Papers

Configuration of immunoglobulin and T cell receptor β and γ genes in acute myeloid leukaemia: Pitfalls in the analysis of 40 cases

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Abstract

Aims: To evaluate the overall incidence of immunoglobulin (Ig) and T cell receptor (TCR) β and γ gene rearrangements in a series of 40 cases of acute myeloid leukaemia (AML) and to determine whether structural modifications of these genes could be correlated with the abnormal expression of lymphoid markers in malignant cells.

Methods: All cases were classified according to the criteria of the FAB group and immunophenotyped with a panel of monoclonal antibodies reactive with myeloid and lymphoid differentiation antigens. DNA analysis was performed by the method of Southern using probes for the Ig J_H, TCR-C β 1, and TCR-J γ 1 regions.

Results: Phenotypic analysis showed that in addition to myeloid markers, 10 cases expressed lymphoid antigens: CD7 in seven (of which three were TdT positive, one CD2 positive, and one CD19 positive) and CD19 in three. Southern blot analysis showed that bands with sizes different from the germ line control were present in the TCR β genes in 11 cases: in six of 30 with pure myeloid phenotype and in five of 10 of those expressing lymphoid markers. A close observation of the size and patterns of those bands, however, showed that they could be artefactual. Indeed, further analysis showed that they were either due to resistant *Eco* RI/*Hind* III sites at the β locus or to plasmid contamination. Rearranged genes were eventually found in only two of the 40 cases: at the Ig J_H region in one of the 30 with pure myeloid phenotype (3.3%) and at the TCR γ genes in one of 10 with lymphoid markers (10%).

Conclusions: These observations showed that Ig/TCR gene rearrangements were rare in this AML series (overall incidence of 5%) and that they were not significantly more common in cases with aberrant expression of lymphoid markers. The size and pattern of the potential non-germline bands that can be found in these loci must be carefully evaluated.

The immunological analysis of leukaemic cells in acute myeloid leukaemia (AML) has been particularly helpful in the characterisation of poorly differentiated cases where the assignment of malignant cells as myeloid is not easy using conventional morphology and cyto-

chemistry.^{1,2} On the other hand, it has shown that leukaemic cells with otherwise unequivocal myeloid features, such as myeloperoxidase expression, may often exhibit nuclear or membrane antigens that in normal haemopoiesis are restricted to B or T lymphoid lineages. Expression of terminal deoxynucleotidyl transferase (TdT), a nuclear enzyme known to be involved in the generation of diversity of Ig and TCR genes,^{3,4} has been reported to occur in 5-10% of AML cases^{1,5-7} and to be associated with the morphologically immature subtypes M0 and M1.² A similar incidence and morphological correlation has also been found for the expression of CD7, a 40 kilodalton protein of unknown function, present on the membrane of normal thymocytes, mature T lymphocytes, T lymphoblastic leukaemias⁸⁻¹¹ and also in a pluripotent progenitor cell capable of multilineage differentiation.^{12,13} The biological importance of these findings is unknown despite the considerable debate it has raised—namely the cellular origin of these leukaemias and their clinical behaviour.^{14,15} The results on the analysis of Ig or TCR genes in these cases have also been controversial. Several reports point to a high incidence of Ig or TCR rearrangements in TdT positive AML.¹⁶⁻¹⁹ Other studies have failed to show such a correlation between the expression of TdT or CD7 and rearrangements at these loci.^{11,20-22} The reasons for this discrepancy are not clear but may be related to analysis of different patient groups (adults rather than children), stringency of criteria for the immunological diagnosis of AML, or even misinterpretation of Southern blot results.

Methods

Forty patients (31 adults and nine children) with newly diagnosed acute leukaemia were studied. The morphological diagnosis of AML was based on the examination of bone marrow and peripheral blood slides stained with May-Grünwald-Giemsa and the cytochemistry reactions, Sudan Black B or myeloperoxidase, and α -naphthyl-acetate esterase.

All cases were classified according to the revised criteria of the FAB classification²³ and were distributed as follows: M1-7; M2-7; M3-1; M4-6; M5a-12; M5b-2; M6-1. In four patients whose leukaemic blast cells were unreactive with SBB, MPO, and ANAE (cases 8, 18, 19 and 29), AML (M0) was diagnosed, based on the reactivity with the myeloid antibodies CD33 or CD13, and the absence of

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lymphoid markers other than TdT or CD7.²⁴ Case 17 was classified as M6 by the presence of typical morphology and the reactivity with a glycophorin antibody in 37% of the blast cells.

IMMUNOLOGICAL STUDIES

The mononuclear cell fraction of peripheral blood or bone marrow was isolated by density gradient centrifugation with Lymphoprep (Nyegaard). Cytocentrifuge slides were prepared for cytochemical reactions and immunocytochemistry.

The panel of monoclonal antibodies used to identify the phenotype of blast cells included the following markers reactive with haemopoietic precursors and myeloid cells: CD34 (My10); HLA-Dr (GRB1); CD33 (My9); CD13 (My7); CD11b (OKM1); CD14 (My4); CD15 (C3D-1); CD41 (J15); CD61 (Y2/51); CD71 (BerT9) and anti-glycophorin. The lymphoid differentiation antigens included TdT (HT-1, HT-3, HT-4 – Dako); for the B lineage, CD19 (HD37), CD22 (To15), and CD10 (CALLA); for the T lineage, CD7 (3A1), CD2 (T9-10), CD3 (T3-4B5), CD1 (NA1/34). The assessment of membrane antigens was performed by conventional immunofluorescence methods. The detection of nuclear TdT and cytoplasmic antigens was carried out by immunocytochemical reactions (indirect immunoperoxidase or APAAP) as previously described in detail.² Each specific marker was scored as positive when in the presence of 20% or more reactive cells, except for TdT and CD19, where a cutoff value of 10% was used.

DNA STUDIES

DNA was extracted from the peripheral blood or bone marrow mononuclear cell fraction according to previously published methods.²⁵ In all samples tested more than 85% of the cells were morphologically malignant. Control germline DNA was obtained from normal peripheral blood granulocytes.

To investigate Ig heavy chain gene rearrangements, overnight digestions (100 IU enzyme/15 µg of high molecular weight DNA) were carried out with *EcoRI*, *HindIII*, *BglII* and *BamHI*. TCR β gene rearrangements were analysed with *EcoRI*, *HindIII*, and *BamHI* and TCR γ with *BamHI*. After digestion, DNA was size fractionated in 0.7–0.8% agarose gel electrophoresis, denatured, neutralised and blotted on to nylon membranes by capillary transfer according to the method of Southern.²⁶

PROBES

The probes used in this study were the following: Ig heavy chain joining region (J_H)—a 2.5 kilobase *EcoRI* genomic fragment containing the 3' half of the heavy chain joining region²⁷; TCR-β constant region—a 0.7 kilobase *HindIII-EcoRI* c-DNA fragment coding for most of the C β1 constant domains, which also hybridises to C β2^{28,29}; and TCR γ joining region—a 0.68 kilobase *HindIII-EcoRI* genomic fragment containing J τ1 which also hybridises to J τ2.³⁰

Probes were ³²P labelled by random priming to a specific activity of more than 5 × 10⁸ cpm/µg. Filters were prehybridised for one hour at 65°C in 6 × SSC, 0.5% SDS, 5 × Denhart's

Table 1 Clinical and haematological characteristics of 40 AML patients

Case No	FAB	Sex	Age	White cell count	% Blasts*	MPO	HLA	CD34	CD13/CD33	TdT	CD19	CD10	CD7	CD2
1	M5a	M	1	600	90	0	91	4	—/66	0	0	0	0	0
2	M2	M	3	120	90	90	38	24	—/52	0	19	2	0	0
3	M1	M	35	25	90	55	65	—	23/50	0	0	0	30	0
4	M5a	F	58	19	60	30	72	47	—/49	0	1	0	11	13
5	M1	F	21	9	90	8	80	62	—/05	70	0	1	29	—
6	M5a	M	20	40	95	0	95	0	—/92	0	8	0	10	—
7	M2	F	62	88	94	27	40	—	—/05	8	6	0	63	72
8	M0	F	8	4	99	1	7	43	50/76	70	0	0	70	3
9	M5a	F	3	70	90	20	50	40	—/60	0	0	0	5	1
10	M5a	M	59	79	70	30	53	23	—/40	—	2	0	1	6
11	M5a	M	19	40	80	0	55	62	—/54	0	4	0	6	5
12	M5a	F	18	180	98	5	50	55	—/72	—	0	0	1	6
13	M5a	F	56	64	84	0	70	0	—/40	0	1	0	5	5
14	M5a	M	19	48	85	0	75	23	—/35	0	0	0	14	4
15	M2	F	9	48	95	90	76	39	—/70	—	35	0	6	8
16	M1	F	66	37	93	90	57	5	—/47	0	3	0	48	9
17	M6	M	77	22	40	30	20	80	—	—	—	—	—	—
18	M0	F	69	5	60	0	78	—	—/50	0	0	0	6	5
19	M0	M	73	2	90	0	60	28	62/02	0	0	0	16	12
20	M2	F	82	200	90	70	60	0	20/56	0	1	0	0	4
21	M3	F	7	19	95	95	4	—	—/85	0	—	0	0	0
22	M5b	F	73	40	50	0	80	—	—/20	0	2	—	4	5
23	M2	F	54	65	87	65	75	31	—/68	—	0	0	3	10
24	M4	F	19	58	85	35	53	—	—/50	0	1	0	5	7
25	M5a	M	4	263	90	0	87	—	—/90	0	0	0	2	6
26	M4	F	65	25	75	25	80	—	—/70	0	0	0	8	11
27	M5b	F	76	3	70	7	82	0	64/—	—	2	0	2	0
28	M4	M	19	38	75	50	70	—	37/22	—	2	0	17	9
29	M0	M	64	32	95	0	85	27	—/32	30	9	7	48	2
30	M1	M	75	122	99	10	87	—	57/18	0	0	0	0	0
31	M1	M	60	40	80	10	80	56	—/—	0	0	0	0	0
32	M1	M	64	37	88	12	87	65	—/13	0	0	—	18	—
33	M5a	F	32	90	72	0	75	—	—/50	—	0	0	—	0
34	M4	F	63	120	86	—	89	7	67/92	0	0	1	62	4
35	M4	F	4	120	70	20	63	52	—/63	—	61	0	21	11
36	M1	M	72	50	90	15	80	—	72/75	—	0	0	12	27
37	M2	F	14	70	80	70	70	—	—/60	0	0	0	3	4
38	M2	M	75	40	95	40	92	—	60/—	—	4	0	4	10
39	M5a	F	54	70	48	3	48	—	32/28	0	3	0	3	11
40	M2	F	45	85	90	78	30	25	—/50	0	0	0	0	0

*Bone marrow.

Table 2 Configuration of Ig(J_H) and TCR- β and TCR- γ genes in 40 AML patients

Case No	FAB	Ig(J_H)				TCR- β			TCR- γ
		EcoRI	HindIII	BglII	BamHI	EcoRI	HindIII	BamHI	BamHI
1	M5a	—	GG	GG	GG	GG/GG (3-2; 2-7)	GG/GG	GG	GG/GG (2-7)
2	M2	GG	—	GG	GG	GG/GG (8)	GG/GG (13)	GG	—
3	M1	—	GG	GG	GG	GG/GG (8)	GG/GG (13)	—	GG/GG
4	M5a	GG	—	—	—	GG/GG	GG/GG	—	GG/GG
5	M1	GG	—	GG	GG	GG/GG (3-2)	GG/GG	—	GG/GG
6	M5a	GG	—	GG	—	GG/GG	GG/GG	—	GG/GG
7	M2	GG	GG	—	—	GG/GG	GG/GG	—	18; 14-5
8	M0	GG	GG	—	—	GG/GG	GG/GG	—	GG/GG
9	M5a	13; 9	8; 5	3-7; 2	—	GG/GG (8; 3-2)	GG/GG	GG	GG/GG
10	M5a	GG	GG	—	GG	GG/GG	GG/GG	GG	GG/GG
11	M5a	GG	GG	—	GG	GG/GG	GG/GG	GG	GG/GG
12	M5a	GG	GG	—	GG	GG/GG	GG/GG (13)	GG	GG/GG
13	M5a	GG	GG	—	GG	GG/GG	GG/GG	GG	GG/GG
14	M5a	GG	GG	—	GG	GG/GG	GG/GG	GG	GG/GG
15	M2	GG	GG	—	GG	GG/GG (8; 3-2)	—	GG	GG/GG
16	M1	GG	GG	—	GG	GG/GG	GG/GG	GG	GG/GG
17	M6	GG	GG	GG	GG	GG/GG	GG/GG	GG	—
18	M0	GG	GG	—	—	GG/GG	GG/GG (13)	GG	GG/GG
19	M0	—	GG	—	—	GG/GG	—	—	GG/GG
20	M2	GG	GG	GG	—	GG/GG	GG/GG	GG	—
21	M3	—	GG	GG	GG	GG/GG	GG/GG	—	—
22	M5b	GG	GG	—	—	GG/GG	—	GG	GG/GG
23	M2	GG	GG	—	—	GG/GG	—	GG	GG/GG
24	M4	—	GG	—	—	GG/GG	—	GG	GG/GG
25	M5a	GG	GG	—	—	GG/GG	—	GG	GG/GG
26	M4	GG	GG	—	—	GG/GG	—	GG	GG/GG
27	M5b	GG	GG	—	—	GG/GG	—	GG	GG/GG
28	M4	—	GG	—	—	—	—	GG	GG/GG
29	M0	GG	GG	—	—	GG/GG	GG/GG	GG	GG/GG
30	M1	—	GG	—	GG	GG/GG	GG/GG	GG	GG/GG
31	M1	GG	—	—	GG	GG/GG	—	GG	—
32	M1	GG	GG	—	—	GG/GG	—	GG	—
33	M5a	—	GG	—	GG	GG/GG	—	GG	—
34	M4	GG	GG	GG	—	GG/GG	GG/GG (13)	GG	GG/GG
35	M4	GG	GG	—	GG	GG/GG (8)	GG/GG	GG	GG/GG
36	M1	GG	GG	—	—	GG/GG	GG/GG (13)	GG	GG/GG
37	M2	GG	GG	—	—	GG/GG	GG/GG (13)	GG	—
38	M4	GG	—	—	GG	GG/GG	—	GG	—
39	M4	GG	GG	—	GG	GG/GG	GG/GG	—	GG/GG
40	M2	GG	GG	GG	GG	GG/GG	—	GG	GG/GG

solution, 100 μ g/ml denatured hsDNA, and hybridised for 18 hours with 2×10^6 cpm/ml of labelled probe. After hybridisation, blots were washed for 2×15 minutes in $2 \times$ SSC, 0.1% SDS, 2×15 minutes in 0.2 SSC, 0.1% SDS at 65°C, and autoradiographed at -70°C with intensifying screens for four to seven days.

Figure 1 Southern blot analysis of the Ig heavy chain region using the J_H probe. Control DNA from normal peripheral blood granulocytes (C). The restriction enzyme used is indicated at the bottom of the lanes. Position and size of the germline fragments are indicated by a line. In case 9 two rearranged bands are visible in the two digests (arrows).

Results

IMMUNOLOGICAL PHENOTYPING

The clinical and haematological features of the 40 AML cases are summarised in table 1. In 10 cases a significant proportion (20% or higher) of blast cells expressed markers that in normal haemopoiesis are regarded as specific for lymphoid lineages. Of these 10 cases, five were morphologically immature (two M0; three M1), and in five the blast cells showed features of maturation (three M2 and two M4). Seven cases were CD7 positive, of which three were also TdT positive (cases 5, 8, and 29). The three TdT positive, CD7 positive AML cases also expressed CD34 and CD13/CD33 except for case 5 which was classified as M1 because of typical morphology and 8% MPO positive blasts; other lymphoid antigens including cytoplasmic CD3 were absent. In case 7, a typical M2 with Auer rods, both CD7 and CD2 were expressed in a high proportion of blasts. Cytoplasmic CD3 as well as TdT were negative. The remaining CD7 positive cases (cases 3, 16 and 34) were negative for all other lymphoid markers. A proportion of 21% CD7 positive cells and 27% CD2 positive cells were also observed in cases 35 and 36, respectively, where the reactivity for these markers with remaining normal T cells could not be ruled out. A significant proportion of leukaemic cells reacting with CD19 was found in three cases (2, 15, and 35).

DNA ANALYSIS

The results on DNA analysis are summarised in table 2. The Ig(J_H) and TCR- β genes were

Figure 2A *Eco RI* digests hybridised with *Cβ1* probe. Bands with a different size from that of the germline control (lines) are indicated with an asterisk. Cases 2, 3, and 5 are shown in repeated digests. The partial restriction enzyme map at the bottom is based on previously published data. The germline fragments detected by *Eco RI* (R), *Hind III* (H), and *Bam HI* (B) are indicated by continuous lines below the map and fragments derived from partly resistant *Eco RI* and *Hind III* (*) by broken lines.

(B) *Eco RI* digests of cases 1 and 9 hybridised with ³²P labelled pUC19 show the presence of plasmid contamination in these samples.

investigated in all cases and the TCR γ genes in all but nine cases.

Ig genes exhibited a germline pattern in all but case 1, a 3 year old child with M5a, which did not express any lymphoid marker. Two rearranged bands were found with three restriction enzymes. *Eco RI* and *Hind III* digests of this case are shown in fig 1. A faint germline band was observed in the *Hind III* digest, indicating that a minor population of cells retained a germline configuration despite the morphological and immunological homogeneity of the blast cells in this case.

The TCR β genes probed with *Cβ1* showed germline bands in 28 cases. In the remaining 12 one or more bands with sizes different from the germline control could be observed—in six out of 29 AML cases without lymphoid markers (20%) and in six out of 10 cases expressing lymphoid antigens (60%). The new bands were observed only in *Eco RI* or *Hind III* digests and had the following characteristics: an 8 kilobase

Eco RI band was found in five different cases (table 2 and fig 2). This band disappeared in different digests of the same samples (cases 2 and 3). Bands of 3.2 and 2.7 kilobases were present in case 1. The former was also visible in cases 5, 9, and 15. New *Eco RI* digests of cases 1 and 9 probed with radiolabelled cloning vector (pUC19) showed that those bands were the result of plasmid contamination (fig 2B). Cases 9 and 15 also had the 8 kilobase band; in the *Hind III* digests a 13 kilobase band was present in cases 2, 3, 12 and 18, 34, 36 and 37 (table 2 and figs 3 and 4). In all these cases the persistence of the 11 and 4 kilobase *Eco RI* and the 7.4 and 3.3 kilobase *Hind III* germline bands was difficult to reconcile with any pattern of β gene rearrangement. The findings with these two enzymes are compatible with partial digestions at the *Eco RI* site close to the 5' end of the *Cβ2* region or at the *Hind III* site that lies within the first intron of *Cβ2* (fig 2).³¹ The *Bam HI* digests (not tested in cases 3 and

Figure 3 *Hind III* digests hybridised with C β 1. A 13 kilobase fragment (*) is seen in cases 2, 3, 12 and 18, due to partial digestion at the *Hind III* site within C β 2. Size of germline fragments is indicated by lines.

5) probed with C β 1 eventually showed that all these cases were in germline configuration at the β locus, thus confirming the artefactual nature of those findings (figs 4 and 5).

The TCR γ genes were in germline configuration in all but case 8 (AML-M0, TdT, and CD7 positive), where two new bands were visible in *Bam HI* digests. This finding is compatible with a diallelic rearrangement for C τ 1 (fig 6). This sample was in germline configuration at the Ig and β genes. In case 1 a 2.7 kilobase band was visible, identical in size with a band found in the *Eco RI* digest of the same case, probed with C β 1.

In summary, we found a total of 25 "non-germ-line" bands in 40 AML samples (table 2), of which 16 were present at the β locus in *Eco RI* or *Hind III* digests. None of these

corresponded to a true rearrangement, as shown by *Bam HI* digests and as suspected by examination of the respective restriction map.^{29 30 32} One additional band at the TCR γ locus was probably the result of a cross-hybridisation artefact. Of the remaining eight bands, six were present at the Ig heavy chain locus in one case (detected by three different restriction enzymes), and two were seen at the TCR γ in another case. Only two out of 40 AML samples had rearranged genes: 1 of 29 of pure myeloid phenotype (3.3%) and 1 of 10 expressing lymphoid markers (10%), a difference which was not significant.

Discussion

The DNA results in this series could, at first

Figure 4 *Hind III* and *Eco RI* digests of cases 34–37, and *Bam HI* digests of cases 35 and 36, hybridised with C β 1 probe. Partial digestions are indicated by * and germline bands by lines.

Figure 5 *Bam HI* digests hybridised with *Cβ1* probe. Only germline fragments are seen in the samples that showed new *Eco RI* or *Hind III* bands (table 2 and figs 2 and 3).

sight, be interpreted as evidence for the presence of rearrangements in TCR β genes in a significant proportion of AML cases expressing markers of lymphoid lineage. A closer analysis of the molecular data, however, would immediately pose the following problems of interpretation: (1) the presence of new *Eco RI* or *Hind III* bands in the β genes that had the same molecular weight in different DNA samples; (2) patterns of "rearrangement" with both enzymes that did not fit with each other.

The first finding would, on its own, be an unexpected pattern for rearrangements occurring in genes with great diversity, unless the "inappropriate" rearrangements in AML cells reflected the use of the same gene segments in different patients. A more likely explanation would obviously be technical artefact. In fact, an analysis of the size of those bands showed

that five of 10 new *Eco RI* fragments were 8 kilobases, even in lanes with some electrophoretic distortion due to DNA overload, and all new *Hind III* bands were 13 kilobases. These sizes would be expected if a partial digestion at the *Eco RI* site 5' to *Cβ2* region or the *Hind III* site within *Cβ2* had occurred³¹ and the fragments from which they originate should not be interpreted as rearrangements. In cases 1, 5, 9 and 15 *Eco RI* fragments of less than 4 kilobases were found. In our view, *Eco RI* bands of less than 4 kilobases do not seem compatible with a rearrangement for *Cβ1* as the distance between the *Eco RI* site 3' to *Cβ1* and the more proximal J $\beta 1$ segment is more than 4 kilobase in germline DNA.^{29,30} Alternative explanations would be rearrangements occurring through a non-looping out deletion mechanism, which has been reported in β genes,³³ or a cytogenetic anomaly involving the β locus or *Eco RI* site polymorphisms. Nevertheless, it seems unlikely that any of the first two phenomena would produce similar bands in different samples. In fact we were able to show that those bands were not caused by true rearrangements as the *Bam HI* digests showed a germline configuration at the β locus in these patients. Although *Eco RI* polymorphisms cannot be ruled out in the absence of a germline control derived from each individual patient, polymorphisms for this enzyme do not seem to occur at this locus.²⁸ As expected, a cross-hybridisation artefact due to plasmid contamination was shown in two samples.

An additional problem in those samples would be the coexistence of strong germline fragments with "non-germline" bands in the *Eco RI* and *Hind III* digests. The intensity of the 11 kilobase germline fragment containing *Cβ1* in *Eco RI* digests, or the 7.3 kilobase germline fragment containing *Cβ2* in *Hind III* digests made it unlikely that a monoallelic rearrangement for these regions had occurred in those patients (figs 2 and 3). Also unlikely would be the presence of a dual cell population, as all the samples analysed had more than 85%

Figure 6 *Bam HI* digests hybridised with TCR $J\gamma$ probe. Case 8 exhibited two rearranged bands indicated by arrows (see text). Case 1 had a 2.7 kilobase faint band (*), also visible in *Eco RI* digests, probed with *Cβ1* (fig 2). Germline bands indicated by lines.

leukaemic blasts, the morphology of which seemed to be homogeneous.

We can conclude, then, that the β genes were in germline configuration in all these patients, despite the presence of restriction fragments that differed from the control DNA in two different digests.

The TCR γ genes were in germline configuration in all cases except case 8, where two faint bands were just visible in *Bam* *HI* digests. This patient, an 8 year old girl, had morphologically immature leukaemia (M0); the blasts expressed TdT and CD7 antigens in a high proportion of cells. The Ig and TCR β genes were in germline configuration. The pattern of bands observed in this case could correspond to a diallelic rearrangement for C τ 1 but, due to the limited number of combinations of the TCR γ genes, does not necessarily indicate a monoclonal population.^{34 35} In normal T cells as well as in almost all T lymphoblastic leukaemias, the β and γ genes are rearranged in the same cells, reflecting the fact that both genes rearrange at the same stage of differentiation in T cell ontogeny.^{25 31 36 37} The rearrangement of γ genes in the presence of germline β genes has nevertheless been reported in TdT positive AML,¹⁶ although that seemed to occur in cases where the Ig genes were also rearranged.

Rearrangement at the Ig heavy chain genes was observed in only one patient (case 9) of our series, a 3 year old child. It was an M5a AML without any lymphoid marker. An *Eco* *RI* or *Hind* *III* polymorphism, due to extremely variable polymorphism in the region 5' to the J cluster,³⁸ was unlikely as two rearranged bands were also visible in *Bgl* *II* digests in this case. The only two rearrangements found in this series occurred in children. In the series published by Foa *et al.*,¹⁶ where a high incidence of Ig gene rearrangements was found in 10 patients with TdT positive AML, eight were children and there was a predominance of monocytic subtypes. In a series of AML in infants Koller *et al.*³⁹ found Ig rearrangements, with germline TCR β and γ genes, in 20% of the patients, although no relation with TdT expression or any particular FAB subtype could be established. It is conceivable that AML in children and infants might intrinsically have biological characteristics which differ from those found in adult AML. Rearrangements in Ig or TCR genes, regardless of the phenotypic profile exhibited by the leukaemic cell, could be just one of these characteristics.

In summary, our results confirm recent reports stating that TdT or CD7 expression in AML blasts is not associated with a high incidence of rearrangements in Ig or TCR genes. These findings are not totally unexpected, especially in terms of CD7 expression in myeloid cells. The expression of this molecule is known to precede TCR gene rearrangement in T cell ontogeny, a fact that suggests that it is not necessarily related to the rearrangement of those genes.^{40 41} On the other hand, it has been shown that normal CD7 positive fetal liver cells are capable of differentiating either into lym-

phoid T or myeloid lineages.¹² Kurtzberg *et al.*¹³ showed the same phenomenon in a series of CD7 positive immature leukaemias. One of the patients had an *in vivo* phenotypic transformation from T cell lineage to myeloid differentiation (with the myeloid blasts retaining the rearrangement of TCR genes). In this context CD7 expression in myeloid blasts in most cases may represent just a relic of an ancestral phenotype. Those few cases of CD7 positive AML that exhibit TCR gene rearrangements might indeed correspond to the expansion of a clonogenic cell that, having transiently passed throughout a T cell pathway of differentiation, has transmitted to its progeny the permanent scar of an irreversible molecular event.

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