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Immunoglobulin Gene Rearrangement in Acute Myelogenous Leukemia[1]

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ABSTRACT

Twenty-one cases of newly diagnosed nonlymphocytic leukemia were subjected to analysis of immunoglobulin gene organization. Although immunoglobulin gene organization is an essential property of B-lineage cells, immunoglobulin gene rearrangement has also been observed in mouse T-cells and occasionally in cultured human T-cell leukemic cells. Here we report the first case of acute myelogenous leukemia in which μ-chain gene rearrangement is demonstrated. This finding provides a further step in our understanding of both normal and abnormal hematopoietic cell differentiation and the heterogeneity of nonlymphocytic leukemia.

INTRODUCTION

ANLL[4] is a heterogeneous disease based on morphological, biochemical, and cytogenetic characteristics; the X-linked enzyme glucose-6-phosphate dehydrogenase; and response to therapy (8, 16). Despite the recent advances in treatment, the overall prognosis of ANLL is still disappointing (16). For this reason, evaluation of parameters related to ontogeny provides new insight into the heterogeneity of the disease.

Detection of immunoglobulin gene rearrangement has become an important procedure in defining the cellular origin of immature cells. Korsmeyer *et al.* (14) demonstrated that the blast cells of common ALL were derived from B-cell precursors when analyzed at the DNA level. Similar findings of immunoglobulin gene rearrangement were also demonstrated in the lymphoid blast crises of chronic granulocytic leukemia (2, 9). To our knowledge, there have been no reports of immunoglobulin gene rearrangement in ANLL. We present a patient with AML in whom immunoglobulin μ-chain gene rearrangement was observed.

MATERIALS AND METHODS

Diagnosis of Nonlymphocytic Leukemia. The diagnosis was based on the morphology and cytochemical staining of bone marrow samples. Cell morphology was examined on Wright-Giemsa-stained smears. The French-American-British scheme was used for the morphological classification of leukemic cells (3). Sudan Black B, peroxidase, nonspecific esterase, and periodic acid-Schiff cytochemical stains were used.

Immunoglobulin Gene Analysis. High-molecular-weight DNA was extracted from the mononuclear cells (obtained from the bone marrow following Ficoll-Hypaque gradient centrifugation) from each case. In the patient described, high-molecular-weight DNA was obtained from bone marrow cells at the time of presentation and when in remission. Genomic DNAs from fibroblasts or thymocytes that have already been shown to have germ line immunoglobulin genes were also used as controls. These genomic DNAs were digested with *Bam*HI, *Eco*RI or *Hind*III restriction endonucleases. Digested DNA was size fractionated by agarose gel electrophoresis and transferred to nitrocellulose paper (22). Such filter-bound DNA fragments were then hybridized to nick-translated [^{32}P]DNA probes of the germ line immunoglobulin genes and visualized on autoradiograms (21). The human immunoglobulin gene probes used include the constant region of μ (Cμ, 1.3-kilobase embryonic *Eco*RI probe), the J_H probe (3-kilobase embryonic *Eco*RI-*Hind*III J_H-containing fragment) (20), κ (Cκ, 2.5-kilobase embryonic *Eco*RI Cκ-containing fragment) (12), and λ (Cλ, 0.8-kilobase embryonic *Eco*RI-*Hind*III Cλ_1-containing fragment) (11) immunoglobulin genes. Cμ germ line clones were kindly provided by Drs. T. H. Rabbits and G. Mattheysens (Laboratory of Molecular Biology, The MRC Centre, Hills Road, Cambridge, United Kingdom) (19), and the Cκ and Cλ germ line clones were given by Dr. P. Leder (Department of Genetics, Harvard Medical School, Boston, MA) (11, 12).

RESULTS

Morphology and Cytochemical Studies. Leukemic cells from the patient (a 15-year-old girl whose initial WBC was 30×10^9/liter with 90% blast cells and bone marrow was completely replaced with leukemic cells) were classified as MI morphology (AML) according to the French-American-British classification and were Sudan Black B positive (25% of the blasts), peroxidase positive (23% of the blasts), periodic acid-Schiff negative, and nonspecific esterase negative (Fig. 1).

Immunoglobulin Gene Analysis. As shown in Fig. 2, the Cμ probe recognized a rearrangement of one μ-chain allele after digestion with *Bam*HI, whereas the other gene was retained in germ line configuration (19-kilobase fragment). This Cμ probe was also capable of detecting germ line configuration in an 11.5-kilobase *Hind*III fragment (the digested fragment contains the switch region but lacks the J_H region). The J_H probe also detected a rearrangement of one μ chain while retaining germ-like configuration of the other allele after *Eco*RI digestion. Both light-chain genes were also in germ line configuration. The Cκ probe detected a 12.5-kilobase *Bam*HI fragment for the germ line κ genes. The Cλ probe recognized 9-, 16.5-, and 19-kilobase *Eco*RI fragments and an additional 21-kilobase fragment for germ line λ genes, corresponding to the type I/II pattern for λ gene polymorphism described by Hieter *et al.* (11).

All other cases, including 6 patients with AML, one acute myelomonocytic leukemia, one acute monoblastic leukemia, 2 cases of juvenile CML of childhood, and 10 cases of adult AML, revealed germ line configuration of immunoglobulin genes (data not shown). In parallel studies, 2 cell lines, K562, derived from a CML patient (18), and HL-60, derived from a promyelocytic leukemia (4), also showed no immunoglobulin gene rearrangements. Twenty-two newly diagnosed patients with common ALL

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[4] The abbrevations used are: ANLL, acute nonlymphocytic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; ALL, acute lymphocytic leukemia.

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showed μ-chain gene rearrangements, consisent with a previous report (14) (data not shown).

DISCUSSION

This detection of immunoglobulin gene rearrangement restricted to a single allele in leukemic blasts from a patient with AML was a surprising finding. This is similar to previous studies in a patient with T-cell ALL (10), human T-cell lines (9, 14), and mouse T-cells (7), where the rearrangement is similarly restricted to a single allele with retention of germ line configuration in the other allele. Using a J_H probe [3-kilobase embryonic *Eco*RI-*Hind*III J_H-containing fragment (20)], a single allelic rearrangement was also detected after *Eco*RI digestion. This is in contrast to our analysis of common ALL where, using the J_H probe, immunoglobulin gene rearrangements were never restricted to a single allele. Whenever *Bam*HI restriction enzyme was used and hybridized to the Cμ probe, restriction polymorphism was quite unlikely since studies of B-precursor leukemic cells and fibroblasts from the same patients gave different results (9, 14). In addition, when the cells from her bone marrow in remission were examined, the Cμ genes were also in germ line configuration.

Based on the developmental hierarchy of immunoglobulin gene rearrangements (15), these findings may suggest that the initial event in immunoglobulin gene organization occurs during an early stage of development of hematopoietic cells where the cells are not only restricted to differentiate along B-lineage pathways but also capable of further differentiation along other pathways. Alternatively, these leukemic cells may have a normal counterpart cell; the proportion of these cells in normal hematopoiesis might be very low, and it is only when leukemic transformation and clonal expansion occurs at this stage of differentiation that such cells may be demonstrated.

A significant body of knowledge of chromosomal abnormalities in leukemia has been accumulated (17, 23). Such findings, together with the presence of human cellular oncogenes (13, 23), suggest that chromosomal abnormalities play an important role in leukemogenesis. It is uncertain whether a precise relationship exists between immunoglobulin heavy-chain gene rearrangement and a chromosomal abnormality in our case. Although no karyotypic analysis was done, to our knowledge, an abnormality of chromosome 14, where immunoglobulin heavy-chain genes are located (5), has not been reported in patients with AML (23). In addition, the experiments with *Hind*III digestion (Fig. 2) detected an 11.5-kilobase fragment that is consistent with germ line configuration. This suggests that the region including the J_H gene was organized without rearrangements in the vicinity of the switch region, where translocation of C-*myc* oncogene is often observed in Burkitt lymphoma (1, 6).

The present case illustrates the value of immunoglobulin gene analysis of leukemic cells and the potential for furthering our understanding of leukemic cell ontogenesis and biological behavior in the various types of leukemias.

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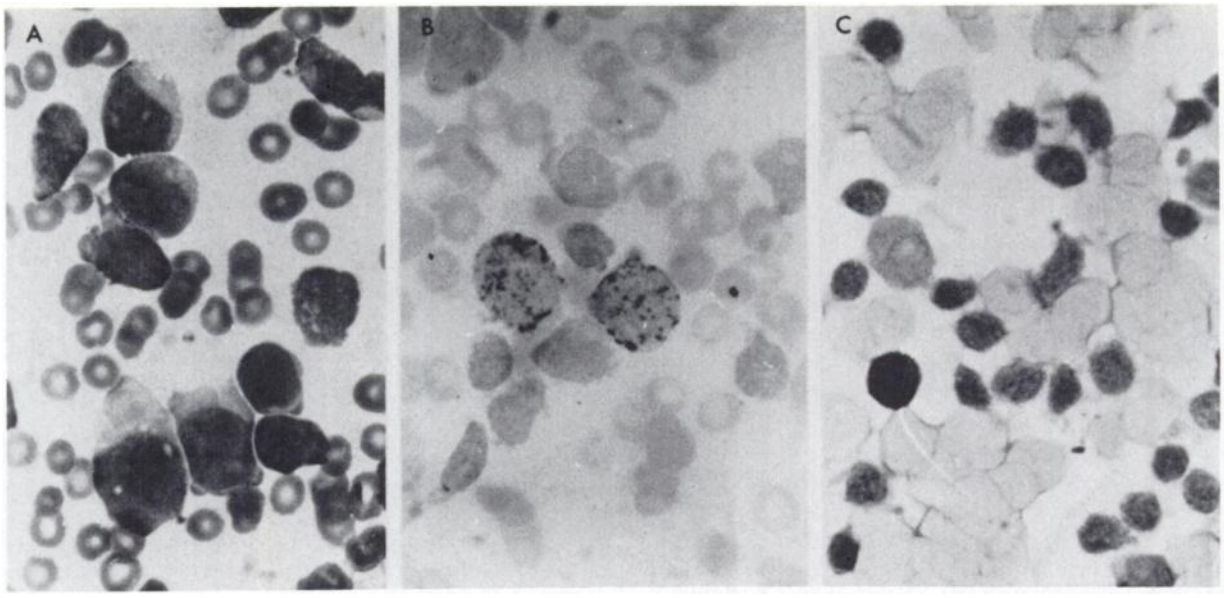

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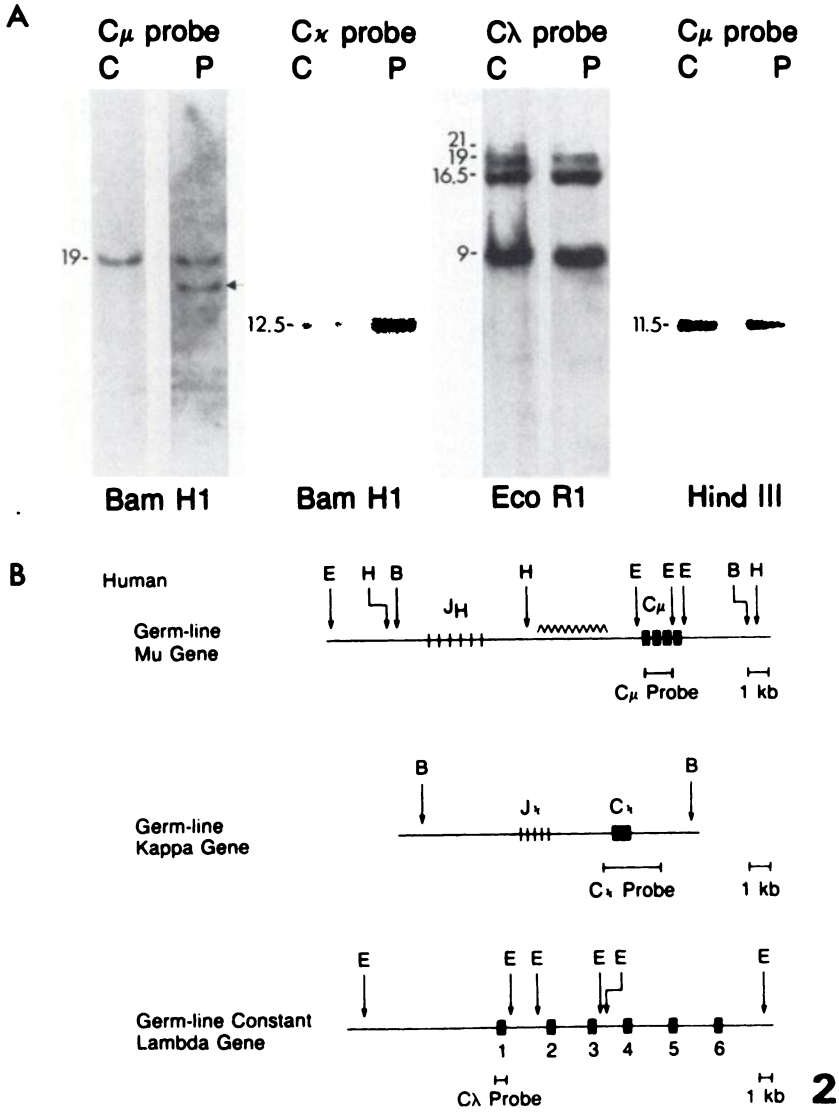

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Fig. 1. *A*, Bone marrow smear with a predominance of myeloblastic cells. Leukemic cells were identified as MI morphology according to the French-American-British classification. Wright-Giemsa, × 1200. *B*, leukemic cells reactive with Sudan Black B. Twenty-five % of cells were stained. Sudan Black B, × 1200. In addition, 23% of the cells stained positively with peroxidase. *C*, periodic acid-Schiff-negative reaction. Periodic acid-Schiff, × 1200.

Fig. 2. *A*, Southern blot analysis to detect germ line and rearranged immunoglobulin genes in patient's leukemic cells (*P*) and control fibroblast DNA (*C*). The Cμ probe, containing a 1.3-kilobase (*kb*) germ line *Eco*RI fragment, was capable of detecting both a germ line configuration (——) in a 19-kilobase *Bam*HI fragment and a rearranged allele (*arrow*). This Cμ probe was also capable of detecting germ line configuration in an 11.5-kilobase *Hind*III fragment. The Cκ probe, containing a 2.5-kilobase embryonic *Eco*RI fragment, could identify a 12.5-kilobase *Bam*HI fragment for germ line κ genes. The Cλ probe, containing a 0.8-kilobase embryonic *Eco*RI-*Hind*III $C\lambda_1$-containing fragment, recognized 9-, 16.5-, and 19-kilobase *Eco*RI fragments and an additional 21-kilobase fragment for germ line λ genes. *Numbers*, fragment sizes in kilobases. *B*, restriction enzyme map of human germ line μ gene and the human immunoglobulin gene probes used in this study. ∿∿ corresponds approximately to the length of the switch μ region. Restriction endonuclease cleavage sites are shown as *E* (*Eco*RI), *H* (*Hind*III), and *B* (*Bam*HI).