed B-Cell Line Sharing the Identical Immunoglobulin Gene Rearrangement With Acute Myeloid Leukemia

By Heinrich H. Gerhartz, Claus R. Bartram, Anand Raghavachar, Helga Schmetzer, Christoph Clemm, Wolfgang Wilmanne, and Eckhard Thiel

Mononuclear cells from a 44-year-old patient with acute myeloid leukemia (AML) gave rise to a spontaneous permanent cell line cultured in suspension. The cell line was shown to be positive for Epstein-Barr virus nuclear antigen (EBNA). As expected, its composite phenotype was of B-cell type with B-cell antigens (CD 20, CD 21) and with monoclonal surface IgM of kappa type, but without detectable IgM secretion. Surprisingly, identical monoclonal rearrangements of the immunoglobulin heavy chain (JH) sequences could be demonstrated in the uncultured bone marrow AML cells and in the cell line that also had kappa light chain gene rearrangement. This is the first case to our knowledge of an EBNA positive B-cell line with identical monoclonal Ig heavy chain rearrangement as detected in myeloblastic leukemia cells.

MATERIAL AND METHODS

Acute myeloid leukemia (AML) is a neoplasm that is generally thought to originate from progenitor cells restricted to the myeloid lineage. Some cases, however, have been identified by G6PDH and karyotypic studies involving both the B cell and the myeloid lineage. Recently, a minority of AMLs have been found to have rearranged immunoglobulin (Ig) heavy chain genes by Southern blot analysis.

Although B cells from AML patients in remission seem to be relatively resistant to Epstein-Barr virus (EBV)-transformation in vitro, the amplification of monoclonal cells during remission of AML has been successfully carried out by infection with EBV in one case. It is not known, however, if the respective monoclonal B cells from AML patients are derived from the same clone as the leukemia cells, or if they represent a normal B-cell line. Moreover, distinct subpopulations of AML cells with specific growth requirements have been demonstrated, which might favor the assumption of an oligoclonal disease with the predominance of a clone with myeloid differentiation capacity.

In an attempt to select and identify those leukemic cells that are responsible for proliferation, we performed long-term suspension cultures stimulated by supernatant of the 5637 cell line, which secretes—among other factors—granulocyte colony stimulating activity (G-CSF). From one case presenting with Ig gene rearrangement at diagnosis a permanent cell line resulted that was further characterized by immunologic and molecular genetic methods.

From the Med Klinik III, Klinikum Großhadern, Munich University, Departments of Pediatrics II and Transfusion Medicine, Ulm University, and the Medical Klinik Innenstadt, Munich University, FRG.

Submitted February 1, 1988; accepted October 17, 1988.

Supported by the Deutsche Forschungsgegellschaft (SFB 324/ A9) and Deutsche Krebshilfe. Helga Schmetzer is supported by the Wilhelm Sander-Stiftung, Neuburg/Donau, FRG.

Address reprint requests to Heinrich H. Gerhartz, MD, Med Klinik III, Klinikum Großhadern, Marchioninistr. 15, D-8000 München 70, FRG.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1989 by Grune & Stratton, Inc.

684

Blood, Vol 73, No 3 (February 15), 1989: pp 684-687
gated anti-human-IgG antibodies (Ortho diagnostics, NJ), stained with 0.1% H2O2 and 0.5 mg/mL diaminobenzidine.

**Determination of unbound Ig.** Immunoglobulin secreted into culture supernatants was kindly determined in a BNA apparatus (Behring, Marburg) by Dr. A. Fache-Moghadam, Munich, according to standard nephelometric techniques.

**DNA analysis.** DNA was extracted from ficollated kryopreserved leukemic cells by standard techniques.16 Fifteen μg DNA was digested with appropriate restriction enzymes (Boehringer, Mannheim), electrophoresed on a 0.7% agarose gel, blotted, and hybridized as described.17 To demonstrate Ig-gene rearrangements, Hind III and Eco RI digests were hybridized to a 2.4 kb Sau 3a probe detecting 10.5 kb and 17 kb germline bands, respectively18 and from Bam HI digests to a c-kappa probe, which detects a 12 kb germline band,19 both probes were kindly provided by Dr. P. Leder. Bcr gene configuration was investigated in BGI II, Hind III and Eco RI digests using a 2 kb BagI II/Hind III 5’ probe.20 After hybridization, the filters were washed under stringent conditions and exposed to XAR-5 film (Kodak, Rochester, NY) using Dupont lightning-Plus intensifying screens for up to 24 hours at −70°C.

**RESULTS**

The 43-year-old male patient was admitted to our hospital with a 4-week history of headache, faintness, and nausea and a leucocyte count of 21 × 10⁹/L, thrombopenia (46 × 10⁹/L), and anemia (Hb 8.8g%). His bone marrow was found to be infiltrated with abnormal myeloblasts (53%) and promyelocytes (15%), leaving 16% neutrophils and 16% erythroid cells in a bone marrow smear. The patient was treated according to a study protocol of the European Organization of Research on Treatment of Cancer (EORTC) employing daunorubicin (50 mg/m²) for three days, cytosine arabinoside (2 × 80 mg/m²) for seven days, and vincristine 2 mg for one day. A control marrow after 4 weeks showed a complete remission. A second course was given for consolidation, followed by two cycles of maintenance treatment. Four months later (8 months from diagnosis) the patient’s marrow was cryopreserved for autologous transplantation (ABMT) while he was still in complete remission (CR), but he relapsed 6 weeks later before ABMT was done. Attempts to induce a second CR with high-dose cytosine-arabinoside plus amsacrine failed and the patient died from progressive leukemia 11 months after the initial diagnosis.

The cytologic and immunologic phenotype of the patient’s bone marrow cells at presentation is shown in Table 1 (first column). The majority of the blasts showed a myelomonocytic morphology (M4 according to the FAB classification) with a positive reaction for POX. Thirty-five percent of the cells reacted with myeloid markers (CD 15 and CD 33), whereas no B-cell marker positive cells were present. Twenty-five percent of the cells reacted with CD 7. The pan T marker CD 7 is known to react with some AML cases.21

Upon suspension culture with or without addition of the 5,637-conditioned medium, the cell numbers initially rose from 2.5 × 10⁶ per flask to 8-12 × 10⁶ per flask in the first week to decline thereafter until the sixth week. In the seventh week, however, stimulated and unstimulated suspension cultures gave rise to a continuously growing cell line (clumps and single cells), which was characterized by the presence of B-cell markers (CD 20, CD 21), CALLA (CD 10), TdT, and surface Ig of the IgM type, whereas no T-cell markers and only very few cells with myeloid markers could be found (Table 1). Both stimulated and unstimulated cultures gave identical results. They were pooled for further examination. The cells were not cloned from single colonies in semi-solid media.

Uncultured cells obtained at relapse from the patient’s BM were similar in phenotype to the cells at presentation. Additionally, a small percentage of B cells and T cells was demonstrable (Table 1).

DNA was extracted from uncultured Ficoll-separated BM cells at presentation and during remission (BM cryopreserved for ABMT) as well as from the cell line 3 months after initiation of the culture and was investigated for Ig gene rearrangements by Southern blot analysis. Both uncultured BM cells at presentation as well as the cell line showed an identical monoclonal rearrangement of Ig heavy chain gene sequences in Hind III as well as Eco RI digests, while BM cells obtained during clinical remission showed a germline configuration (Fig 1). A kappa light chain gene rearrangement was found in the cell line, whereas light chain genes remained in germ line position in DNA extracted from initial and remission bone marrow cells (Fig 2).

Immunoglobulin could not be detected in culture supernatant by a nephelometric method (data not shown). Obviously, the cell line did not secrete substantial amounts of immunoglobulins, although the cells carried IgM on their surface and both heavy and light chain genes had undergone rearrangement.

Investigations with antibodies against EBV-related antigens (EBNA and virus capsid antigen [VCA]) revealed that the cultured cells were positive for both antigens, as concordant results were achieved by direct immunofluorescence and in western blot analysis (data not shown).

When cells of this B-cell line were plated in semisolid agar
Fig 2. Southern blot analysis using a kappa light chain specific probe that detects a 12 kb germline band in Bam HI digests. A rearrangement (arrow) was found in the cell line (2), but not at presentation (1) or in remission (3) BM cells.

The findings of the case described here, however, make the outgrowth of B cells unrelated to the AML very unlikely. First, the patients’ BM cells at presentation and the EBV-positive B-cell line had identical rearrangements of immunoglobulin heavy chain genes (Fig 1). In this connection the rearranged light chain gene of the B-cell line can be taken as a marker of affiliation to the initial AML clone. Second, the phenotype of the cell line as well as the absence of detectable Ig-secretion, which is rare in normal EBV-induced B-cell lines, indicates that the cell line is composed of relatively immature B cells. This assumption is further supported by the high proportion of cells positive for J5 (CD 10). Another explanation for this finding, however, might be the high sensitivity of the enzyme-immunoassay compared with the indirect immunofluorescence technique.

When we accept the identical JH chain gene rearrangement of the cell line as a marker of affiliation to the initial AML clone, then it is a surprising finding that an EBV-susceptible subclone did arise with a phenotype of B cells and a likewise completed genotype as reflected by additional light chain gene rearrangement. The possibility that this case represented a primary myeloid crisis of CML with lymphoid differentiation is ruled out by the fact that no Philadelphia chromosome could be demonstrated in the cell line and by lack of a bcr gene rearrangement in DNA obtained from primary leukemic cells and from the cell line.

Taken altogether, this case of AML with its respective cell line further supports the notion that the target cell for transformation in AML can be a progenitor cell that is capable of both myeloid- and B-cell differentiation. Immor-
talization of the cells by EBV obviously forced the cells along the B-cell lineage. Possibly such a mechanism might play a role in cases of AML in which lymphatic subclones can be demonstrated. However, the alternative explanation, namely a selection process from a very small subpopulation (<1%) of cells with both myeloid- and B-cell properties cannot be excluded, because the cells were cultured in suspension and not from single clones in semisolid medium.

REFERENCES


ACKNOWLEDGMENT

The authors thank Dr. H. Wolf, Max-von-Pettenkofer-Institute, Munich University, for the investigations of EBV-related antigens in the cell line. Dr. A. Fateh-Mogadham, Institute for Clinical Chemistry, Klinikum Großhadern, Munich University, kindly determined Ig concentrations in the cell culture supernatants. The skilful technical assistance of D. Knauss-Dittmann, B. Höning and C. Tell is gratefully acknowledged.
Spontaneous Epstein-Barr virus transformed B-cell line sharing the identical immunoglobulin gene rearrangement with acute myeloid leukemia

HH Gerhartz, CR Bartram, A Raghavachar, H Schmetzer, C Clemm, W Wilmans and E Thiel