TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE IN AKR LEUKEMIC CELLS AND LACK OF RELATION OF ENZYME ACTIVITY TO CELL CYCLE PHASE

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SUMMARY: Terminal deoxynucleotidyl transferase activity in mice, as in other animals, is normally confined to the thymus and the bone marrow (5, 7). In leukemic AKR mice, terminal transferase activity was also detected in leukemic cells. The specific activity of terminal transferase in these cells as a function of cell cycle phase was determined using AKR leukemic cells separated according to their position in the cell cycle by velocity sedimentation through a sucrose gradient at unit gravity. Normalization of enzyme activity to either total number of cells or to total protein in the crude homogenate failed to reveal a relationship of enzyme activity to cell cycle phase.

Since the demonstration of terminal deoxynucleotidyl transferase (EC 2.7.7.31 deoxyribonucleoside triphosphate: DNA nucleotidyl exotransferase) in the circulating cells of a patient with acute lymphoblastic leukemia (1), there has been a renewed interest in the biology of this unique enzyme in both normal and abnormal cells (2-6). Originally detected only in the thymus glands of numerous species (7), its presence in low levels has recently been reported in normal human (2, 6), mouse (5), rat (8) and rabbit (9) bone marrow.

Taken as a whole, the present evidence (1-9) suggests that terminal transferase has some function in the development of cells which give rise

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to T-lymphocytes. However, the biological role of terminal transferase on a molecular level remains unknown, although it has been suggested that it could be a somatic mutagen (10).

A biological role for DNA polymerase α (see reference 11 for a summary of the nomenclature of eukaryotic DNA polymerases) in DNA replication was suggested by its increase in populations of growing as opposed to resting cells (12, 13). Persuasive evidence in support of such a role for DNA polymerase α has been provided by its increase in mouse L cells (14), synchronized HeLa cells (15) and fractionated AKR leukemic cells (Barr, R.D., Sarin, P., Sarna, G. and Perry, S., ms. in preparation) during the S phase of the cell cycle. Because the level of terminal transferase per gram of thymus tissue has been shown to increase during the development of the fetal calf (7), we have studied the relationship of terminal transferase to the cell cycle.

Normal cells exhibiting terminal transferase activity are found in a very heterogeneous environment (the thymus and the bone marrow) and are not available as homogeneous populations which can be manipulated to obtain cells in various phases of the cell cycle. However homogeneous AKR leukemic cells, which have been found to be terminal transferase positive, can be easily obtained for study. As shown in Table 1, pre-leukemic AKR mice, like normal mice of other strains, have terminal transferase in their thymocytes but not in their splenic cells. In leukemic AKR mice, the tumor cells in the thymus, as well as those in the spleen and nodes, have terminal transferase activity. Terminal transferase positive cells can therefore be obtained, as a homogeneous population, from AKR thymus glands which are totally replaced with tumor cells. Furthermore, they can be fractionated according to their position in the cell cycle (16).

Leukemic AKR mice were obtained from Microbiological Associates, Bethesda, Maryland. Single cell suspensions of leukemic cells were

Table 1. Terminal Deoxynucleotidyl Transferase Levels in AKR Tissues

Tissue	Enzyme Units/10^8 Cells	
	Pre-leukemic (5.5 weeks)	Leukemic (7-12 months)
Thymus	0.48 (0.29-0.58)	0.24 (0.23-0.25)
Lymph nodes	N.D.	0.21 (0.10-0.30)
Spleen	<0.0002	0.29 (0.02-0.52)

Cell suspensions were prepared and studied as previously described (5). Suspensions from leukemic mice were prepared from 2-7 animals which had greater than 50% lymphoblasts in their peripheral blood. The cell suspensions from these animals were composed of greater than 80% blast-like cells. Activity shown represents the average of all determinations, with the range shown in parenthesis. N.D. = not determined.

prepared (16) and fractionated, according to their position in the cell cycle, by velocity sedimentation through a sucrose gradient at unit gravity (17). This technique separates cells more on the basis of differences in size than of differences in density (18). In a previous report (19) it has been shown that fractions 9 and 10 are composed mainly of cells in the G_0 and G_1 phases of the cell cycle, and that increasing fraction number reflects progress through the cell cycle with fractions 26-30 composed of cells in the G_2 and M phases of the cycle.

The cell samples, separated by velocity sedimentation, were independently homogenized, chromatographed on phosphocellulose, and assayed for terminal transferase. The results, which are summarized in Table 2 show that there appears to be no relationship between terminal transferase activity per 10^8 cells and cell cycle phase. Expression of the results as enzyme units per milligram of protein, likewise revealed no relationship of enzyme activity to proliferative status (data not shown). Two peaks of activity were observed in each sample: one eluting at 0.22 M

Table 2. Terminal Transferase Activity in Fractionated AKR Leukemic Cells

Fraction Number	Enzyme Units/ 10^8 Cells	Fraction Number	Enzyme Units/ 10^8 Cells
9-10	0.070	19-20	0.160
11-12	0.090	21-22	0.100
13-14	0.120	23-25	0.090
15-16	0.080	26-30	0.090
17-18	0.080		

Fractionated cell suspensions were collected in 30 x 50 ml aliquots from each sedimentation chamber (16), harvested by centrifugation, and stored at -70°C. Cells were extracted and terminal transferase assays performed as previously described (6) with the following modifications: the detergent treated crude homogenate was made 1.5 M KCl, stirred at 4°C for 2 hours and dialyzed against TEM + 50 mM KCl before high speed centrifugation, and the assay reaction was scaled up to a final volume of 0.4 ml using 20 µg/ml of oligo$(dA)_{12-18}$.

KCl (Peak I) and one eluting at 0.29 M KCl (Peak II) corresponding to the peaks previously observed in normal murine thymocytes (5). The ratio of Peak I to Peak II varied slightly from approximately 0.5 to 1.0, but was consistently higher than that observed when normal murine thymocytes are used as starting material and showed no relation to cell cycle phase. Other murine tumor cells which express terminal transferase activity (e.g. cells from animals with Moloney leukemia virus induced tumors) also show an increased Peak I to Peak II ratio (Silverstone, A. and Kung, P.C., unpublished observations).

Terminal transferase is present in AKR leukemic cells and the level of enzyme activity is unrelated to cell cycle phase. In the light of these data, it seems unlikely that terminal transferase plays a role in normal DNA replication in cells which express this enzyme. However, it is clearly possible that other factors, which would not be detected in our system such as compartmentalization of the enzyme or availability

of the primer, might be the major determinants of its biological activity.

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