

## T CELL RECEPTOR AND IMMUNOGLOBULIN GENE REARRANGEMENTS IN ACUTE MYELOBLASTIC LEUKEMIA

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The nature of the blast cells in acute myeloblastic leukemia (AML)<sup>1</sup> has been examined extensively at the level of the phenotype. Morphology, using standard staining procedures such as Wright-Giemsa, is now supplemented with histochemical (1) or immunological methods (2-4) that detect markers associated with developmental stage or lineage. Morphological studies usually disclosed blasts that were homogeneous within each patient, although sufficient patient-to-patient variation was detected to provide a basis for the FAB classification (5). These observations were consistent with a view of AML similar to the blocked differentiation hypothesis developed from analysis of blasts in acute lymphoblastic leukemia (ALL) (6). In contrast, studies of chemically or immunologically defined markers revealed striking heterogeneity. In addition to markers associated with immaturity, such as HLA-DR (7), markers of the three lineages of myelopoiesis were encountered regularly. In some instances, lymphoid differentiation markers were found (8) in AML blasts.

The most complex phenotypes were those where markers of more than one lineage were detected simultaneously in individual blast cells (8-11). We have called this phenotype lineage infidelity (9), using the term to contrast with the concept that differentiation programs are followed faithfully in blast cells until some block is reached. We preferred this view over one that postulated biclonal disease, since, where genetic markers of clonality have been used, AML was found to be predominantly clonal (12).

Regardless of the interpretation, phenotypic studies are limited by the specificity and sensitivity of the reagents used to define markers. It is now feasible to examine leukemic blasts at the level of the genome. In both B cell and T cell malignancies, gene rearrangements have been found (13-15) similar to those that are considered to be first steps in the expression of Ig or T cell receptor (TCR) proteins. Further Ig gene rearrangements have been reported (16) in cell lines considered to be of myeloid origin. These genetic methods have advantages

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<sup>1</sup> *Abbreviations used in this paper:* AML, acute myeloblastic leukemia; TCR, T cell receptor for antigen.

over phenotypic markers; their lineage specificity is widely accepted, and in addition, they provide evidence of clonality (14).

In this paper we report findings in blasts from 24 AML patients. DNA from the cells was probed with cDNA from the  $\beta$  chain of TCR and Ig H and L chain probes. In three instances, rearrangements of the TCR were detected. Blasts of one of these patients also had Ig chain gene rearrangements.

## Materials and Methods

### *Patient Population*

Peripheral blood samples were collected from 24 patients with a diagnosis of AML based on conventional morphological and cytochemical criteria. The clinical characteristics of these patients were similar to those for other series from this institution (17, 18). The morphological classifications include FAB class 1–6, a mean age of 53.4 (median 53, range 18–82); a mean peripheral blast count of  $34,700 \times 10^6$  per liter (median 28,000 range 120–250,000). Blast cell phenotypes were determined as described by Smith et al. (9); the mAb My-906, a kind gift from Dr. Griffin of Dana Farber Institute, Boston, MA was added to the panel of immunological reagents. This antibody detects an antigen associated with granulopoietic differentiation (19). As in other studies (8–11) there was a marked patient-to-patient and blast-to-blast phenotypic variation. Single cells simultaneously expressing markers of more than one lineage were detected in 8 of 24 patients.

DNA from the blasts of three patients was not in germline configuration. The clinical features and blast cell characteristics of these three patients are summarized below.

*Patient 1.* A 66-y-old man presented with increasing shortness of breath. The hematological findings on admission were: hemoglobin, 94 g/liter; white blood cells,  $375,000 \times 10^6$  per liter (80% blasts); platelet count,  $76,000 \times 10^6$  per liter. The blast cells were considered M4; they were peroxidase positive (30%), esterases positive (25%), and expressed My-1 (18%), OKM1 (38%), MO2 (35%), My-906 (71%), or spectrin (1%). Doubly-marked cells (0.3%) were present, simultaneously expressing spectrin and My-906. The blast cells were not positive for T cell-associated surface markers or terminal deoxynucleotide transferase. Surface and cytoplasmic Ig were absent. The patient underwent leukapheresis but died within several hours of admission with extensive pulmonary and central nervous system leukemic infiltration.

*Patient 2.* A 66-y-old man presented with 4-mo history of pancytopenia and recurrent sore throat. On admission, the hematological findings were: hemoglobin, 84 g/liter; white blood cells,  $2,800 \times 10^6$  per liter (8% blasts); platelets,  $45,000 \times 10^6$  per liter. The bone marrow was hypercellular, with 42% blasts. The erythroid precursors were bizarre with megaloblastic and binucleate cells. The blast cells were considered M6; they were myeloperoxidase positive and expressed My-906 (6%), MO2 (9%), spectrin (19%), OKT3 (0.5%) and OKT8 (0.5%). Doubly-marked cells (0.1%) were detected expressing simultaneously spectrin and MO2, and spectrin and OKT8. This patient was treated with high-dose cytosine arabinoside according to the protocol now under test in this Institute (20, 21). He died 2 mo later with septicemia without achieving a remission.

*Patient 3.* A 66-y-old woman presented with sore throat. On admission, the hematological findings were: hemoglobin, 99 g/liter; white blood cells,  $2,700 \times 10^6$  per liter (64% blasts); platelets,  $236,000 \times 10^6$  per liter. The marrow was hypercellular, with 74% blasts. The blast cells were considered M1. They were myeloperoxidase positive and expressed My-7 (63%), My-906 (39%), and terminal deoxynucleotide transferase (13%). Doubly-marked cells (1.5%) were present, simultaneously expressing terminal deoxynucleotide transferase and My-7. The cells were not positive for T cell-associated surface antigens or surface and cytoplasmic Ig. The patient was treated with high-dose cytosine arabinoside. She died 6 mo later without ever achieving a complete remission.

### *Southern Blot Analysis*

A T cell-depleted, blast cell-enriched fraction was obtained from each peripheral blood sample by the two-stage procedure described previously (22). DNA was extracted from

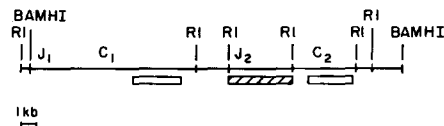


FIGURE 1. Schematic representation of the relationship of the hybridization region (white box) of the  $\beta$ -C probe to  $\beta$ -TCR DNA. The probe reacts with both  $C_1$  and  $C_2$  regions. As deduced from the restriction sites shown, this probe will detect a single 23.5 kb band in germline DNA digested with Bam HI and two bands, 11 and 4 kb, respectively, with Eco RI digestion. The hatched box shows the relationship of the  $JC_2$  probe to  $\beta$ -TCR DNA.

these populations by proteinase K digestion and phenol/chloroform extraction. Each DNA sample (10  $\mu$ g) was digested with 50 U of the restriction enzymes Bam HI and Eco RI in appropriate buffers at 37°C for at least 6 h to ensure complete digestion (23). The resulting DNA fragments were separated according to size by agarose gel (0.8%) electrophoresis in 40 mmol/liter Tris-acetate, 1 mmol/liter EDTA, pH 8.0. Hind III-digested  $\lambda$  phage DNA molecular weight markers were included on every gel. The sized-separated DNA fragments were transferred from agarose gel to Genatran filters (Schleicher and Schuell, Keene, NH) as described by Southern (24). The filters were prehybridized in solutions containing 50% (vol/vol) formamide, 5 $\times$  SSC, 0.05 M  $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$  (pH 6.7), 5 $\times$  Denhardt's, and 400  $\mu$ g/ml sonicated salmon sperm DNA at 42°C for 12–18 h. DNA on Genatran filters was hybridized to  $^{32}\text{P}$ -labeled DNA probes in hybridization solutions consisting of 50% (vol/vol) formamide, 5 $\times$  SSC, 0.02 M  $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$  (pH 6.7) 1 $\times$  Denhardt's, and 200  $\mu$ g/ml sonicated salmon sperm DNA at 42°C for 36–48 h. The filters were washed to a final solution of 0.1 $\times$  SSC at 65°C for 1 h, and autoradiographed at  $-70^\circ\text{C}$  with an intensifying screen.

#### Preparation of Labeled Probe DNA

A probe ( $\beta$ -C) was prepared from TCR  $\beta$  chain cDNA by nick-translating a 0.2 kb cDNA Ava I–Eco RV fragment. This fragment contains the constant ( $C_1$ ) region of the  $\beta$ -TCR gene, and crossreacts with the second constant region ( $C_2$ ). The hybridization regions of this probe to the TCR DNA are shown schematically in Fig. 1. As may be deduced from the restriction sites shown in Fig. 1, this probe will detect a single 23.5 kb fragment in germline DNA digested with Bam HI (Fig. 2a, lanes 4 and 5). With Eco RI digestion, the germline pattern consists of two bands, the 11 kb band represents the first constant region ( $C_1$ ), while the 4 kb band represents the second constant region ( $C_2$ ) (Fig. 2b, lane 4).

The  $JC_2$  probe consists of a 4 kb Eco RI fragment of the  $J_2$  region. This probe detects a 4 kb band in germline DNA digested with Eco RI (Fig. 2c, lane 3). It is useful in analyzing rearrangement into the  $C_2$  region.

The  $J\mu$  probe consists of a 3 kb cloned Eco RI–Hind III fragment, which contains the J region of the H chain Ig gene. In a Bam HI-digested germline DNA, this probe detects a single 18 kb band.

The  $C_k$  probe is a 1 kb Eco RI fragment from the C region of the  $\kappa$  L chain. This probe will detect a 12 kb band in germline configuration with Bam HI digestion.

#### Northern Blot and Dot Blot Analyses

RNA analysis was carried out on RNA samples from patients whose DNA showed a rearranged configuration detected with one of the above probes.

Total cellular RNA was extracted by guanidium thiocyanate/CsCl method (25). 10  $\mu$ g of each RNA sample was separated according to size by electrophoresis in 1.1% agarose gel. The size-separated fragments were transferred to Genatran filters by Northern techniques, prehybridized in solution containing 50% formamide (vol/vol), 5 $\times$  SSC, 0.05 M  $\text{Na}_2\text{HPO}_4$  (pH 6.7), 5 $\times$  Denhardt's solution, and 400  $\mu$ g/ml sonicated salmon sperm DNA, and hybridized to radiolabeled [ $^{32}\text{P}$ ]DNA  $\beta$ -C and  $C\mu$  probes described above, washed, and autoradiographed. RNA from T cell lines (Jurkat) and B cell line (RPMI-3638) were used as control.

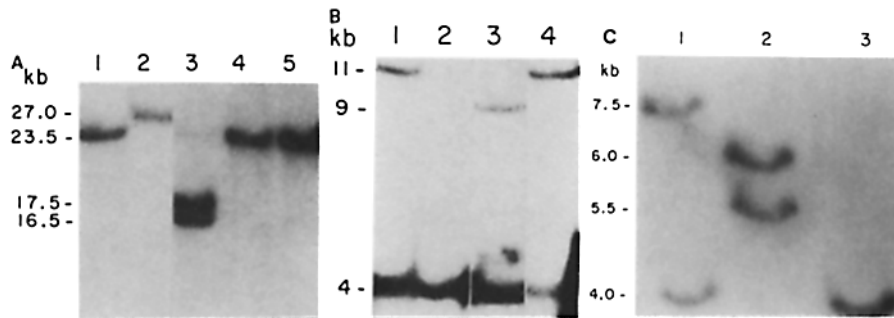


FIGURE 2. *A*, Bam HI digestion and hybridization to [ $^{32}$ P]-labeled  $\beta$ -C probe. Lane 1, DNA from patient 3 consisted of a single 23.5 kb band. Lane 2, DNA from patient 2. There is a 27.0 kb band. No germline band is detected. Lane 3, DNA from patient 1. The germline band is missing. There are two rearrangement bands; 17.5 and 16.5 kb, respectively. Lanes 4 and 5, Germline configuration consisting of a single 23.5 kb band. *B*, Eco RI digestion and hybridization to  $^{32}$ P-labeled  $\beta$ -C probe. Lane 1, DNA from patient 3. Two bands, 11 and 4 kb, were seen. The 11 kb band is less dense than the 4 kb band. Lane 2, DNA from patient 1. The 11 kb band is missing, only the 4 kb band is detected. Lane 3, DNA from patient 2. No germline 11 kb band is detected. There is a 9 kb band in addition to the 4 kb band. Lane 4, Germline configuration consisting of two bands, 11 kb and 4 kb, respectively. *C*, Eco RI digestion and hybridization to JC<sub>2</sub> probe. Lane 1, DNA from patient 3. The 4 kb germline band as well as a rearranged 7.5 kb band were detected. Lane 2, DNA from patient 1. The germline band is missing. Two rearranged bands, 6.0 and 5.5 kb, were detected. Lane 3, Germline configuration consisting of a single 4 kb band.

RNA samples obtained from  $10^5$ ,  $5 \times 10^4$ ,  $2.5 \times 10^4$ ,  $1.25 \times 10^4$ ,  $0.6 \times 10^4$  patients' cells were spotted onto Genatran filters, prehybridized for 8–12 h, then hybridized with [ $^{32}$ P]DNA  $\beta$ -C probe, washed, and autoradiographed.

### Cytogenetics

Blast-enriched populations were cultured in  $\alpha$ -MEM with HTB9-conditioned medium (26) as a source of growth factor for 72 h (27). Chromosome harvests were initiated by incubation with colchicine (0.02  $\mu$ g/ml) followed by treatment with hypotonic KCl (0.075 M) and fixation three times with 3:1 methanol/glacial acetic acid. Chromosome spreads were prepared following standard methods, and identified using trypsin G-banding (28).

## Results

### $\beta$ -TCR Rearrangement

DNA samples from 24 AML patients were analyzed. DNA samples from 22 patients showed a 23.5 kb band with the  $\beta$ -C probe and Bam HI digestion (Fig. 2a, lanes 4 and 5). In 21 patients, two bands, 11 and 4 kb, respectively, were seen with  $\beta$ -C probe and Eco RI digestion (Fig. 2b, lane 4). DNA from 22 patients, digested with Eco RI and probed with the JC<sub>2</sub> probe showed a 4 kb band (Fig. 2c, lane 3). Thus, in the blast cell DNA of 21 of 24 patients, convincing evidence of rearrangement was not found.

*Patient 1.* DNA from this patient, when digested with either Bam HI (Fig. 2a, lane 3) or Eco RI (Fig. 2b, lane 2) and analyzed with  $\beta$ -C probe was not in germline configuration. The 23.5 kb germline band was missing in the Southern blot of the Bam HI digest, and there were two rearranged bands of 17.5 and 16.5 kb, respectively. This configuration suggests rearrangement of both  $\beta$ -TCR loci. With Eco RI digestion, the 11 kb band corresponding to the C<sub>1</sub> region was

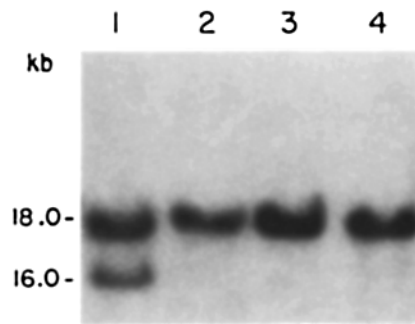


FIGURE 3. Bam HI digestion and hybridization with  $J\mu$  probe. Lane 1, DNA sample from patient 1 showed an additional 16.0 kb band as well as the 18.0 kb germline band. Lanes 2-4, DNA samples from other patients; only the 18.0 kb germline band was detected.

absent; only the 4 kb band, corresponding to  $C_2$  region, was detected. This configuration is consistent with deletion of  $C_1$  regions from both loci. With the  $JC_2$  probe and Eco RI digestion, two rearranged bands, 6.0 kb and 5.5 kb, were present; the 4 kb germline band was absent (Fig. 2c, lane 2). This configuration suggests rearrangement into both  $C_2$  loci.

*Patient 2.* With Bam HI digestion and  $\beta$ -C probe (Fig. 2a, lane 2), there is a single 27 kb rearranged band. Since the germline band was not detected, and there is only one rearrangement band, this configuration is consistent with deletion of one  $\beta$ -TCR locus and rearrangement of the remaining locus. This is confirmed by Eco RI digestion. In this case, no germline 11 kb band was detected, a rearranged 9 kb band was seen in addition to the 4 kb germline band. This shows rearrangement into  $C_1$  locus.

*Patient 3.* With Bam HI digestion and  $\beta$ -C probe (Fig. 2a, lane 1) there is a single 23.5 kb band. With Eco RI digestion (Fig. 2b, lane 1), two bands, 11 and 4 kb, respectively, were seen (Fig. 2b, lane 1). The 11 kb band is less dense than the 4 kb band; this is consistent with deletion of one  $C_1$  locus. With the  $JC_2$  probe and Eco RI digestion, a 4 kb germline band and a rearranged 7.5 kb band were seen (Fig. 2c, lane 1), confirming rearrangement into one  $C_2$  locus with deletion of one  $C_1$  locus.

### *Ig Rearrangement*

When digested with Bam HI and hybridized with  $^{32}P$ -labeled  $J\mu$  probe, DNA samples from 23 of 24 patients showed a single germline 18.0 kb band. (Fig. 3, lanes 2-4). Patient 1's DNA showed an additional 16 kb band in addition to the 18 kb germline band (Fig. 3, lane 1). No rearrangement of the  $\kappa$  chain was detected with the  $C_k$  probe in any patient's sample.

*Dot blot analysis.* RNA samples from patient 1, patient 2, and patient 3 expressed a  $\beta$ -TCR gene transcript (Fig. 4a, lanes 3 and 4).

*Northern blot analysis.* RNA samples from patient 1 expressed a 1.0 kb  $\beta$ -TCR mRNA, while RNA from Jurkat cell line expressed the complete 1.3 kb  $\beta$ -TCR gene transcript as well as the 1.0 kb transcript (Fig. 4b). No  $\mu$  H chain Ig mRNA could be detected by Northern blot analysis (Fig. 4c).

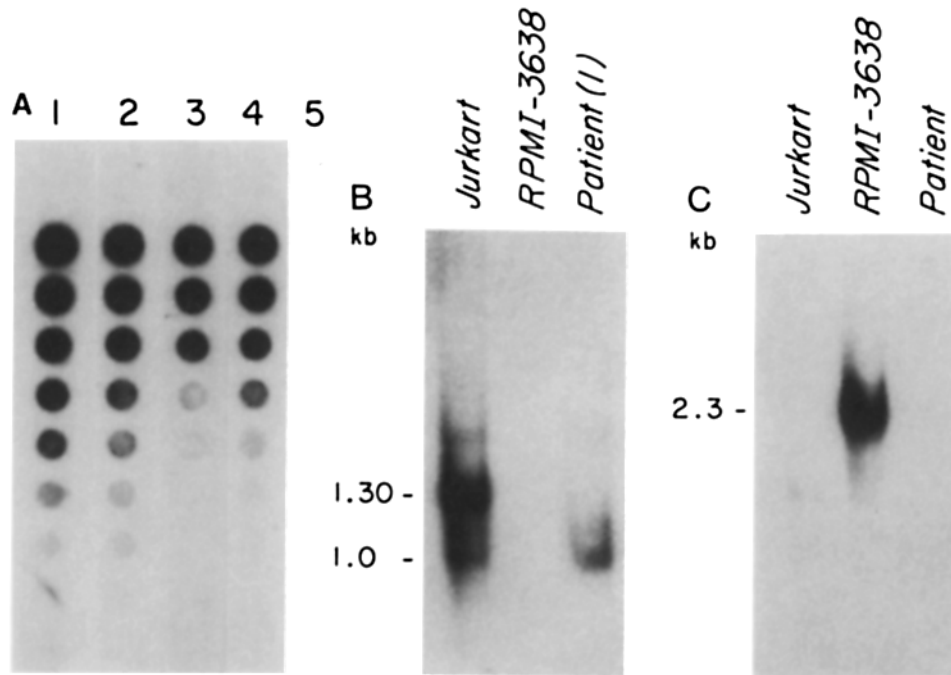


FIGURE 4. A, Dot blot analysis is shown of RNA from patients with T-ALL (lanes 1 and 2) patient 1 (lane 3), patient 2 (lane 4), expressed  $\beta$ -TCR gene transcript. No transcript was detected in RNA from a patient with B-CLL (lane 5). Data from patient 3 was not shown. B, Northern Blot analysis is shown of RNA from the Jurkat cell line (T cell line) expressed 1.30 kb  $\beta$ -TCR gene transcript and an incomplete 1 kb message on Northern blot analysis. RNA from patient 1 expressed an incomplete 1.0 kb message. No  $\beta$ -TCR transcript was detected in RPMI-3638 (B cell line). C, A 2.30 kb Ig H chain transcript was expressed in RPMI-3638 RNA. RNA from Jurkat cell line and patient 1 did not express an Ig gene message transcript.

#### Karyotypic Analysis

The karyotypes of patient 1 and patient 2 are shown in Fig. 5, *a* and *b*, respectively.

In patient 1, a 46 XY, 7:12 translocation karyotype was found. In patient 2, the cells were hypodiploid with consistent loss of chromosomes 7, 17, 22, and Y; a 1:5 translocation and three marker chromosomes were also identified. In patient 3, a karyotypic analysis was not done.

#### Discussion

Rearrangements of Ig genes are generally considered to be lineage-specific events, reflecting the commitment of stem cells to differentiation along the B lymphocyte lineage. Such rearrangements, while common in cases of human B cell malignancy, are a rare event in non-B cell neoplasia (29). Similarly, in T cell malignancies, rearrangement of the  $\beta$ -TCR genes have been detected (15).

In this study, we analyzed the organization and expression of the Ig H and L chain genes as well as the  $\beta$ -TCR gene in 24 patients with a diagnosis of AML. The blast cells from all these patients fulfilled the morphological and cytochemical criteria of AML by FAB classification. Moreover, when studied with a panel

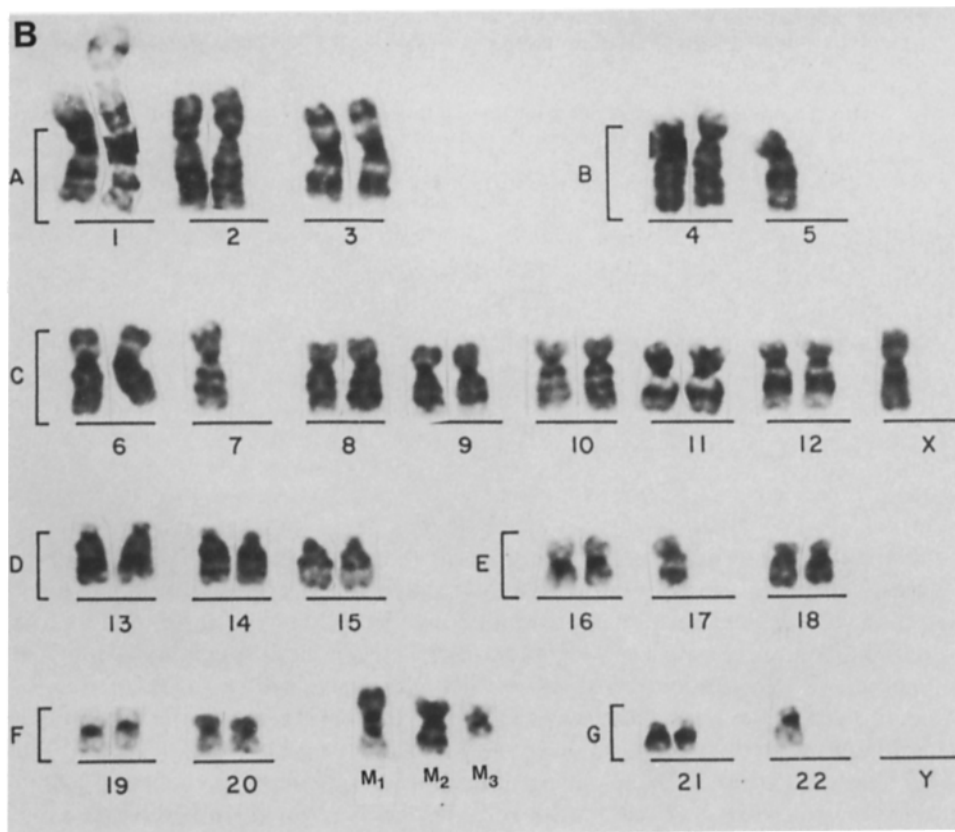
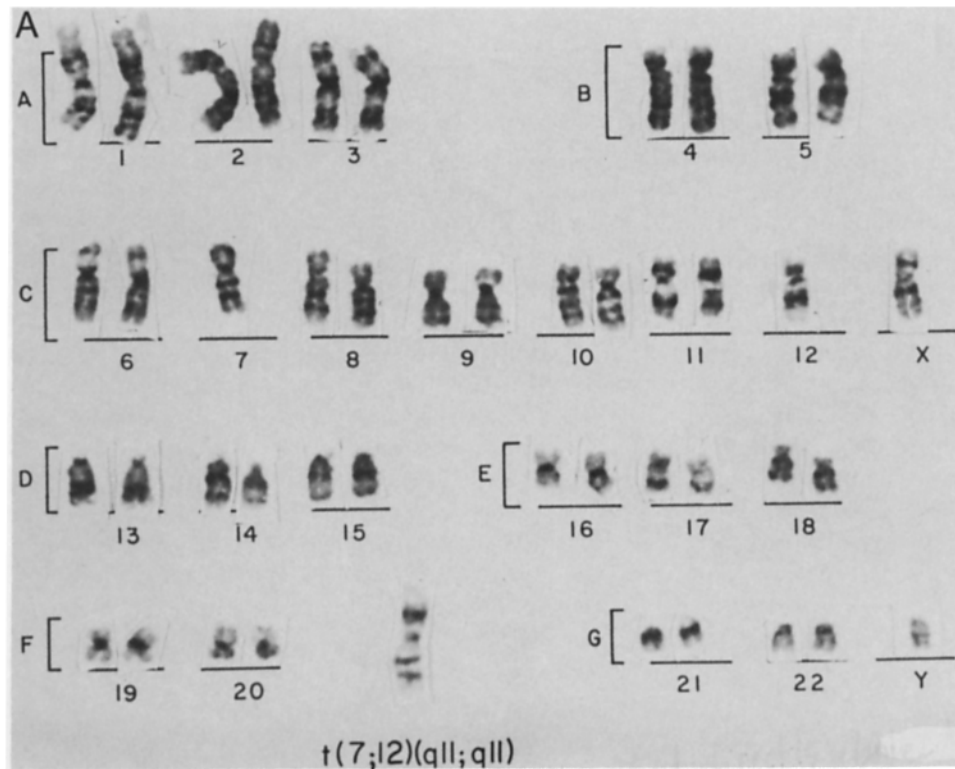


FIGURE 5. *A*, Karyotype of patient 1. There is a 7:12 translocation. *B*, Karyotype of patient 2. The cells are hypodiploid. There were consistent 1:5 translocations, deletions of Y chromosomes, and monosomies in 7, 17, 22. Three marker chromosomes were detected.

of immunological reagents, blast cells from these patients expressed one or more markers associated with the myeloid lineages. Cells from some of these patients simultaneously expressed markers of more than one myeloid lineage, (intra-lineage infidelity) or markers associated with both lymphoid and myeloid lineages (interlineage infidelity). Abnormalities of  $\beta$ -TCR gene were seen in DNA samples from 3 of 24 patients; in addition, changes were found in Ig genes of patient 1. While other mechanisms (for example random deletion) cannot be excluded, the observations are most readily explained by gene rearrangement. It seems unlikely that three random events, affecting the leukemic population in different patients, would mimic the changes firmly associated with the rearrangements that occur during lymphopoiesis. The frequency of rearrangements in AML blasts detected here is a minimal estimate, since analysis with more probes and different restriction enzymes might detect further abnormalities.

The rearranged configurations differed in these three patients, indicating that in myeloid leukemia, rearrangements of the  $\beta$ -TCR or Ig genes may have the same diverse pattern as in lymphoid malignancies (14). On Northern blot analysis, patient 1 RNA expressed an incomplete 1.0 kb  $\beta$ -TCR transcript, which is reminiscent of the joining of the D, J, and C regions of the  $\beta$ -TCR gene (DJC transcript) (30), or a JC transcript (31); no Ig H chain mRNA was detected despite a rearranged J<sub>H</sub> band. These findings suggested that, at least in myeloid leukemia, rearrangement of the  $\beta$ -TCR or Ig gene loci does not always imply expression of a functional mRNA transcript. This is supported by the absence of T cell surface antigens or surface or cytoplasmic Ig in this patient's blast cells. In contrast, in patient 2, T cell-associated markers were found on occasional blast cells.

DNA samples from two patients showed only the rearranged configuration. These data are consistent with the view that the individual patient's leukemic cells had the same gene rearrangements and therefore were clonal, despite considerable heterogeneity in surface phenotypes. It follows that the genetic markers associated with lymphoid differentiation were present in the same cells that express phenotypic markers associated with granulopoiesis. In patient 3, an apparent germline configuration was detected with the  $\beta$ -C probe and Bam HI digestion, but rearrangement into the C<sub>2</sub> locus was demonstrated with JC<sub>2</sub> probe following Eco RI digestion. These molecular findings for patient 3, while compatible with a single clonal population, cannot be considered as conclusive as those for the previous two patients, since the normal bands evident in the Southern blots could be derived for a second coexisting population.

Cytogenetic analysis revealed abnormal karyotypes in two patients with rearrangement of  $\beta$ -TCR gene. Patient 1 showed a 7:12 translocation, while patient 2's blast cells were hypodiploid, with 1:5 translocation, deletion of chromosomes 7, 17, 22, and Y, as well as three additional marker chromosomes. The  $\beta$ -TCR gene is located on band q<sup>35</sup> of chromosome 7 (32) and the karyotypes of both of these patients showed an abnormality involving chromosome 7; however, the breakpoint in patient 1 is distant from the  $\beta$ -TCR gene locus. In patient 2, the rearranged DNA configuration suggested deletion of one  $\beta$ -TCR locus, this is consistent with the loss of one chromosome 7.

The significance of  $\beta$ -TCR or Ig gene rearrangement in myeloid leukemia



remains to be determined. The restriction patterns observed in at least two patients provide evidence that the disease was clonal; therefore in these instances the coexistence of two clones, as reported recently by Siegelman et al. (33) in lymphoma, is not a probable basis for the findings. Rather, events within a single leukemic clone require explanation. Stem cell phenotypes and their patterns of predetermined differentiation are not known. It is possible, therefore, that the association of  $\beta$ -TCR and Ig gene rearrangements with predetermined myelopoiesis may not be abnormal. This view is supported by our failure to find complete VDJ joining.

The restriction enzyme patterns are consistent with DJ joining, an event that might occur without leading to lineage commitment. It is also possible that leukemic transformation itself may be able to confer myelopoietic specificity on stem cells that have already undergone some gene rearrangement. Nonetheless, our findings are consistent with the concept of lineage infidelity. Indeed, the hypothesis of abnormal gene expression in leukemia predicts that unusual molecular configurations will be encountered. There are now numerous examples of mutations, gene amplifications, and rearrangements in leukemia (34–36). Further direct studies of gene expression are needed if these molecular findings are to be related unequivocally to leukemic patterns of growth and maturation.

### Summary

The organization and expression of the  $\beta$  chain of T cell antigen receptor gene ( $\beta$ -TCR) and Ig H and L chain genes were analyzed by Southern blot technique in 24 patients with a diagnosis of acute myeloblastic leukemia (AML). Rearrangements of the  $\beta$ -TCR genes were seen in DNA samples from 3 of the 24 patients. One of these three patients also showed rearrangement of the Ig H chain gene. RNA samples from all three patients expressed a  $\beta$ -TCR gene transcript on dot blot analysis. However, on Northern blot analysis, one patient expressed an incomplete 1.0 kb transcript and no Ig H chain mRNA, despite a rearranged configuration. The karyotypes of two of these patients showed abnormalities involving chromosome 7. Rearrangements of T cell antigen receptor genes may occur in nonlymphoid malignancy, and is consistent with the concept of lineage infidelity in AML.

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