Correspondence

Accepted: December 18, 1984

Acta haemat. 1985;73:124-125

Terminal Deoxynucleotidyl Transferase Positivity in Neuroblastoma

A.	Anne	O'Meara
F.	Fin	Breatnach
T.	Theresa	Finn
E.	Emer	Lawlor

Dublin

Anne O'Meara, Fin Breatnach, Theresa Finn, Emer Lawlor, Children's Research Centre, Our Lady's Hospital for Sick Children, Crumlin, Dublin 12 (Republic of Ireland)

Since the discovery of the enzyme terminal deoxynucleotidyl transferase (TdT) in calf thymus [1] this 'marker' has come to play a major role in the interpretation and classification of the leukaemias [2]. We would like, however, to report strong TdT positivity in marrow aspirate of a child with a non-haematological malignancy. A 5-year-old girl was admitted with a 3-week history of lethargy and flitting aches. Physical examination revealed obvious pallor and a low grade fever. There were no other abnormal physical findings. Haematological investigations included HB 9.6 g/dl, WCC $3.9 \times 10^9/l$ of which 42% were neutrophils, 54% lymphocytes, 2% monocytes and 2% eosinophils; platelet count was $177 \times 10^9/l$. May-Grünwald-Giemsa staining of initial marrow aspirate showed reduction in erythroid and megakaryocytic cell lines, but no evidence of a malignant process. Repeat marrow aspirate demonstrated malignant cells distributed both singly and in clumps permeating and replacing normal marrow elements. The cell morphology was consistent with acute lymphatic leukaemia (ALL) of L2 type using the FAB classification. Periodic acid Schiff and Sudan black stains were negative and the acid phosphatase stain revealed an atypical diffuse granular positivity. Immunophenotyping of the marrow cells with a panel of monoclonal antibodies gave the following result: UCHL-1- (P. Beverly), Ia⁺, cALLA (J5)-, OKT6⁺, VIM-D5⁺ and VIB-C5⁺ (W. Knapp), My7⁺ and My9⁺ (J. Griffin) anti-monocyte-, sIg⁺. A TdT immunofluorescence (IF) assay (Bethesda Research Laboratories) of bone marrow smear revealed greater than 90% positivity of bone marrow cells. A tentative diagnosis of ALL was made, probably of early T cell lineage. Subsequent clinical investigation, however, proved this not to be the case. A skeletal survey was normal apart from multiple lucent areas in the skull vault and there was downward and lateral displacement of the left kidney on IVP. CT scan of abdomen confirmed a small calcified mass (not seen on plain film) in the upper left quadrant extending medially and downward. 24-hour urinary estimation of VMA was normal. A repeat marrow aspirate reacted strongly with monoclonal antibody UJ13A [3], indicating presence in the marrow of cells of neuroectodermal origin. A diagnosis of stage IV neuroblastoma was thus confirmed. The child was commenced on three weekly courses of vincristine, cyclophosphamide, cisplatin and VM26. After two courses, the marrow was free of neuroblastoma cells and removal of the primary tumour was carried out after a further four courses. Histology of the tumour confirmed a

well-differentiated neuroblastoma which did not demonstrate TdT positivity using the IF technique. The enzyme TdT catalyzes the addition of deoxynucleoside triphosphates to the 3'-OH of oligo- or polydeoxynucleoside initiators without template instruction [4]. The enzyme is normally present in high levels only in the thymus, but low levels are also found in bone marrow [5] and brain [6]. Some authors have demonstrated low levels of TdT activity in neuroblastoma cell lines while others have reported negative results in neuroblastoma tissue and marrow infiltrated with neuroblastoma [7]. Human brain TdT has been reported to have the same physical and molecular properties and to be antigenically identical with TdT of human leukaemia cells and calf thymus [6]. Presence of TdT is detectable by straightforward enzyme analysis or by monospecific antibody prepared against a homogeneous protein antigen [5]. Since the quantitative test for TdT did not aid in the classification of ALL and is costly and complicated to perform, it is no longer used routinely in many centres. TdT by IF assay, on the other hand, is a convenient and rapid technique that enables easy-

Terminal Deoxynucleotidyl Transferase Positivity in Neuroblastoma

125

transferase levels and membrane phenotypes in diagnosis of acute leukaemia. *Lancet* ii: 520-522 (1977).

Kemshead, J.T.; Goldman, A.; Fritschy, J.; Malpas, J.S.; Pritchard, J.: Use of panels of monoclonal antibodies in the differential diagnosis of neuroblastoma and lymphoblastic disorders. *Lancet* i: 12-14(1983).

Bollum, F.J.: Terminal deoxynucleotidyl transferase; in Boyer, The enzymes, vol. 10; 3rd ed., pp. 145-171 (Academic Press, New York 1974).

Bollum, F.J.: Terminal deoxynucleotidyl transferase as a haematopoietic cell marker. A review. *Blood* 54: 1203-1215 (1979).

Norton, P.; Viola, M.V.: Terminal deoxynucleotidyl transferase (TdT): an enzyme specific to thymus and brain. *Clin. Res.* 26: 354A(1978).

Greenwood, M.F.; Coleman, M.S.; Hutton, J.J.; Lampkin, B.; Krill, C.; Bollum, F.J.; Holland, P.: Terminal deoxynucleotidyl transferase distribution in neoplastic and haematopoietic cells. *J. clin. Invest.* 59: 889-899 (1977).

Hutton, J.J.; Coleman, M.S.; Keneklis, T.D.; Bollum, F.J.: Terminal deoxynucleotidyl transferase as a tumor cell marker in leukaemia and lymphoma. Results from 1,000 patients. *Adv. med. Oncol.* 4: 165-175(1979).

Murphy, S.; Jaffe, E.S.: Terminal transferase activity and lymphoblastic neoplasms. *New Engl. J. Med.* 311: 1373-1374 (1984).

er access to TdT determinations than enzymatic assay [5]. Most authors report complete concurrence between both assays though others [8] have highlighted a small number of exceptions to this rule in ALL. They proposed that the discrepancies between percentage of IF cells and quantitative enzymatic activity suggest 4 heterogeneity among cells in content of TdT as well as the possibility of antigenically reactive enzyme that is 5 enzymatically inactive. It is concluded that immuno-cytochemical techniques are the methods of choice in 6 terms of both specificity and sensitivity [9]. Our observations demonstrate that TdT is not necessarily haemopoietic precursor cell-specific. The observation in this patient of TdT positivity in marrow which was subsequently negative in the primary tumour could indicate either: (a) chemotherapy altered the antigenicity of the tumour or (b) neuroblasts infiltrating marrow may be phenotypically different from the tumour of origin. Since the differential diagnosis of acute leu-

9 leukaemia in childhood often includes neuroblastoma, these findings suggest that TdT positivity alone is insufficient immunologically to distinguish between these malignancies.

References

Bollum, F.J.: Oligodeoxyribonucleotide primers for calf thymus polymerase. J. biol. Chem. 235: PC18 (1960).

Hoffbrand, A.V.; Ganeshaguru, K.; Janossy, G.; Greaves, M.F.; Catovsky, D.; Woodruff, R.K.: Terminal deoxynucleotidyl