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Terminal deoxynucleotidyl transferase activity in childhood and adult acute lymphoblastic leukaemia

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SUMMARY The measurement of terminal deoxynucleotidyl transferase (TDT) activity in leukaemic blasts of 26 cases of acute lymphoblastic leukaemia (ALL) (13 children < 14 years, 13 adults > 14 years) demonstrated significantly greater activities of the enzyme in a proportion of the adults. The predominant cytological sub-type in the adult patients was L2 (FAB classification) whereas L1 cytological sub-type dominated in the childhood group. There was no relation between TDT values and FAB sub-type but the highest activities in the childhood group were seen in patients assessed at the time of relapse. We conclude that continued use of quantitative TDT estimations may provide useful information in further characterising the currently recognised cytological and immunological sub-types in ALL.

Terminal deoxynucleotidyl transferase (TDT) is a DNA polymerase enzyme which is normally only present in significant amounts in cortical thymocytes.[1] The discovery that the enzyme was present in lymphoblasts in acute lymphoblastic leukaemia (ALL) has led to the routine estimation of the enzyme as an accurate haemopoetic cell marker in ALL and lymphoblastic lymphomas.[1-5] Normally biochemical estimations of the enzyme are carried out on cells obtained from blood and bone marrow in ALL and expressed quantitatively as units of enzyme activity per 10^8 white cells. Previous authors have not been impressed by the differences in absolute values of the enzyme in children and adults with ALL.[1 6] In assessment of 26 cases, TDT estimations were performed on bone marrow or blood samples with greater than 90% blasts and significant differences were obtained between the children and adult groups. This finding is of importance in the context of the known different prognosis of the adult and childhood disease.

Patients and methods

Twenty-six patients with ALL consisting of 13 adults and 13 children were studied. The diagnosis of ALL was made on examination of Romanowsky-stained slides of blood and bone marrow together with the FAB histological classification system.[7] The biochemical estimation of TDT activity was used as a confirmatory test of the diagnosis. Cells were examined for surface immunoglobulin by direct immunofluorescence (B cells) and for spontaneous rosette formation with sheep erythrocytes (T cells). The TDT estimation was performed on samples obtained from bone marrow (20) in the majority of cases or peripheral blood (6) containing greater than 90% blasts as assessed by morphological means. Peripheral blood was assessed in those patients in whom sufficient marrow was not available. Nineteen cases were tested at the time of diagnosis; the remainder (7) were samples obtained at the initial relapse. Positive control samples were obtained from child thymus removed at routine paediatric cardiothoracic surgery. Blood and marrow samples available in routine haematological practice were also assayed. These included 10 patients with B cell chronic lymphatic leukaemia (peripheral blood), eight patients with myeloid leukaemia (relapse marrow), six patients with Hodgkin's disease (uninvolved marrow and blood), 10 patients with non-Hodgkin's lymphoma of the non-lymphoblastic type (marrow) and 34 control marrows from patients with non-malignant haematological disorders. Subsequently, the first remission bone marrows were available for assessment in 10 of the ALL patients.

METHOD FOR THE TDT ASSAY

Blood or marrow mononuclear cells were purified on a Ficoll Triosil gradient, counted and stored at −20°C within 6 h of collection at a concentration of 1-2 × 10^7 cells/50 μl extraction buffer (0·25 *M*

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potassium phosphate pH 7·5 containing 1 mmol beta-mercaptoethanol). The enzyme assay was then carried out using the method of Hoffbrand *et al.* (1977).[6] Briefly, the cells were frozen and thawed three times and the extract spun at 100 000 *g* for 60 min; 10 μl of the supernatant was then immediately assayed at 37°C for 1 h in a reaction mixture of 60 μl containing 0·025 units poly-d (adenine) as primer (P/L Biochemical Co, Milwaukee, USA), 83 μmol/l 3H-deoxyguanasine triphosphate (Radiochemical Labs, Amersham) as substrate, 42 mmol/l tris-HCl (pH 7·5), 42 mmol/l potassium chloride, 4·2 mmol/l dithiothreitol (DTT, Sigma) and 80 μmol/l manganous chloride. After incubation, 45 μl reaction mixture was transferred on to filter discs presoaked with 0·1 ml tetrasodiumpyrophosphate. The discs were dried and then washed in 10% and 5% trichloroacetic acid respectively, before drying and counting in 10 ml scintillation fluid in a beta-scintillation counter. Values were calculated in relation to the total protein content of the sample. The results were expressed as units per 10^8 cells, one being equivalent to the polymerisation by TDT of 1 nmol of nucleotide in one hour.[6] The specificity of TDT activity was assessed by a series of estimations including the inhibition of the enzyme activity by the addition of 50 μmol of ATP to the reaction mixture. The ATP caused complete disappearance of all TDT activity excluding the possibility that high activities occurred as a result of non-specific DNA polymerase activity. Experiments assessing TDT activity after storage up to 48 h at 4°C or room temperature did not significantly alter the absolute TDT values.

Results

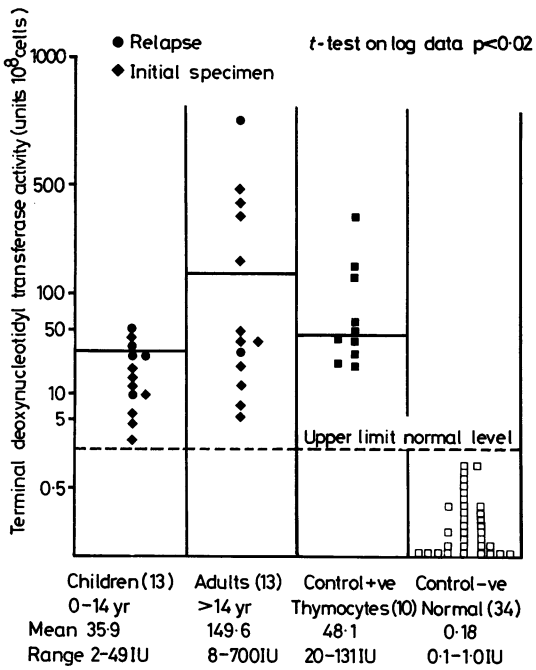
The values for TDT estimation are shown in Table 1a and b and demonstrated in the Figure. The majority of adult patients were classified as FAB sub-group L2 and the five patients with the highest

Table 1a *Levels of TDT activity in thirteen cases of adult ALL; the predominant histological type was L2 (9/13) (FAB classification). Eight cases were non B non T and five cases were not tested. There was no relation between the patients' age or FAB classification to levels of TDT activities in the adult group. Patients SM and SP had TDT estimations performed on peripheral blood*

Name	*Age*	*FAB histological class*	*% T*	*% B*	*Non B Non T %*	*Relapse (R)*	*TDT/10^8 WBC (IU)*
SM	52	Unclass	5	<1	95		47
RO	19	L2	8	2	90		378
WA	28	L2	<1	<1	>99	R	33
WR	28	L1	ND	ND	—		191
ROB	31	L2	ND	ND	—		11
BA	31	L1	<1	<1	>98		25
BE	23	L2	ND	ND	—	R	700
AN	59	L2	ND	ND	—		9
RU	68	L2	<1	<1	>98		8
AI	57	Unclass	14	18	68		42
OA	15	L2	10	19	71		42
BR	15	L2	<1	<1	>99		189
SP	15	L2	ND	ND	—		271

Table 1b *Levels of TDT activity in thirteen cases of childhood ALL; the predominant histological type in the childhood group was L1 (FAB classification). 4/5 of the cases assessed at the time of first relapse (R) showed TDT activities greater than 30 units in comparison to only 1/8 patients showing greater than 30 units at the time of presentation. Patients SJ, CR, DO and BUR had TDT estimations performed on peripheral blood*

Name	*Age*	*FAB histological class*	*% T*	*% B*	*Non B Non T %*	*Relapse (R)*	*TDT/10^8 WBC (IU)*
WI	5	L1	5	7	88	R	49
FI	5	L1	21	10	69		4
HA	5	L2	ND	ND	—	R	34
SJ	1·1	L1	6	6	81		2
BO	11	L1	<1	<1	>99		45
ALL	7	L1	9	15	76	R	9
DA	8	L2	ND	ND	—	R	34
CR	2	—	9	9	82		12
BU	8	L2	3	2	95		7·3
TR	9	L1	ND	ND	—	R	42
DO	2	L1	<1	<1	>99		22
DAV	3	Unclass	<1	<1	>99		19·3
BUR	1·5	L1	ND	ND	—		9·9



Comparison of TDT values occurring in adult and childhood ALL. There appear to be two sub-groups of adult ALL with different TDT values as assessed by biochemical estimation of the enzyme. The positive control thymocytes give a scatter of values between the adult and childhood ALL ranges.

activities in the series were shown to be of the L2 type. However, an equal number of adults with L2 histology also had lower activities of TDT. In contrast, the childhood group was predominantly of L1 histological classification and none of the children had TDT values greater than 100 IU though the highest values seen in the childhood group were in samples from those patients in relapse. It was not possible to find any difference in TDT activities between the L1 and L2 cases due to insufficient data. Because of the different proportions of L1/L2 cases in adults and children (significant using a Fisher Exact test, $p = 0{\cdot}03$) any investigation of L1/L2 difference should be carried out within the separate groups. An overall analysis could not distinguish a difference in TDT activities as resulting from the L1/L2 levels rather than the adult/child difference. The Figure indicates that in this series the mean TDT activity in adults, 149·6 IU, was significantly different from that of the childhood group, 35·9 IU, at the 0·02 level using a *t* test on logs of TDT values. In addition, the difference between adult and child TDT activities using the Wilcoxon rank sum test was found to be significant ($p < 0{\cdot}05$). When the six estimations performed on peripheral blood were removed the difference between adult and childhood groups, while still being of a similar magnitude, was no longer significant as a result of reduction of the sample size. Conversely, removal of the estimations performed on relapse specimens, whilst reducing the numbers, still showed a significant difference between the two groups.

The values obtained from thymocytes showed activities of TDT between the values found in adult and childhood ALL. Control marrow values demonstrated less than 1 IU of TDT activity and the values for patients with chronic lymphatic leukaemia, acute myeloid leukaemia, Hodgkin's disease and non-Hodgkin's lymphoma were also within the normal range. The ten ALL patients who had TDT assessment at the time of initial marrow remission had unmeasurable TDT activities. Within the group of adult patients there appears to be a number of patients who have TDT activities which resemble those in the childhood group and the mean value is enhanced by five patients who have markedly different activities from the remainder. This may indicate that in some adult patients the cell type involved may be a more primitive cell giving rise to much larger TDT activity per cell.

Discussion

In this report we have drawn attention to the fact that in our adult ALL cases, TDT estimation appeared to show two sub-populations. Whether those cases with high terminal transferase values in the adult group or those with L1 or L2 cytological sub-types will be seen to be those who have an unfavourable prognosis we are not yet in a position to say. Nevertheless, it is tempting to speculate that tumours with markedly increased TDT may well arise at a different stage of development of the primitive lymphoid series than the "common" acute lymphoblastic leukaemia of childhood. The assessment of surface markers using antibodies against "common"-ALL antigen, HLA (homologous leucocytic antibody)—D locus related antigen and antigen in relation to quantitative TDT estimations have not revealed differences between TDT activities in immunological sub-groups of ALL.[2 8] These antisera were unavailable in our preliminary study. Janossy *et al.*[2] did not find any relation between TDT activities and surface marker phenotype in 15 adult ALL patients tested. 60% of these cases were "null" ALL—that is, common-ALL antigen negative, and 40% were common-ALL positive.

Previous authors[1 6] have not been impressed by absolute differences in TDT between adults and childhood ALL. Bollum,[1] in a review of data from

over 1000 cases, indicated the estimation of TDT activity in 118 children with ALL showed a mean activity of 85 IU whilst in assessment of only nine adults in that series, the mean activity was higher at 181 IU. Pangalis and Beutler,[9] in a group of adults, have demonstrated a TDT range of 4·7 to 2500 IU with their method but indicated that absolute values in TDT did not seem to relate to ultimate prognosis in their patients.

We would conclude from this preliminary series that a careful survey of quantitative TDT activity in ALL may reveal in adults a biological sub-population showing a higher concentration of TDT than childhood ALL. It would appear from previous work that such a high TDT sub-group would not correspond to common-ALL or "null" ALL[2 8] found in adults, but may form a completely separate sub-group independent of immunological sub-types. We consider that the biochemical estimation of TDT may still have an important part to play in the detailed classification of adult ALL and it would be inadvisable to rely totally on qualitative recognition of TDT by antibody techniques.

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