

### Immunoglobulin $\mu$ -Chain Gene Rearrangement in a Patient with T Cell Acute Lymphoblastic Leukemia

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**A**bstract. A 6-yr-old girl with T cell acute lymphoblastic leukemia (ALL) is described. She had a mediastinal mass and her leukemic cells expressed T cell-associated antigens (Leu 1+, OKT3+, OKT9+, and OKT10+). When we examined genomic DNA from the leukemic cells, we detected Ig  $\mu$ -chain gene rearrangement with germ-line configuration of light chain genes. As reported recently, detecting Ig gene rearrangement has become an important procedure for further classifying B cell precursor cells. This case, however, suggests that there is also heterogeneity among patients with T cell ALL, not only at the level of cell surface phenotypes, but also at the level of the Ig gene. These findings have major implications when we consider both the ontogenesis of these leukemic cells and the normal differentiation of human lymphocytes.

#### Introduction

Surface marker studies have made a major contribution to the understanding of acute lymphocytic leukemia (ALL)<sup>1</sup> and have demonstrated the heterogeneity of this disease (1–4). The three major subgroups of ALL, T cell, B cell, and common (non-T

cell, non-B cell) ALL, are generally defined by immunological markers. Heterogeneity within each subgroup can be further delineated by using T and B cell-associated monoclonal antibodies and studied by defining a hierarchy of Ig gene rearrangements. The majority of non-T, non-B ALLs seem committed to B cell differentiation, based on the expression of B cell-associated antigens (B-1, BA-1) and Ig gene analysis (5–7). The T cell origin of ALL, which is defined by the presence of the receptor for sheep erythrocytes and/or T cell-associated antigens, is also a heterogeneous group, particularly when seen within a framework of normal T cell differentiation (8–11). Theoretically, non-B cell lineage cells should retain germ-line configuration of their Ig genes. We present the case of a patient with T ALL in whom Ig  $\mu$ -chain gene rearrangement was also observed.

**Case report.** A 6-yr-old girl noted unilateral and progressive swelling of her neck. When admitted, she had hepatosplenomegaly and cervical lymphadenopathy. At that time, the white blood cell count was  $42 \times 10^9$ /liter, with 28% polys, 12% bands, 18% eosinophils, 12% lymphocytes, 2% monocytes, 3% myelocytes, and 25% blast cells. The hemoglobin was 14.2 g/dl and the platelet count was  $18.1 \times 10^9$ /liter. A chest x-ray revealed a large bilateral mediastinal mass. Bone marrow examination showed 80% replacement with lymphoblasts. The patient was treated on the modified LSA<sub>2</sub>/L<sub>2</sub> protocol and entered complete remission (12). She has no evidence of recurrent disease at the time of this report.

#### Methods

**Cell morphology and cytochemistry.** Cell morphology was examined on Wright-Giemsa stained smears. The French-American-British scheme was used for the morphological classification of leukemic cells (13). Sudan black B, peroxidase, nonspecific esterase, and periodic acid-Schiff (PAS) cytochemical stains were used.

**Immunologic studies.** The E-rosette assay was performed as described previously (14). Surface Ig (slg) was detected with fluoresceine isothiocyanate-conjugated goat anti-human Ig (polyvalent; Meloy Laboratories

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1. *Abbreviations used in this paper:* ALL, acute lymphoblastic leukemia; C, constant region; CALLA, common acute lymphoblastic leukemia antigen; cIg, cytoplasmic immunoglobulin; J<sub>H</sub>, heavy-chain joining gene; kb, kilobase; PAS, periodic acid-Schiff; slg, surface immunoglobulin.

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Inc., Springfield, VA). Cytoplasmic Ig (cIg) was detected with fluoresceine isothiocyanate-conjugated goat anti-human  $\mu$ -chain (Meloy Laboratories, Inc.) after the leukemic cells had been prepared by cytocentrifugation and fixed in ethanol and acetic acid (15). The monoclonal antibodies designated OKT3, OKT4, OKT6, OKT8, OKT9, OKT10, and B-1 were obtained from Ortho Pharmaceutical Corp. (Raritan, NJ), and Leu 1 was obtained from Becton, Dickinson and Co. (Sunnyvale, CA) (8, 9, 16, 17). Anti-Ia antibody, 21w4, was kindly provided by Dr. M. Letarte (The Hospital for Sick Children, Toronto) (18); BA-3 ([common ALL antigen] CALLA) and BA-2 antibody were provided by Dr. T. LeBien (University of Minnesota, Minneapolis, MN) (19). Cells were examined by fluorescence microscopy and by flow cytometry with the EPICS V system (Coulter Electronics Inc., Hialeah, FL).

**Immunoglobulin gene analysis.** High molecular weight DNA was extracted from the mononuclear cells (obtained from the bone marrow by Ficoll-Hypaque gradient centrifugation). Genomic DNAs from fibroblasts or thymocytes that have already been shown to have germ-line Ig genes were used as controls. These genomic DNAs were digested with BamH I or EcoR I restriction endonuclease, both of which are known to permit the demonstration of both rearranged and germ-line configuration of Ig genes (5, 7, 20–22). Digested DNA was size-fractionated by agarose gel electrophoresis and transferred to a nitrocellulose filter (23). Such filter-bound DNA fragments were then hybridized to nick-translated [ $^{32}$ P]DNA probes of the germ-line Ig genes and visualized on autoradiograms (24). The human Ig gene probes used are the constant (C) region of  $\mu$ -Ig genes ( $C\mu$ , 1.3-kilobase (kb) embryonic EcoR I probe) (20),  $\kappa$ -Ig genes ( $C\kappa$ , 2.5-kb embryonic EcoR I  $C\kappa$ -containing fragment) (21), and  $\lambda$ -Ig genes ( $C\lambda$ , 0.8-kb embryonic EcoR I-Hind III  $C\lambda$ -containing fragment) (22).  $C\mu$  germ-line clones were kindly provided by Drs. T. H. Rabbitts and G. Mattheysens (Laboratory of Molecular Biology, The Medical Research Council Centre, Cambridge, U. K.) (25) and the  $C\kappa$  and  $C\lambda$  germ-line clones by Dr. P. Leder (Department of Genetics, Harvard Medical School, Boston, MA) (21, 22).

## Results

**Morphology and cytochemical and immunological studies.** The patient's leukemic cells were classified as L1 morphology according to the French-American-British system and were PAS<sup>+</sup>, Sudan black B<sup>+</sup>, and nonspecific esterase<sup>−</sup>. The cells did not form E-rosettes and were negative when tested for sIg or cIg. The cells stained positively for the surface antigens Leu 1, OKT3, OKT9, and OKT10 but were negative for OKT4, OKT6, OKT8, Ia, CALLA, B-1, and BA-2 (Table I).

**Ig gene analysis.** The  $C\mu$  probe recognized a rearrangement of one  $C\mu$  allele when genomic DNA was digested with BamH I, whereas the other gene was retained in germ-line configuration (Fig. 1 A). This probe identified a 19-kb BamH I fragment in germ-line DNA. In addition, there was no restriction polymorphism. In parallel studies, 15 cases of non-T, non-B ALL, identified as Ia<sup>+</sup> and CALLA<sup>+</sup>, showed Ig  $\mu$ -chain gene rearrangement. Two additional cases of T ALL and six preparations of thymocytes from different individuals retained the germ-line configuration of  $C\mu$  genes.

Both  $C\kappa$  and  $C\lambda$  genes of this patient were present in germ-line configuration. The  $C\kappa$  probe consistently detected a 12.5-

Table I. Results of Immunological and Cytochemical Studies

Immunological markers		Cytochemistry	
	% fluorescent cells		
Ia	0	PAS	+
CALLA	0	SB	−
sIg	0	Peroxidase	−
cIg	0	NSE	−
B-1	6		
BA-2	4		
Leu 1	92		
OKT 3	60		
OKT 4	0		
OKT 6	0		
OKT 8	0		
OKT 9	95		
OKT 10	98		
	% rosettes		
E-rosettes	0.5		

SB, Sudan black B; NSE, nonspecific esterase.

kb BamH I fragment for the germ-line  $\kappa$ -genes (Fig. 1 A). The  $C\lambda$  probe recognized 9-, 16.5-, and 19-kb EcoR I fragments and an additional 21-kb fragment for germ-line  $\lambda$ -genes, corresponding to the type I/II pattern for  $\lambda$ -genes polymorphism described by Hieter et al. (22). To define rearrangements in the vicinity of the switch region instead of the heavy-chain joining gene ( $J_H$ ) region, Hind III-digested fragments were used. When the T ALL genomic DNA was digested with Hind III and hybridized to the  $C\mu$  probe, an 11.5-kb fragment that contained the switch region and lacked the  $J_H$  region was identified as germ-line  $C\mu$  genes (Fig. 1 B).

## Discussion

The detection of Ig gene rearrangements in leukemic cells, as reported by Korsmeyer et al. (7), has become an important procedure for further classification of non-T, non-B ALL and for our understanding of leukemic cell lineage. According to their report, cells from all 25 cases of non-T, non-B ALL—which displayed Ia (25/25), CALLA (18/25), BA-1 (20/24), and BA-2 (22/24) but lacked T cell markers and surface Ig—showed rearranged Ig genes, indicating that these cells are already committed to B cell lineage at the gene level. Our results in 15 newly diagnosed patients with non-T, non-B ALL (Ia<sup>+</sup>, CALLA<sup>+</sup>, with or without B-1 and BA-2) showed  $\mu$ -gene rearrangements and were consistent with their findings (data not shown). In contrast, none of the 11 T cell ALLs in the original study revealed Ig gene rearrangement; only in the T lymphoblastoid cell-line of HSB-2 was  $\mu$ -chain gene rearrangement observed (7).

The patient presented here was thought to have a fairly

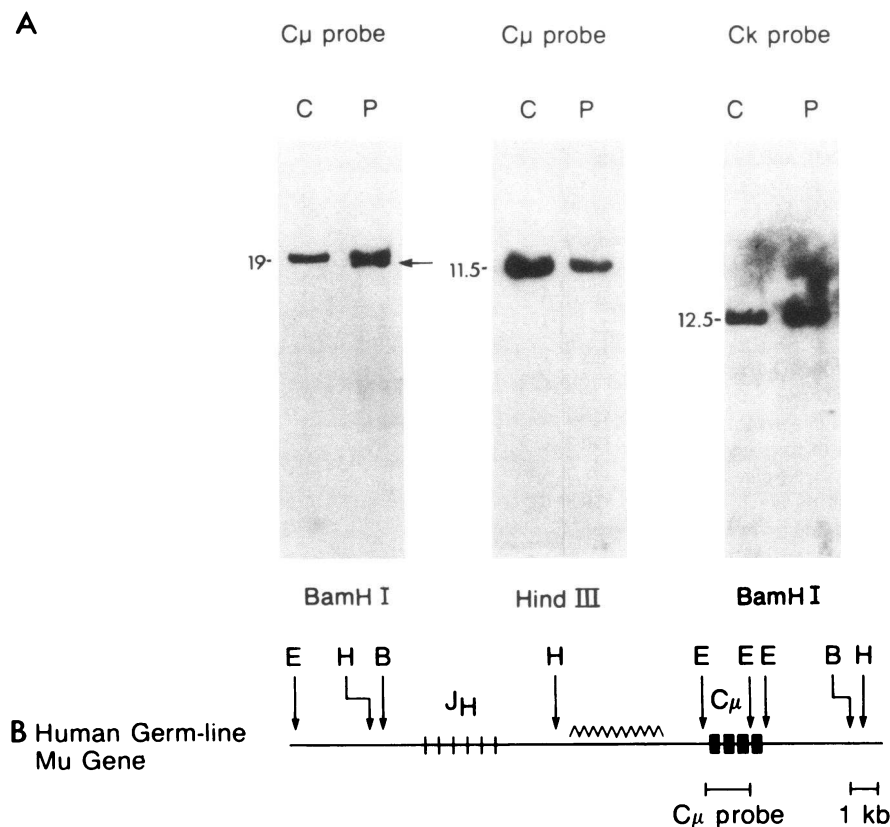


Figure 1. (A) Southern blot analysis to detect germ-line and rearranged Ig genes in the patient's leukemic cells (P) or control fibroblast DNA (C). The  $C\mu$  probe that contained a 1.3-kb germ-line EcoR I fragment could detect a germ-line gene in a 19-kb BamH I fragment and rearranged allele (arrow). This  $C\mu$  probe also could detect a germ-line gene in an 11.5-kb Hind III fragment. The  $C\kappa$  probe could identify a 12.5-kb BamH I fragment for germ-line  $\kappa$ -genes. Numbers indicate fragment sizes in kilobases. (B) Restriction enzyme map of human germ-line  $\mu$ -gene. The wavy line corresponds approximately to the length of the switch  $\mu$ -region. The restriction endonuclease cleavage sites are shown as E (EcoR I), H (Hind III), and B (BamH I).

typical case of childhood T cell ALL. Although her leukemic cells failed to form E-rosettes, she had a mediastinal mass and her leukemic cells were classified as L1 morphology and they stained with PAS. The cells expressed the surface antigens recognized by Leu 1, OKT3, and OKT10 and were negative for B cell-associated antigens; this is consistent with their emergence at the mature thymocyte stage (8–11). The additional lack of  $T_4$  and  $T_8$  antigens may correlate with the derivation of the cell from a  $T_3^+$ ,  $T_4^-$ ,  $T_8^-$  subset of T cells (8). The results of Ig gene analysis showed one  $\mu$ -chain gene rearrangement with germ-line configuration of the other allele and  $C\kappa$  and  $C\lambda$  genes. As previously reported, when genomic DNA was digested with BamH I and hybridized to the  $C\mu$  probe, BamH I restriction polymorphism was quite unlikely (7, 27). Two additional cases of T ALL and six experiments that used normal thymocytes were in germ-line configuration of their Ig genes. Although Ig gene rearrangement frequently occurs in mouse T cells (26), it seems to be unusual in human T cells. To our knowledge, this represents the first example of  $\mu$ -gene rearrangement detected in a newly diagnosed patient with T ALL, although there are a few reports of such rearrangements in cultured human T cell lines (7, 27).

When the cellular origin of this leukemic cell is considered,

it is difficult to accommodate the phenotypic appearance of the leukemic cells by surface marker analysis and Ig gene rearrangement studies. Leukemic blasts that express mixed or dual markers have been reported previously (28–30). Three explanations have been offered to explain mixed leukemia, including derepression of genetic material during neoplastic transformation, neoplastic expansion of a population of normal cells that express both markers, and transformation of a stem cell before the divergence of differentiation pathways. The most likely explanation in the present case is that leukemic transformation might have occurred at the level of the oligopotent progenitor cells, where cells are committed to B cell lineage differentiation, but that the cells failed to assemble complexed Ig genes correctly or were influenced by certain events of differentiation during the early stages of B cell differentiation. The rearrangements described in our case and in the reported T cell lines (7, 27) are always on one allele, which supports this hypothesis.

Recently, evidence for the translocation of the cellular gene homologous to the oncogene of avian retrovirus MC29 in Burkitt lymphomas and its rearrangement in the vicinity of the switch region has been reported (31, 32). One question that arises from these findings is whether Ig gene rearrangement in our case is

real or whether it represents oncogene rearrangements near the switch region. To answer this question, we digested the patient's genomic DNA using the Hind III restriction enzyme and hybridized it with the  $C\mu$  probe. This hybridized fragment contains the switch region but not the  $J_H$  region (Fig. 1 B). In this experiment, the patient's sample revealed the same germ-line configuration as the control (Fig. 1 A), indicating that the rearrangement was not near the switch region.

The present case illustrates the value of Ig gene analysis of leukemic cells and the potential to further our understanding of leukemic cell ontogenesis. The differences among the various subgroups defined in these ways may provide new insights into the heterogeneity of leukemia and may be correlated with differences in prognosis and response to treatment.

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