

REARRANGEMENTS OF IMMUNOGLOBULIN AND T CELL
RECEPTOR β AND γ GENES ARE ASSOCIATED WITH
TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE
EXPRESSION IN ACUTE MYELOID LEUKEMIA

BY ROBERT FOA,* GIULIA CASORATI,[‡] MARIA CRISTINA GIUBELLINO,*
GIUSEPPE BASSO,[§] RAFFAELLA SCHIRÒ,[¶] GIOVANNI PIZZOLO,[¶]
FRANCESCO LAURIA,** MARIE-PAULE LEFRANC,^{‡‡}
TERENCE H. RABBITS,^{§§} AND NICOLA MIGONE[‡]

*From the *Dipartimento di Scienze Biomediche e Oncologia Umana, Sezione di Clinica Medica;
‡Istituto di Genetica Medica and Centro CNR Immunogenetica e Istocompatibilità, University of
Torino, 10126 Torino; §Dipartimento di Pediatria, University of Padova, 35128 Padova;
¶Clinica Pediatrica, University of Milano, Ente Ospedaliero Monza, 20052 Monza; ¶Cattedra
di Ematologia, University of Verona, 37134 Verona; **Istituto di Ematologia L. & A.
Seragnoli, University of Bologna, 40138 Bologna, Italy; the ‡‡Laboratoire d'Immunogénétique,
Université des Sciences et Techniques du Languedoc, 34060 Montpellier Cedex, France; and
the §§Medical Research Council Laboratory of Molecular Biology,
Cambridge, United Kingdom CB2 2QH*

In the classification of acute leukemias, the nuclear enzyme terminal deoxy-nucleotidyl transferase (TdT)¹ is a marker commonly used to differentiate between the lymphoid or myeloid origin of the neoplastic clone. Common, T, and null acute lymphoblastic leukemias (ALL) are characteristically TdT⁺, reflecting the proliferation of immature lymphoid cells, while TdT is usually negative in acute myeloid leukemia (AML). However, it is well documented that a small proportion (5–10%) of AML are TdT⁺ (1, 2). The explanation for this unexpected, though consistent finding, has remained uncertain.

The analysis of DNA rearrangements at the Ig and T cell receptor (TCR) chain regions has proved a valuable approach toward a more accurate definition of the cell lineage, clonal origin, and stage of differentiation of acute and chronic B and T lymphoid leukemias (3–12). In the same way, the T cell rearranging γ gene (TRG- γ) is an indicator of clonal proliferation characteristic of human T cells since in all forms of T cell leukemia so far reported, a TRG- γ rearrangement occurs (13–15).

To better characterize the distinctive features between TdT⁻ and TdT⁺ AML, and possibly to define the cell lineage of the latter cases, we have studied at the

This work was supported by Centro Nazionale delle Ricerche, Progetto Finalizzato "Oncologia," grant 85.02160.44, Rome. M. C. Giubellino received a fellowship from the G. Ghirelli Foundation. Address correspondence to Robert Foa, M.D., Dipartimento di Scienze Biomediche e Oncologia Umana, Sezione di Clinica Medica, Via Genova 3, 10126 Torino, Italy.

¹ *Abbreviations used in this paper:* ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; EM, electron microscopy; TdT, terminal deoxynucleotidyl transferase; TCR, T cell receptor; TRG, T cell rearranging gene; WBC, white blood cells.

molecular level a series of 52 AML, 10 of which were TdT⁺. The leukemic population was typed for markers of DNA rearrangements both at the H (Igh) and L (Ig κ , Ig λ) chain Ig genes, the TCR- β chain locus, and the TRG- γ region. A highly significant correlation between TdT expression and DNA rearrangement at the Igh locus was found; 8 of the 10 TdT⁺ AML carried new configurations at the Igh region, while this occurred only in 2 of 42 TdT⁻ cases. Furthermore, clear or suggestive signs of TCR- β and/or TRG- γ gene rearrangement appeared restricted to the TdT⁺, Igh-rearranged group. These findings suggest that most TdT⁺ AML appear characterized by the expansion of a very early cell population molecularly committed along the lymphoid differentiation pathway, irrespective of the persistent expression of myeloid-related features.

Materials and Methods

Patient Population. 52 AML patients were included in the study, including 10 TdT⁺ AML patients. The diagnosis of AML was based on morphological and cytochemical criteria; myeloperoxidase was assessed in all cases with light microscopy, and in two TdT⁺ AML (patients 5 and 8), also with electron microscopy (EM). Diagnosis was also based on the absence of the common ALL-associated antigen, as well as other B (OKB2, BA1, B1) and T (Leu9, T1, T11, T6, T3, T4, T8) cell-related markers (except for patient 6, see below, who showed a biphenotypic picture), and/or, in the TdT⁺ cases, also on the expression of one or more antigens recognized by anti-myeloid mAbs. For this latter purpose the following reagents were used (partly commercial and partly obtained from the 2nd International Workshop, Boston, MA, 1984): My4, My7, My9 (Coulter Immunology, Hialeah, FL), Mo5 (16), NHL30.5 (17), LeuM1 (Becton Dickinson & Co., Sunnyvale, CA), UCHM1 (Techno Genetics, Torino, Italy), MG1 (Techno Genetics), OKM1 (Ortho Diagnostic Systems, Inc., Westwood, MA), anti-lysozyme antiserum (muramidase; Dako Corp., Copenhagen, Denmark). All immunological characterizations were carried out on the bone marrow or, less frequently, on the peripheral blood leukemic population after fractionation on a Lymphoprep (Nyegaard A/C, Oslo, Norway) gradient. The indirect immunofluorescence technique used has been described in detail elsewhere (18). For nuclear TdT, cytocentrifuge spreads were fixed in cold methanol for 30 min, transferred to cold PBS for 30 min, and stained using a rabbit anti-TdT and, as a second layer, a FITC-F(ab')₂ goat anti-rabbit IgG (both purchased from Bethesda Research Laboratories, Gaithersburg, MD). The myeloid affiliation of the 10 TdT⁺ patients and the clinical outcome of each patient were as follows.

Patient 1. A 9-yr-old girl with a white blood cell (WBC) count of 3.99×10^{10} per liter was classified as M1 according to the FAB classification. The blasts were myeloperoxidase-positive (48%) and 89% expressed the OKM1 antigen; 52% were TdT⁺. The patient went into complete remission with an anti-myeloid protocol, but relapsed after 8 mo. She underwent an allogeneic bone marrow transplantation, which was followed by a second marrow relapse 7 mo later. The child was then refractory to treatment and died 5 mo later.

Patient 2. A 45-yr-old woman with a WBC count of 6×10^9 per liter was classified as M5. 52% of the blasts were muramidase-positive; 45 and 40% expressed the My9 and My7 antigens, respectively, while 80% were TdT⁺. She was treated with an anti-myeloid regimen and she died during induction.

Patient 3. A 3-mo-old girl with a WBC count of 6.5×10^{11} per liter was classified as M5. 2% of the blasts were myeloperoxidase-positive; 70% were recognized by My9, 65% by NHL30.5, 70% by UCHM1, and 69% by Mo5. TdT was positive in 72% of the elements. A partial remission was obtained after 14 mo of treatment with anti-myeloid and anti-lymphoid cytotoxic agents. 1 mo later the child showed a complete relapse.

Patient 4. A 12-yr-old girl with a WBC count of 1.1×10^{10} per liter was classified as M1. 40% of the blasts reacted with LeuM1, 30% with NHL30.5, 62% with Mo5, and 23% with UCHM1. TdT was positive in 18% of the blasts. Double labeling showed that

TdT⁺ cells were also Mo5⁺. Complete remission was obtained with Vincristine, Adriamycin, and Prednisone. 11 mo later, however, the child relapsed.

Patient 5. A 2-mo-old girl with a WBC count of 1.1×10^{12} per liter was classified as M5. 2% of the blasts were myeloperoxidase-positive as shown by light microscopy; this was further confirmed by EM. 66% of the blasts were positive for My7, 50% for My9, 69% for Mo5, and 74% for LeuM1. 71% of the elements were TdT⁺. Despite the rotation of different cytotoxic agents the child died without achieving a complete remission.

Patient 6. A 13-yr-old girl with a WBC count of 1.98×10^{11} per liter was classified as L1 (50%)/M5 (50%). This patient showed a biphenotypic picture: one myeloid, documented by the reactivity with My9 (41%) and MG1 (48%), and one lymphoid, according to the expression of J5 (common ALL antigen; 45%), OKB2 (44%), and BA1 (41%). 90% of the elements (both myeloid and lymphoid) were TdT⁺. The child was refractory to all forms of treatment and died without obtaining a complete remission.

Patient 7. A 41-yr-old man with a WBC count of 2.1×10^{10} per liter was classified as M4. Myeloperoxidase was positive in 40% of the blasts and nonspecific esterase in 90%. 95% of the elements were TdT⁺. The patient, treated with Adriamycin, Ara-C, 6-Thioguanine, and Vincristine was in complete remission 18 mo after diagnosis.

Patient 8. A 4-yr-old boy with a WBC count of 2×10^{11} per liter was classified as M5. The blasts were myeloperoxidase-positive as shown by EM, and 20% were muramidase-positive, 80% My9⁺, and 79% My4⁺. 80% of the elements were TdT⁺. Complete remission was achieved after 1 yr of combined chemotherapy with anti-lymphoid and anti-myeloid agents. He then underwent an autotransplant, but relapsed soon after.

Patient 9. An 8-yr-old girl with a WBC count of 4×10^{10} per liter was classified as M1. 54% of the blasts were myeloperoxidase-positive, 36% were OKM1⁺, and 38% were recognized by My7. 26% of the elements were TdT⁺. Double labeling suggested that TdT was not present on the myeloperoxidase-positive cells. The child was induced with an anti-myeloid regimen, was in remission for 2 mo, then relapsed and died.

Patient 10. A 7-yr-old girl with a WBC count of 2×10^{10} per liter was classified as M1. 25% of the blasts were myeloperoxidase-positive, 9% were My9⁺, and 15% reacted with OKM1. 25% of the elements were TdT⁺. Double labeling showed that myeloperoxidase-positive cells were TdT⁻. The child, treated with an anti-myeloid protocol, died during induction.

DNA Analysis. High molecular weight DNA was extracted from the leukemic cell population separated as described above. After digestion with the appropriate restriction enzymes (see legend to Table I) and electrophoresis on 0.8–0.6% agarose gel, DNA was blotted on nitrocellulose paper and hybridized to ³²P nick-translated probes as already described (4). The Igh, Igx, Igl, TCR-β, and TRG-γ regions were examined by means of the following probes: Igh, 3.3-kb Eco RI–Hind III fragment containing the 3' half of the Igh joining (J_H) elements (19); Igx, 2.7-kb Eco RI fragment isolated from the recombinant phage Huk (20), carrying the region coding for the constant domain of the κ light chain gene (Cκ); Igl, 1.2-kb Bam HI fragment derived from the recombinant phage Huλ5 (21), containing the constant Cλ2 gene that encodes the Kern⁻, Oz⁻ isotype; TCR-β, 400-bp Bgl II cDNA fragment derived from JUR-β2 clone (22) coding for most of the Cβ2 constant domains; TRG-γ, 700-bp Hind III–Eco RI genomic fragment containing the joining (Jγ1) region, ahead of the Cγ1 segment (23). The latter three probes hybridize equally well to the other highly homologous members of their gene families (i.e., to Cλ1 and Cλ3–9, Cβ1, and TRG Jγ2, respectively). The maps of all these gene regions have been reported in detail in the above cited references.

Results

Ig Gene Analysis. In 8 of 10 TdT⁺ AML a rearrangement of the Igh gene region was found (Fig. 1, Table I). In one of the latter cases (Table I, patient 2), a reorganization of the κ light chain was also documented (Fig. 2). The two germline TdT⁺ AML (Table I, patients 9 and 10) showed relatively low per-

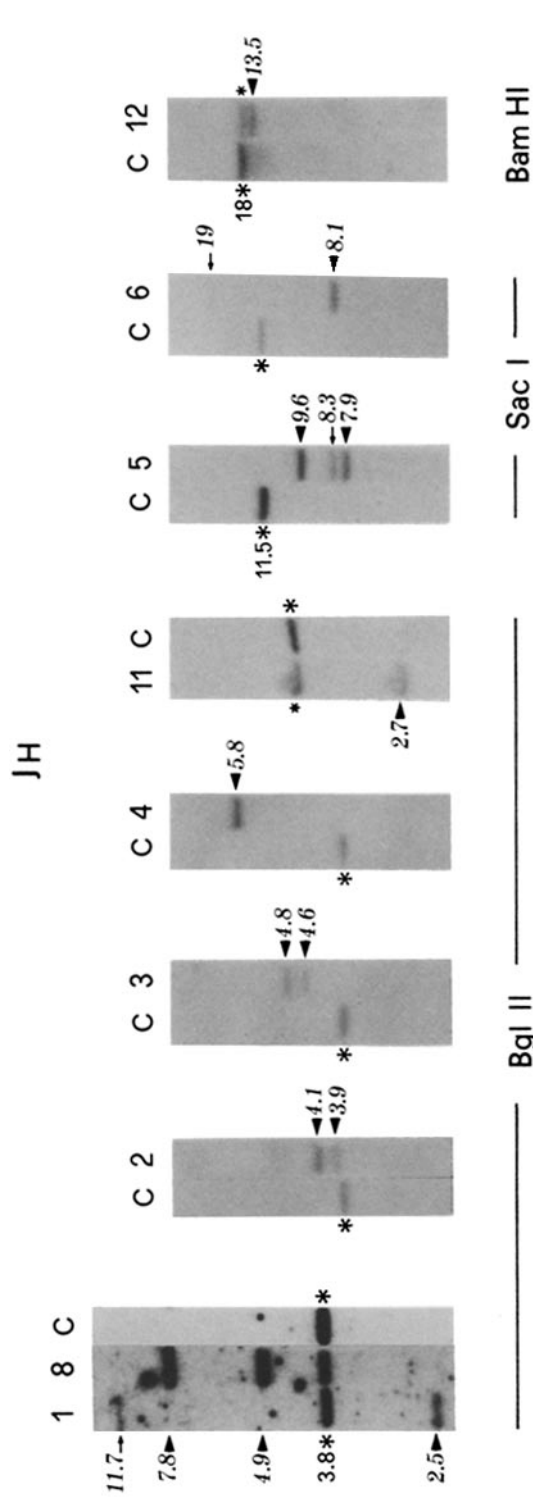


FIGURE 1. Southern blot hybridization to the J_H probe of seven representative TdT^+ (patients 1-6 and 8) and two TdT^- (patients 11 and 12) AML cases after digestion with Bgl II (patients 1-4, 8, 11), Sac I (patients 5 and 6) and Bam HI (patient 12). Lane numbers refer to individual samples as coded in Table 1; (C) represents unrearranged, control DNA. The size of the rearranged and germline fragments, indicated with arrows and asterisks, respectively, are in kilobases. In a short-run gel, patient 4 did show a second rearranged band of 1.3 kb.

TABLE I
*Analysis at the Igh, Igκ, Igλ, TCR-β, and TRG-γ Gene Loci in 10 TdT⁺ and 2
 Rearranged TdT⁻ AML*

Patient	TdT	MPO*	Igh [‡]		Igκ [‡]	Igλ		TCR-β [‡]	TRG-γ [‡]	
			Configura- tion	Restriction enzyme used		Configu- ration	Restriction enzyme used		Configura- tion	Restriction enzyme used
1	52	48	Grr	Bgl II	G	—	—	GR	GR/G	Bam HI, Eco RI
2	80	0	RR	Bgl II, Eco RI	GR	—	—	G	G/G	Bam HI, Eco RI
3	72	2	RR	Bgl II	G	—	—	G	G/G	Bam HI, Eco RI
4	18	0	RR	Bgl II	G	—	—	G	G/G	Bam HI, Eco RI
5	71	2	RRr	Bgl II, Sac I	G	—	—	G	G/G + r	Bam HI, Eco RI, Sac I
6 [†]	90	0	RR	Sac I, Hind III	G	—	—	G	G/G	Bam HI, Sac I, Hind III
7	95	40	RR	Eco RI, Sac I	—	—	—	—	G/G + RRR	Eco RI
8	80	0	GRR	Bgl II	G	—	—	G	GR/G	Bam HI, Eco RI
9	26	54	G	Bgl II, Bam HI, Eco RI, Sac I, Pst I, Hind III	G	G	Eco RI, Hind III	G	G/G	Bam HI, Eco RI
10	25	25	G	Bgl II, Bam HI, Pst I, Hind III	G	G	Eco RI	G	G/G	Bam HI, Eco RI
11**	0	80	GR	Bgl II, Eco RI, Sac I, Pst I	GR	G	Eco RI	—	G/G	Eco RI
12 ^{‡‡}	0	0	GR	Bam HI, Eco RI, Sac I, Hind III	GR	G	Eco RI, Hind III	G	G/G	Bam HI, Eco RI

* Myeloperoxidase at light microscopy.

‡ G, germline; R, rearranged; r, rearranged fragments of faint intensity.

‡ Only restriction enzyme Bam HI used.

† A tentative assignment of the TRG-γ "genotype" differentiating between Cγ1 and Cγ2 gene involvement is shown: Cγ1 "genotype" is shown on the left side of the shell, and Cγ2 on the right. The three Rs added to the G/G TRG-γ genotype of patient 7 indicate that three conspicuous, rearranged fragments were found over an apparently typical germline background (see Fig. 3 and text for details).

† The 90% TdT expression and the absence of a germline Igh fragment suggest that the apparent biphenotypic population (myeloid and lymphoid, see Results) are most likely derived from a common precursor.

** Patient 11 was classified as M1; in addition to myeloperoxidase, the leukemic cells were also sudan black-positive, and negative for B and T lymphoid markers.

‡‡ Patient 12 was classified as M4; 20% of the elements were naphthyl acetate esterase-positive; 10% also showed nonspecific esterase positivity (sensitive to sodium fluoride). 80% and 10% of the blasts were MG1⁺ and OKM1⁺, respectively, while they were negative for B and T lymphoid markers.

centages of TdT⁺ cells (26% and 25%). Double staining indicated that TdT was in fact not expressed on the blasts carrying myeloid markers.

40 of the 42 TdT⁻ AML showed the expected germline configuration of the Igh region. The two patients that carried a J_H rearrangement also showed a reorganization of the κ light chain gene. The difference in the incidence of J_H rearrangements between TdT⁺ and TdT⁻ AML was statistically highly significant ($p = 2.5 \times 10^{-6}$). Whenever possible, the rearranged configurations were confirmed with different restriction enzymes to rule out polymorphisms (see Table I). Possible evidence of the latter phenomenon was observed only with the

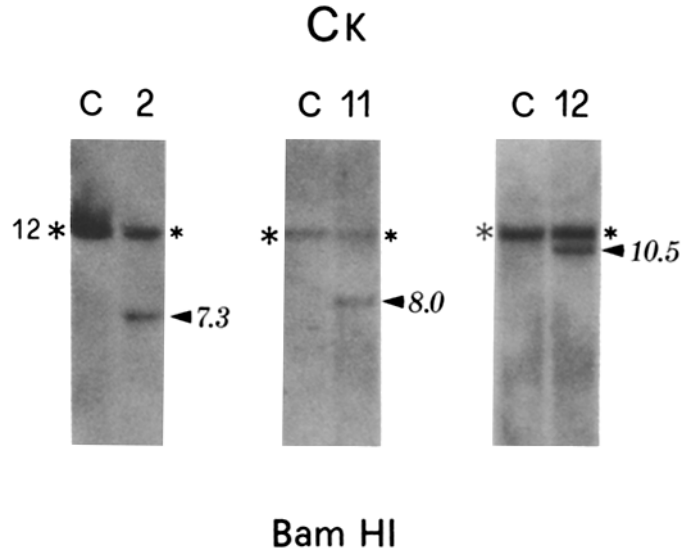


FIGURE 2. Southern blot analysis of the three AML (patients 2, 11, and 12) exhibiting a $C\kappa$ rearrangement. In all cases, Bam HI restriction enzyme was used. The sizes in kilobase of germline (asterisks) and rearranged (arrows) bands are indicated. C, control DNA.

J_H probe in patients 9 and 11. These two patients showed a discordant pattern respectively with one of the six (Hind III) and one of the four (Bgl II) restriction enzymes used. All together, 10 of the 52 AML tested with the J_H probe and 3 of the 46 AML studied with the $C\kappa$ probe presented a novel DNA configuration at the respective loci (Table I). However, an atypical rearranged pattern was observed in 3 of the 10 above-mentioned patients, i.e., more than the two expected J_H -hybridizing fragments were found in patients 1, 5, and 8 (see Fig. 1). No $Ig\lambda$ gene rearrangement could be identified among the 26 samples studied, including two with a $C\kappa$ rearrangement.

TCR- β and TRG- γ Gene Analysis. The TCR- β and TRG- γ loci were analyzed in 47 and 51 patients, respectively. While the great majority of samples showed no signs of TCR gene rearrangement, four patients (1, 5, 7, and 8) presented suggestive evidence of TCR- β and/or TRG- γ gene involvement. The pattern of gene rearrangement varied markedly among these four patients, either in the number or in the intensity of the novel bands. The novel Bam HI and Eco RI fragments detected by the TRG- γ probe had the expected size of the most common $V\gamma$ gene-containing segments (14, 15, and our unpublished observations) and did not fit with an incomplete digestion pattern. Indeed, an apparently typical clonal pattern of rearrangement could be recognized in patients 1 and 8, in whom either both TCR- β and TRG- γ or TRG- γ only were respectively involved. In these two samples, the novel rearranged fragments gave a hybridization signal not dissimilar to the one shown by the unrearranged counterparts (Fig. 3). In the two remaining samples, the rearranged bands appeared too faint (patient 5) or there were too many (patient 7) to account for a clonal involvement of the entire neoplastic cell population. All four patients with true or accessory

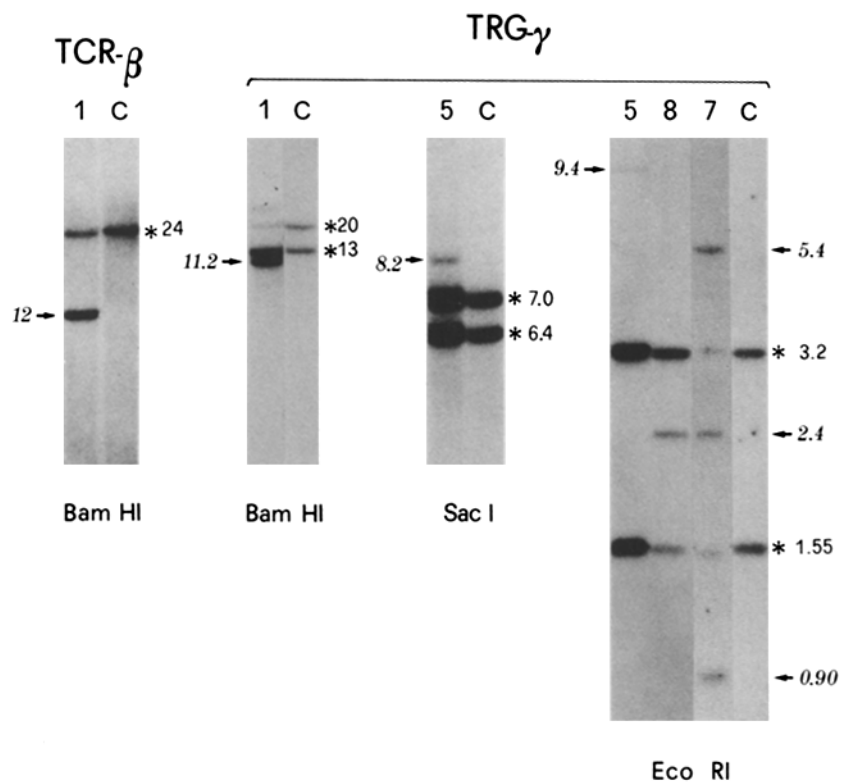


FIGURE 3. TCR- β and TRG- γ gene configuration, after Southern blot hybridization with the respective probes, of four rearranged TdT⁺ AML (patients 1, 5, 7, and 8). (C) contain unrearranged control DNA. The two TCR- β constant genes, C β 1 and C β 2, are both located within the 24-kb Bam HI germline fragment; the two J γ gene segments, J γ 1 and J γ 2, migrate on different fragments after Bam HI, Sac I, and Eco RI digestion, i. e., the J γ 1 on the 20-, 6.4-, and 1.55-kb; the J γ 2 on the 13-, 7.0-, and 3.2-kb fragment, respectively (13, 23, and our unpublished observations). In patient 1, the reduced signal of the higher molecular weight bands can be ascribed to partial DNA degradation. The TRG- γ pattern of the latter case shows an intensity ratio between the novel 11.2- and the 13-kb germline band of ~ 0.5 , which is consistent with a germline configuration at both C γ 2 gene regions. Whether a second C γ 1 allele is or is not rearranged can not be clearly established because of the just-mentioned partial degradation of the higher molecular weight fragments. However, the presence of a residual signal at the 20-kb germline position seems to favor the involvement of only one C γ 1 allele.

signs of TCR- β or TRG- γ gene rearrangement were encountered among the J_H-rearranged TdT⁺ AML.

Discussion

The cell origin of the rare cases of TdT⁺ AML has remained a matter of debate. This issue, along with the biological implications related to the significance of the inappropriate expression of TdT in apparently myeloid blasts, has clinical relevance, especially in view of the overall bad prognoses of these patients and their poor response to treatment (2). The enzyme TdT, associated with the early stages of normal and malignant T and B cell differentiation, is found in bone marrow and thymus lymphoid cells, as well as in T, common, and null ALL

(1); on the other hand, TdT is absent in the great majority of AML (1, 2), thus representing a valuable and widely exploited diagnostic marker between lymphoid and myeloid acute leukemias. Functionally, this enzyme catalyzes the polymerization of deoxynucleotides on the 3'-hydroxyl terminus of an oligonucleotide primer in the absence of a template (24). Although its function during the ontogeny of the lymphoid lineage is not yet clear, it has been suggested that it may have an ancillary role in the D-J_H or V-DJ_H recombination (25). Studies of murine pre-B cell lines transformed by Abelson murine leukemia virus (26, 27) are consistent with the suggestion that the enzyme may be responsible for the addition of extra nucleotides (the so-called N regions) at the junctions of V, D, and J segments.

In this study, we have analyzed the DNA configuration at the Ig H and L, TCR- β , and TRG- γ loci in a group of 52 patients with AML, which included 10 TdT⁺ cases. The myeloid affiliation of the TdT⁺ cases was based on morphological, cytochemical, and immunological features (myeloperoxidase positivity and/or expression of one or more myeloid-associated antigens), together with the absence of B and T cell-related markers. Our data show a significant correlation between Igh rearrangement and TdT expression. 8 of the 10 J_H-rearranged cases belonged in fact to the TdT⁺ group, while only 2 of the 42 TdT⁻ cases showed evidence of J_H rearrangement ($p = 2.5 \times 10^{-6}$). This latter finding confirms that an Igh involvement in classical TdT⁻ AML is indeed a very rare event (28-30).

A reorganization of the Ig κ region could also be demonstrated in 3 of the 46 AML tested (1 TdT⁺ and 2 TdT⁻). As expected, in all three the Igh locus was also rearranged. Thus, it appears that the rare Ig rearrangements observed in non-B leukemias are not confined only to the Igh locus but may also take place at the κ L chain gene region, the involvement of which is conventionally associated with a more mature level of differentiation.

The data obtained with the TCR- β and TRG- γ DNA probes show that these loci may be rearranged in AML (4 of 52; patients 1, 5, 7, and 8), the rearrangements being in all cases restricted to the TdT⁺ subgroup carrying a novel J_H configuration. While in patients 1 and 8 the TCR- β and/or TRG- γ gene organization points to a typical monoclonal pattern, in the remaining two the TRG- γ novel fragments appeared too faint (patient 5) or there were too many (patient 7) to be compatible with a monoclonal situation. It is of interest that a similar heterogeneity, both in number and intensity of rearranged fragments, was also observed after analysis with the J_H probe in three of the above four mentioned patients (No. 1, 5, and 8). The coexistence in patients 1 and 8 of three J_H-containing fragments, one of which is germline (with only one chromosome rearranged at the TCR- β and/or TRG- γ loci), may have different explanations. A trisomy of chromosome 14, a J_H duplication due to unequal chromatid exchanges, or a mixture of two cell populations, one carrying the B and one the T markers of rearrangement, may account for these findings. Even in the case of the latter possibility, i.e., biclonality, myeloid markers would be present on both these cell populations (see Results). In the other two samples with both Igh and TRG- γ clonal markers (patients 5 and 7), the absence of any germline J_H-containing bands implies the existence of a clone(s) carrying both regions rear-

ranged within the same cell. Again, aneuploidy or unequal sister chromatid exchange may be responsible for the additional rearranged bands. Should this be the case, the hypothesis of oligoclonality would be questionable, while the concept of a concomitant involvement within a single cell of the Igh and TRG- γ loci would still hold.

Can these findings help to unravel the apparently conflicting TdT expression in leukemic blasts exhibiting myeloid features? The highly significant correlation between TdT positivity and Igh rearrangement reported here supports the view that the expression of the enzyme TdT may indeed be coupled to the activity of the V-(D)-J-specific recombinase/s (25–27). The evidence that both Igh and TRG- γ rearrangements positively correlate with TdT expression favors the concept that a unique enzymatic machinery might be involved in the rearrangement of these B and T cell-specific loci (27).

This study suggests a predominant lymphoid commitment of TdT⁺ AML. The pattern of genomic configuration, i.e., J_H gene rearrangement sometimes associated with a TCR- β and/or TRG- γ gene reorganization, is similar to that found in non-T-ALL (12, 31 and our unpublished observations) and seems therefore to point to a B cell affiliation for most of these cases. It is conceivable that in these patients the target of the leukemic event is represented by a very early TdT⁺ progenitor cell that is molecularly and not yet phenotypically B lineage oriented, but that is also capable of expressing some myeloid-related features, possibly due to a transient lineage spill-over before reaching a more restricted lymphoid expression. These findings suggest that the expression of TdT in apparently myeloid blasts does not appear due to the expression of abnormal differentiation (lineage infidelity) (32). As suggested by Greaves et al. (33), we favor the hypothesis that in the normal process of early hemopoietic differentiation, a certain degree of promiscuity may occur so that some markers, normally restricted to different cell lineages, may be transiently coexpressed. This is further confirmed by patient 6, who showed an Igh rearrangement with no evidence of germline bands (and 90% TdT positivity), despite a clear biphenotypic pattern (myeloid and lymphoid), and by patient 8, who recently relapsed, and who expressed B cell-related antigens, having lost all myeloid features (our unpublished data). While in most TdT⁺ J_H-rearranged AML the expression of myeloid markers may be a temporary event retained by lymphoid committed blasts, in the two germline TdT⁺ patients, the lack of clonal markers points to the highly immature stage of cell differentiation, such that a firm conclusion on the cell origin of these proliferations can not be reached.

Finally, the heterogenous phenotypic and molecular potential of the leukemic population documented by this study may lead to an understanding of the difficulties in the treatment of these patients, and their frequent refractiveness to the commonly used cytotoxic agents.

Summary

The cell origin of the rare terminal deoxynucleotidyl transferase (TdT)-positive acute myeloid leukemias (AML) was investigated at the molecular level, by examining the configuration of the Ig H (Igh) and L (Ig κ , Ig λ) chain gene regions, and of the T cell receptor (TCR) β and T cell rearranging (TRG) γ loci.

In 8 of the 10 TdT⁺ AML analyzed (classified as myeloid according to morphological and cytochemical criteria, and to the reactivity with one or more anti-myeloid mAbs), a rearrangement of the Igh chain gene was found. In TdT⁻ AML, evidence of an Igh gene reorganization was instead observed only in 2 of the 42 patients studied. Furthermore, evidence of TCR- β and/or TRG- γ gene rearrangement was observed in four AML, all of which belonged to the Igh-rearranged TdT⁺ group. In three cases (one TdT⁺ and two TdT⁻), the Ig κ L chain gene was also in a rearranged position. These findings demonstrate a highly significant correlation between TdT expression and DNA rearrangements at the Igh and TCR chain gene regions and support the view that this enzyme plays an important role in the V-(D)-J recombination machinery. Overall, the genomic configuration, i.e., J_H gene rearrangement sometimes coupled to a κ L chain and TCR gene reorganization, similar to that found in non-T-ALL, suggests that in most cases of TdT⁺ AML, the neoplastic clone, despite the expression of myeloid-related features, is characterized by cells molecularly committed along the B cell lineage.

We thank Dr. T. Honjo for the gift of the H24 phage, Dr. P. Leder for the Huk and Hu λ 5 phages, and Dr. T. Mak for the TCR- β cDNA probe. We also thank Olga Varetto for excellent technical assistance.

Received for publication 14 July 1986 and in revised form 20 October 1986.

References

1. Coleman, M. S., and J. J. Hutton. 1981. Terminal transferase. *In* The leukemic Cell. D. Catovsky, editor. Churchill-Livingstone, London, United Kingdom. 203-219.
2. Jani, P., W. Verbi, M. F. Greaves, D. Bevan, and F. Bollum. 1983. Terminal deoxynucleotidyl transferase in acute myeloid leukaemia. *Leuk. Res.* 7:17.
3. Korsmeyer, S. J., A. Arnold, A. Bakhshi, J. V. Ravetch, U. Siebenlist, P. A. Hieter, S. O. Sharrow, T. LeBien, J. H. Kersey, D. G. Poplack, P. Leder, and T. A. Waldmann. 1983. Immunoglobulin gene rearrangement and cell surface antigen expression in acute lymphocytic leukemias of T cell and B cell precursor origins. *J. Clin. Invest.* 71:301.
4. Foa, R., N. Migone, M. Saitta, M. T. Fierro, M. C. Giubellino, P. Lusso, L. Cordero di Montezemolo, R. Miniero, and F. Lauria. 1984. Different stages of B cell differentiation in non-T acute lymphoblastic leukemia. *J. Clin. Invest.* 74:1756.
5. Nadler, L. M., S. J. Korsmeyer, K. C. Anderson, A. W. Boyd, B. Slaughenhaupt, E. Park, J. Jensen, F. Coral, R. J. Mayer, S. E. Sallan, J. Ritz, and S. F. Schlossman. 1984. B cell origin of non-T cell acute lymphoblastic leukemia. A model for discrete stages of neoplastic and normal pre-B cell differentiation. *J. Clin. Invest.* 74:332.
6. Foa, R., N. Migone, G. Basso, G. Cattoretti, G. Pizzolo, F. Lauria, G. Casorati, M. C. Giubellino, F. Capuzzo, A. Cantu'-Rajoldi, P. Lusso, A. O. Carbonara, and F. Gavosto. 1986. Molecular and immunological evidence of B-cell commitment in "null" acute lymphoblastic leukaemia. *Int. J. Cancer.* 38:317.
7. Waldmann, T. A., M. M. Davis, K. F. Bongiovanni, and S. J. Korsmeyer. 1985. Rearrangements of genes for the antigen receptor on T cells as markers of lineage and clonality in human lymphoid neoplasms. *N. Engl. J. Med.* 313:776.
8. Rabbitts, T. H., A. Stinson, A. Forster, L. Foroni, L. Luzzatto, D. Catovsky, L. Hammarstrom, C. I. E. Smith, D. Jones, A. Karpas, J. Minowada, and A. M. R.

- Taylor. 1985. Heterogeneity of T-cell β -chain gene rearrangement in human leukemias and lymphomas. *EMBO (Eur. Mol. Biol. Organ.) J.* 4:2217.
9. Aisenberg, A. C., T. G. Krontiris, T. W. Mak, and B. M. Wilkes. 1985. Rearrangement of the gene for the beta chain of the T-cell receptor in T-cell chronic lymphocytic leukemia and related disorders. *N. Engl. J. Med.* 313:529.
 10. Rambaldi, A., P.-G. Pelicci, P. Allavena, D. M. Knowles II, S. Rossini, R. Bassan, T. Barbui, R. Dalla-Favera, and A. Mantovani. 1985. T cell receptor β chain gene rearrangements in lymphoproliferative disorders of large granular lymphocytes/natural killer cells. *J. Exp. Med.* 162:2156.
 11. Foa, R., P.-G. Pelicci, N. Migone, F. Lauria, G. Pizzolo, F. Flug, D. M. Knowles II, and R. Dalla-Favera. 1986. Analysis of T-cell receptor beta chain ($T\beta$) gene rearrangements demonstrates the monoclonal nature of T-cell chronic lymphoproliferative disorders. *Blood.* 67:247.
 12. Foa, R., N. Migone, and F. Gavosto. 1986. Immunological and molecular classification of acute lymphoblastic leukaemia. *Haematologica.* 71:277.
 13. Lefranc, M.-P., and T. H. Rabbitts. 1985. Two tandemly organized human genes encoding the T-cell γ constant-region sequences show multiple rearrangement in different T-cell types. *Nature (Lond.)*. 316:464.
 14. Lefranc, M.-P., A. Forster, R. Baer, M. A. Stinson, and T. H. Rabbitts. 1986. Diversity and rearrangement of the human T cell rearranging γ genes: nine germ-line variable genes belonging to two sugroups. *Cell.* 45:237.
 15. Quertermous, T., C. Murre, D. Dialynas, A. D. Duby, J. L. Strominger, T. A. Waldmann, and J. G. Seidman. 1986. Human T-cell γ chain genes: organization, diversity, and rearrangement. *Science (Wash. DC)*. 231:252.
 16. Todd III, R. F., J. A. Roach, and M. A. Arnout. 1985. The modulated expression of Mo5, a human myelomonocytic plasma membrane antigen. *Blood.* 65:964.
 17. Askews, D. S., A. C. Eaves, and F. Takei. 1985. NHL 305: a monoclonal antibody reactive with an acute myeloid leukemia (AML)-associated antigen. *Leuk. Res.* 9:135.
 18. Foa, R., L. Baldini, G. Cattoretti, P. Foa, M. Gobbi, F. Lauria, E. Madon, G. Maserà, R. Miniero, G. Paolucci, P. Paolucci, G. Perona, G. Pizzolo, M. Saitta, and F. Gavosto. 1985. Multimarker phenotypic characterization of adult and childhood acute lymphoblastic leukaemia: an Italian multicentre study. *Br. J. Haematol.* 61:251.
 19. Migone, N., J. Feder, H. Cann, B. van West, J. Hwang, N. Takahashi, T. Honjo, A. Piazza, and L. L. Cavalli-Sforza. 1983. Multiple DNA fragment polymorphisms associated with immunoglobulin μ chain switch-like regions in man. *Proc. Natl. Acad. Sci. USA.* 80:467.
 20. Hieter, P. A., J. V. Maizel, and P. Leder. 1982. Evolution of human immunoglobulin KJ region genes. *J. Biol. Chem.* 257:1516.
 21. Hieter, P. A., G. F. Hollis, S. J. Korsmeyer, T. A. Waldmann, and P. Leder. 1981. The clustered arrangement of immunoglobulin lambda light chain constant region genes in man. *Nature (Lond.)*. 294:536.
 22. Yoshikai, Y., D. Anatoniou, S. P. Clark, Y. Yanagi, R. Sangster, P. Van den Elsen, C. Terhorst, and T. W. Mak. 1984. Sequence and expression of transcripts of the human T-cell receptor β -chain genes. *Nature (Lond.)*. 312:521.
 23. Lefranc, M.-P., A. Forster, and T. H. Rabbitts. 1985. Two distinct T cell γ variable region genes rearranged in human DNA. *Nature (Lond.)*. 319:420.
 24. Bollum, F. J. 1978. Terminal deoxynucleotidyl transferase: biological studies. *Adv. Enzymol.* 47:347.
 25. Alt, F. W., and D. Baltimore. 1982. Joining of immunoglobulin heavy chain gene segments: implications for a chromosome with evidence of three D-JH fusions. *Proc. Natl. Acad. Sci. USA.* 79:4118.

26. Desiderio, S. V., G. D. Yancopoulos, M. Paskind, E. Thomas, M. A. Boss, N. Landau, F. W. Alt, and D. Baltimore. 1984. Insertion of N regions into heavy-chain genes is correlated with expression of terminal deoxytransferase in B cells. *Nature (Lond.)*. 311:752.
27. Yancopoulos, G. D., T. K. Blackwell, H. Suh, L. Hood, and F. Alt. 1986. Introduced T cell receptor variable region gene segments recombine in pre-B cells: evidence that B and T cells use a common recombinase. *Cell*. 44:251.
28. Ha, K., M. Minden, N. Hozumi, and E. W. Gelfand. 1984. Immunoglobulin gene rearrangement in acute myelogenous leukemia. *Cancer Res.* 44:4658.
29. Rovigatti, U., J. Mirro, G. Kitchingman, G. Dahl, J. Ochs, S. Murphy, and S. Stass. 1984. Heavy chain immunoglobulin gene rearrangement in acute nonlymphocytic leukemia. *Blood*. 63:1023.
30. Cheng, G. Y., M. D. Minden, B. Toyonaga, T. W. Mak, and E. A. McCulloch. 1986. T cell receptor and immunoglobulin gene rearrangements in acute myeloblastic leukemia. *J. Exp. Med.* 163:414.
31. Pelicci, P.-G., D. M. Knowles II, and R. Dalla Favera. 1985. Lymphoid tumors displaying rearrangements of both immunoglobulin and T cell receptor genes. *J. Exp. Med.* 162:1015.
32. Smith, L. J., J. E. Curtis, H. A. Messner, J. S. Senn, H. Furthmayr, and E. A. McCulloch. 1983. Lineage infidelity in acute leukemia. *Blood*. 61:1138.
33. Greaves, M. F., L. C. Chan, A. J. W. Furley, S. M. Watta, and H. V. Molgaard. 1986. Lineage promiscuity in hemopoietic differentiation and leukemia. *Blood*. 67:1.