Papers

Heterogeneity of T cell lymphoblastic leukaemias

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Abstract

Twenty eight out of 170 consecutive cases of acute lymphoblastic leukaemia (ALL) were examined. They were of T cell origin, with the following distribution: seven (28%) cases had pre-T or pro-thymic features; nine (36%) cases showed early thymocytic features, six (24%) had cortical features; and three (12%) had a "mature" phenotype. The remaining three cases could not be subclassified. A striking finding was that pre-T ALL differed from intrathymic ALL not only in the absence of both E rosettes and intrathymic differentiation antigens, but also in the expression of two non-lineage specific antigens HLA-DR and CD10. Both antigens appear in the bone marrow from the very first stages of lymphoid differentiation, implying that the origin for pre-T ALL is bone marrow. A comparison of the clinical features of pre-T and thymic ALL showed that pre-T ALL disease showed a pattern more similar to non-T ALL disease: a lower incidence of mediastinal mass, absence of extrahaematopoietic disease, lower white cell counts and haemoglobin concentrations, and a higher incidence of bone pain. No obvious difference in response to treatment was apparent.

The results show that T-ALL is not only a heterogeneous immunological group but also suggest that it may have different origins: bone marrow for pre-T ALL and the thymus for thymic ALL.

T cell acute lymphoblastic leukaemia (T-ALL) comprises 15% to 25% of all cases of ALL.^{1,2} It affects more men than women and is characterised by the presence of mediastinal mass, an extrahaematopoietic infiltrate, higher white cell counts and a lower incidence of anaemia and thrombocytopenia.³⁻⁵

The systematic use of the pan-T antigen CD7, which appears on the surface of T cells the moment differentiation begins, even before T β rearrangement,^{6,7} and, more recently, the detection of intracytoplasmatic CD3⁸ has pointed to the T cell origin of a group of ALL, named pre-T ALL, which unlike intrathymic T ALL phenotypes, are E rosette negative. Few studies, however, have looked into whether this immunophenotypic heterogeneity might be related to different clinical features and outcome.^{9,10} Little is also known about whether these maturational subgroups

also differ in other non-T cell differentiation antigens, which might indicate a different origin or functional activity of neoplastic cells.

The aim of this study was (1) to analyse the possible clinical and biological diversity among different maturational T ALL features, with special reference to the immunophenotypic and clinical differences among cases of pre-T and intrathymic ALL, and (2) to discover whether such differences might indicate a bone marrow origin for pre-T disease, using non-T ALL disease as a reference.

Methods

One hundred and seventy patients (82 children and 87 adults, aged 2 to 79 years) diagnosed as "de novo" ALL according to morphological and cytochemical criteria,¹¹ were included in this study. All patients were treated with three similar regimens, and although there were small differences in drug dose and schedules, all three regimens included vincristine, prednisone, L-asparaginase and daunorubicin. There were no differences either in the distribution of clinical and immunological features nor in the response to treatment and survival among the three groups.

Mononuclear cells isolated by Ficoll-Hypaque density gradient centrifugation were analysed for sheep erythrocyte rosette formation, and by direct immunofluorescence for surface and cytoplasmatic immunoglobulins, and by indirect immunofluorescence with a TdT antiserum and a panel of monoclonal antibodies. The specificities of the monoclonal antibodies were as follows: B-cell lineage: B4 (CD19), B1 (CD20); T-cell lineage: 3A1 (CD7), OKT11 (CD2), Cris1/Leu1 (CD5), OKT6 (CD1), OKT3 (CD3), OKT4 (CD4), OKT8 (CD8); non-lineage specific markers: FMC8 (CD9), BI-3C5 (CD34), OKT10 (CD38), OKT9 (CD71), GRT2 (CD45), GRT22 (CD45R); and myeloid markers: My7 (CD13), My9 (CD33). The reactivity and specificity of these monoclonal antibodies in ALL have been reported previously.¹²⁻¹⁴ A marker was considered positive when it was expressed in more than 20% of blast cells. We defined as pre-T or prothymic ALL those cases of T-cell ALL (CD5+ or CD7+) that lacked E/CD2 as well as other intrathymic differentiation markers (CD1, CD4, CD8, surface CD3), B lineage, and myeloid markers.

The clinical and biological data analysed included age, sex, presence of infection, bleeding, bone pain, general symptoms

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Table 1 Immunological classification of T-ALL (n = 28)*

Stage	Markers											Cases
	TdT	Ia	E/CD2	CD7	CD5	CD38	CD71	CD1	CD4	CD8	sCD3	No (%)
Pre-T	+	+	—	+	—	±	—	—	—	—	—	7 (28)
Early	+	—	+	+	+	±	+	—	—	—	—	9 (36)
Cortical	+	—	+	+	+	±	—	+	+	+	—	6 (24)
Mature	+	—	+	+	+	±	—	—	±	±	+	3 (12)

*Three thymocytic cases without subclassification.

(malaise, loss of appetite, weight loss), hepatomegaly, splenomegaly, lymphadenopathy, mediastinal mass, central nervous system or other infiltrations, haemoglobin concentration, white cell counts, including the percentage and absolute number of blasts, the number of platelets, erythrocyte sedimentation rate, morphology according to FAB criteria,¹¹ cytochemistry (periodic acid Schiff, acid phosphatase, and Sudan black or peroxidase stainings), serum immunoglobulins, serum aspartate transaminase, serum alanine amino transferase, alkaline phosphatase, lactate dehydrogenase, uric acid, calcium and phosphorus activities.

A descriptive study was performed using the BMDP 2D program.¹⁵ Results were expressed as mean (SD) and medians for continuous parameters, and frequency distributions for discrete variables. Both differential clinical features and univariate analysis of prognostic factors regarding the complete remission rate were performed with contingency tables¹⁶ that included the following statistical tests: χ^2 test with Yates' correction, Fisher's exact test, and the test for trend—BMDP 4F program.¹⁵ Survival curves were plotted according to the method of Kaplan and Meier¹⁷ and compared using the Mantel-Cox¹⁸ and Breslow¹⁹ tests—BMDP 1L program. Multivariate analyses of prognostic factors were also performed on the whole series, both on complete remission rate using the multiple linear logistic regression model²⁰—BMDP LR program—and on survival by the stepwise proportional hazard regression model²¹—BMDP 2L program.²¹

Results

IMMUNOLOGICAL MARKERS

Of 170 ALL cases, 102 (60%) showed common

features; 27 (16%) were null-ALL; 27 (16%) T-ALL, and seven (4%) had a pre-B or a B phenotype seven (4%). Twenty five out of the 28 T ALL cases could be ascribed to one of the four major T immature phenotypes: pre-T or prothymic, n = 7; early thymic, n = 9; cortical, n = 6 and late n = 3 (table 1). Of the remaining three cases, one was considered to have a special phenotype because of the absence of TdT and CD7 but its capacity to form E rosettes, the presence of CD5, and the negativity of B cell markers indicated a T cell origin. Moreover, its morphology, together with the absence of mature T cell antigens, ruled out mature T cell lymphoproliferative disease. The other two cases, although displaying a typical immature T phenotype, could not be ascribed to a specific intrathymic phenotype due to insufficient number of cells. B cell markers (CD19, CD20, CIg, SIg), as well as myeloid markers (CD33 or CD13) were negative in all cases of T cell ALL. Cytoplasmic CD3 was positive in the only prothymic case studied.

A comparison of the expression of non-lineage specific antigens in pre-T as opposed to intrathymic ALL (table 2) showed that nearly half the pre-T cases were HLA-DR positive, while the intrathymic cases were consistently negative. Only three of these latter cases (one from each intrathymic stage) expressed CD10, while this was detected in 65 (71%) of the pre-T patients. No other differences were seen in the remaining non-lineage specific markers analysed. The reactivity of these antigens in non-T ALL is also shown as a reference value (table 2). During intrathymic differentiation expression of CD9, CD38, CD45 and CD45R was not associated with any particular maturation subgroup.

CLINICAL FEATURES

Tables 3 and 4 show the main clinical and biological characteristics of pre-T and thymic ALL which are also contrasted with those of non-T ALL. A comparison of the entire group of T ALL with non-T cases shows that the former group was characterised by the presence of mediastinal mass (p = 0.0001), extrahaematopoietic disease (p = 0.002), a lower incidence of anaemia (p = 0.01), higher white cell counts (p = 0.0002) and focal acid phosphatase positive blasts (p = 0.0000).

Pre-T ALL differed from intrathymic ALL in that it predominantly affected males and was associated with a greater degree of bone pain, a lower incidence of mediastinal mass, the absence of extrahaematopoietic infiltrates, and lower haemoglobin and lactate dehydrogenase

Table 2 Non-lineage specific markers: Pre-T ALL v thymic ALL

	Pre-T (n = 7)	Thymic (n = 21)	Non-T (n = 141)
Ia	43%* 70 (35)	0%	98% 81 (28)
CD10	71% 66 (35)	15% 40 (25)	76% 75 (27)
CD34	0%	14%† 53	56% 61 (30)
CD9	50% 42 (20)	50% 40 (23)	87% 73 (28)
CD45	100% 92 (4)	100% 88 (8)	87% 61 (28)
CD45R	50% 98 (15)	50% 67 (20)	69% 50 (20)

Results expressed as percentage of positive cases.

In parentheses mean (standard deviation) of percentage of positive blasts.

*Two of the three positive cases were also CD10 positive.

†CD34 positive in one early thymic case.

Table 3 Clinical features: Pre-T ALL v thymic ALL

	Thymic (n = 21)	Pre-T (n = 7)	Non-T (n = 141)
Age Mean (SD)	20 (12)	21 (15)	22 (22)
> 15 years	50%	57%	49%
Sex: percentage of men	67%	86%	62%
Bleeding	60%	60%	47%
Infection	43%	60%	40%
Bone pain	9%	50%	46%
Hepatomegaly	41%	50%	59%
Splenomegaly	71%	40%	60%
Lymphadenopathy	77%	80%	65%
Mediastinal mass	67%	33%	3%
CNS infiltrate	21%	0%	5%
Other infiltrate	36%	0%	6%

Results expressed as percentage of positive cases.

concentrations and white cell counts; the proportion of blast cells in the bone marrow was higher. All the cases of pre-T ALL had L1 morphology compared with 60% of intrathymic ALL (tables 3 and 4).

Of the intrathymic ALL cases, the cortical stage was noteworthy because of its higher infiltrative tendency in terms of liver enlargement (50% compared with 29% in the early and 0% in the late stages), enlarged spleen (100% v 71% in early and 0% in the late stage) and lymphadenopathy (100% v 71% and 33% in early and late stages, respectively), and higher white cell counts (75%, 56%, and 33% of cases with more than $100 \times 10^9/l$ in cortical, early, and late stages, respectively). As in pre-T ALL, all the cortical cases had L1 morphology. On the other hand, none had either infections or bleeding compared with the more mature T ALL stage in which all patients had both complications.

OUTCOME

The patients with T ALL showed a slightly shorter median survival than the non-T ALL group (figure). When cases of pre-T ALL were compared with the intrathymic ALL group survival was similar (19 v 13 months respectively (table 5, figure). Among the intrathymic ALL group those cases with cortical features had a better prognosis, 33 months followed by the early thymic cases with 15 months of survival; patients in the late stage had the poorest outcome with eight months.

The presence of CALLA antigen in T ALL was associated with higher complete remission

Figure Survival in cases of pre-T, thymic, and non-T ALL.

rates (88% v 60%) and a longer survival (33 v 17 months), although the differences were not significant.

Discussion

Systematic use of the CD7 monoclonal antibody has identified a group of ALL of T cell origin, pre-T ALL, that would otherwise have been classified as null-ALL. It has recently been shown that CD3 can also be detected in the cytoplasm but not on the cell surface in almost all pre-T ALL cases, and in all the intrathymic ALL phenotypes.^{8,10} In our series pre-T ALL represented 25% of T ALL cases, a similar incidence (24%) to that reported by Thiel *et al*¹⁰ and Catovsky *et al* (28%),⁵ and higher than that of Foa *et al* (10%).¹

Thiel *et al* include as pre-T ALL cases that are E-rosette negative but which have the sheep rosette receptor CD2, detected by monoclonal antibodies such as OKT11, and which show T cell differentiation antigens such as surface CD3 (T3) and CD1 (T6).¹⁰ In our study the cases that expressed either surface CD3 or CD1 or the presence of the CD2 receptor were not considered pre-T ALL. In our opinion the classification of a case of ALL of T cell origin in the pre-T ALL subgroup cannot only be based on the negativity of E-rosettes but should also be reflected by the absence of intrathymic differentiation antigens, such as CD1 and sCD3, because their presence indicates an intrathymic maturational stage, independent of the reactivity with the sheep red blood cells.

In our series the patients with pre-T ALL also differ from the cases of intrathymic ALL because they are usually CD10 positive and may express HLA-DR antigens, a finding also reported by Thiel *et al*.¹⁰ Both antigens appear in the bone marrow from the earliest stages of haematopoietic differentiation, and although they may appear in several other different

Table 4 Clinical features: Pre-T ALL v thymic ALL

	Thymic (n = 21)	Pre-T (n = 7)	Non-T (n = 141)
Haemoglobin Mean (SD)	11.5 (3.9)	9.0 (1.2)	8.6 (2.6)
< 9 g/dl	29%	40%	61%
White cell counts:			
> 100 × 10 ⁹ /l	127 (123)	95 (111)	35 (55)
> 100 × 10 ⁹ /l	63%	29%	8%
Platelets:			
< 40 × 10 ⁹ /l	84 (85)	96 (71)	83 (95)
< 40 × 10 ⁹ /l	33%	40%	37%
Blasts:			
> 75%	73 (25)	67 (35)	57 (27)
> 75%	59%	50%	29%
Bone marrow blasts:			
> 90%	82 (13)	97 (2)	87 (16)
> 90%	14%	100%	44%
FAB: %L2	40%	0%	40%
PAS†	62%	60%	63%
Focal acid phosphatase	85%‡	100%	6%
Lactate dehydrogenase	1350 (1208)	629 (477)	970 (1173)
> 1000 IU/l	36%	20%	29%
Uric acid	4.9 (2.4)	5.3 (3.3)	6.1 (4.3)
> 8 mg/dl	8%	8%	17%

Results expressed as percentage of positive cases.

†Myeloperoxidase staining was negative in all cases.

‡Two negative cases.

Table 5 Outcome of Pre-T v thymic ALL

	Complete remission rate	Relapses	Survival (months of median)
Pre-T (n = 7)	57%	33%	18.6
Thymic (n = 21)	69%	42%	12.6

p < 0.5

locations and lineages,¹⁴ they are not expressed by the normal intrathymic cells, whereas in the normal human bone marrow there are cells positive for TdT, HLA-DR and a T-cell antigen.⁸ Thus the presence of HLA-DR and CD10 antigens suggests not only a more immature stage but also a bone marrow origin for pre-T ALL.

It has been reported that up to 26% of acute myeloblastic leukaemias (AML) express CD7.^{22,23} The prevalence of CD7 positivity was particularly significant in the less differentiated subtypes M0 and M1.²³ These myeloid leukaemias often expressed other lymphoid markers and frequently had rearranged T cell receptor β chain gene. These results suggest that the malignant transformation occurred in an early multipotential CD7 positive progenitor cell.

Cytoplasmic CD3 was only used in one prothymic ALL case. We believe that this marker is most useful for differentiating a CD7 positive AML, which is CD3 negative, from a pre-T ALL, which is usually CD3 positive.^{8,10} Other markers, such as the absence of CD33 and CD13 myeloid antigens, and the negativity of myeloperoxidase staining, precluded a myeloid origin for the cases that we have classified as pre-T ALL.

Most of the previously described differential features for T and non-T ALL reflect their different origin—thymus and bone marrow, respectively. The pre-T subgroup, however, whose clinical features have not been analysed separately, is included within the T ALL group. One of our aims was to analyse the existence of differences between pre-T and thymic ALL cases and whether these differences might reflect a bone marrow origin as well. The rare incidence in the pre-T ALL subgroup of clinical features typically associated with T ALL, such as mediastinal mass, extra-haematopoietic disease, and high white cell counts, would suggest that patients with pre-T ALL are related to the non-T ALL cases rather than to the T ALL group. The higher proportion of blast cells in bone marrow and the greater incidence of both anaemia and bone pain would also support a bone marrow origin for pre-T ALL blasts.

Little is known about the prognosis of pre-T ALL because it has usually been included either in the null ALL group or analysed together with the remaining T-cell ALL. Recently, Thiel *et al* reported that complete remission rates, survival, and disease-free survival are significantly lower for pre-T ALL patients compared with E-rosette positive T ALL patients.¹⁰ Our results indicate a similar outcome for both groups, and in fact a slightly longer survival was observed in pre-T ALL patients. The low incidence of the appearance of the pre-T phenotype however, makes it difficult to draw any definitive conclusions about its prognostic importance in our series.

In intrathymic ALL those patients with

cortical features had the best outcome, while those with a mature phenotype had the poorest, both in terms of complete remission rate and survival. Crist *et al* found a lower complete remission rate for the immature phenotype but there were no differences for disease-free survival among the three maturational stages.⁹

These data indicate that the clinical heterogeneity observed in patients with T ALL probably reflects the existence of different entities of distinct origin. The use of precise immunophenotypical criteria for their definition and larger series of patients will probably shed further light on the heterogeneity of these leukaemias and should permit the use of more specific treatment.

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