TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE AND DNA POLYMERASE IN CLASSES OF CELLS FROM RAT THYMUS*

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SUMMARY

Cells from rat thymus have been fractionated on albumin density gradients. The activities of terminal deoxynucleotidyl transferase, DNA dependent DNA polymerase, thymidine kinase, and the rate of incorporation of thymidine into DNA vary among the classes of cells. Terminal transferase activity present in thymus is quantitatively recovered from the gradient and is highest in fractions rich in small lymphoid cells or thymocytes.

INTRODUCTION

The existence of a deoxynucleotide polymerizing activity, terminal deoxynucleotidyl transferase (TdT), separate from DNA polymerase has been known for some time (1,2,3). In a broad survey of tissues from various animals, TdT activity was found exclusively in vertebrate thymus and was not detected in bursa Fabricius, Peyer's patches, spleen, lymph nodes, circulating lymphocytes or a variety of other organs (4). A recent report (5) confirmed these observations, but reported the presence of TdT in leukemic cells of childhood acute lymphoblastic leukemia. We have found high levels of TdT in leukemic cells from an adult with myelomonocytic leukemia and have detected low levels of the enzyme in normal human bone marrow (unpublished results). All of these findings stimulated our interest in isolating different classes of cells from thymus and characterizing them with respect to the

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types of nucleotide polymerizing activities they contain. Our results indicate that rat thymus is composed of several biochemically distinct types of cells.

METHODS

Fractionation of Cells. Minced thymus from weanling rats was forced through a #40 nylon mesh screen. The cells were washed in RPMI 1640 tissue culture medium (Gibco) and counted. Fraction V bovine serum albumin (BSA) was desalted by dialysis and dissolved in phosphate buffered saline. An 8 ml discontinuous density gradient (8 layers) containing 19-33% BSA was prepared, similar to the linear gradients used by Shortman to fractionate lymphoid cells (6). Approximately 10^9 cells from thymus were suspended in 2 ml of 17% BSA and layered on top of the gradient. The gradient tube was then centrifuged at 1000 x g for 35 min at 4° C. The seven distinct layers of cells which formed were removed separately with a 1 ml syringe. They were washed 4 times in 0.85% saline and then suspended in RPMI 1640 culture medium for counting of nucleated cells. Detergent was added to cell suspensions to eliminate erythrocytes before counting on the Coulter Counter.

Thymidine Incorporation. ^{3}H-thymidine (5µCi) was added to 10^6 cells from each gradient fraction in one ml RPMI 1640. After incubation at 37° C for 30 min the thymidine pulse was stopped by the addition of cold 5% trichloracetic acid. The cells were collected on Whatman GF/C disks, washed with 5% TCA, ethanol, ether and dried for scintillation counting.

Enzyme Assays. Pelleted cells from each gradient fraction were suspended in TKM-sucrose (7) at 10^8 cells per ml and sonicated for 4 min at 0° C. Cell breakage was roughly 95%. The crude sonicates were centrifuged at 100,000 x g for 60 min, and assayed for TdT, high

molecular weight DNA polymerase, and thymidine kinase. The initiator for TdT was $d(pA)_{\overline{50}}$ with ^{3}H-dGTP polymerization (4). High molecular weight DNA polymerase was assayed using activated DNA as template (7). One unit of transferase or polymerase activity equals one nmole nucleotide polymerized per hour. The thymidine kinase reaction mixture contained 25 µl of soluble supernatant fraction, 0.1 M Tris, pH 8.0, 6 mM 3-phosphoglycerate, 5 mM $MgCl_2$, 5 mM ATP, and 20 µCi ^{3}H-thymidine (1.9 Ci/mmole). The reaction was incubated at 35° C for 30 min and then placed in ice. From each reaction 0.1 ml was spotted onto a 1.5 x 24 inch DE-81 strip and developed in 4 M formic acid. The chromatograms were cut into 1 cm sections and solubilized overnight in NCS (Nuclear Chicago) at 37° C. The solubilized disks were suspended in toluene-Triton scintillation fluid. Radioactivity in dTMP, dTDP and dTTP was summed for total kinase activity.

RESULTS

Distribution of cells. Table I illustrates a typical distribution of cells on the BSA density gradient. Cell separations on these gradients are not discrete. Each fraction from the gradient was seen to be heterogeneous by microscopy. The proportions of various sizes of lymphoid cells differs through the gradient with larger cells tending to concentrate near the top of the gradient (Fraction 1) in agreement with findings of Shortman (6). Cells were well dispersed and few clumps were seen. Erythrocytes were concentrated in fractions 4 and 5 visually demonstrating the resolving power of the gradient. Fractions 3 to 5 generally contain 65% or more of the total nucleated cells and at least 90% of these were small lymphoid cells (thymocytes). Mitotic figures were rare in all fractions. A pellet is present at the bottom of the tube and contains cellular debris and connective tissue.

Table I. Distribution of Cells on the BSA Gradient

Fraction number	Nucleated cell count
1 Top	0.12×10^8
2	0.40×10^8
3	0.93×10^8
4	1.8×10^8
5	2.3×10^8
6	1.9×10^8
7 Bottom	0.40×10^8

Polymerase Assays. Greater than 90% of the TdT and DNA polymerase activities present in extracts of unfractionated thymus were recovered in fractionated cell suspensions. The distribution of these enzymes in cells from the BSA gradient is shown in Fig. 1. It should be noted that activity is expressed as units per 10^8 cells. TdT activity per cell is highest in fractions 4, 5 and 6. This is the central region of the gradient where most of the small lymphoid cells (thymocytes) band. Ten-fold differences in activity per cell are observed. Fraction 1 contains many of the large lymphoid cells and very little TdT. The high molecular weight DNA polymerase is more broadly distributed over the cells in the gradient and differences in DNA polymerase per cell are less marked (3 to 4 fold). Neither TdT nor DNA polymerase could be detected in the pellet at the bottom of the gradient tube.

Thymidine Incorporation. Thymidine incorporation into acid insoluble product was used as a measure of DNA synthesis in cells from the BSA gradient (Fig. 1). The rate of thymidine incorporation was 5 times as high in fractions 1 and 7 as in fraction 5 where the major class of small thymocytes was located. This result shows that the rate of thymidine incorporation does not parallel the activity of DNA polymerase or TdT. We then measured the activity of thymidine kinase in cells from the gradient, since this enzyme influences the size and rate of labeling of the intracellular pool of thymidine deoxynucleotides.

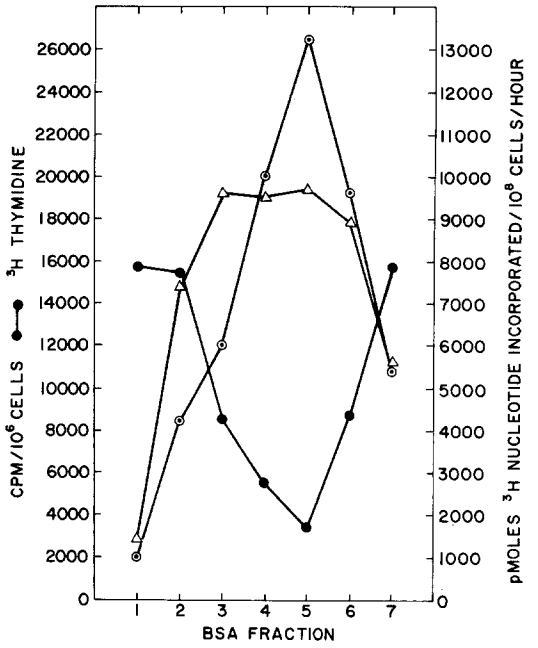

Figure 1. Biochemical activities in thymic cells fractionated on a BSA density gradient. ●—● , incorporation of ^{3}H-thymidine into acid insoluble product; Δ—Δ , activity of high molecular weight DNA polymerase; ⊙—⊙ , activity of terminal deoxynucleotidyl transferase.

Thymidine kinase activity paralleled the rate of thymidine incorporation with typical activities of 16 nmoles dTMP formed/10^{8} cells/hr in fraction 1 and 5.0 in fraction 5. The intracellular nucleotide pools in the gradient fractions have not been determined.

DISCUSSION

Cells from rat thymus can be fractionated on BSA density gradients. Previous studies (6) have demonstrated that fractions from such gradients are heterogeneous both in morphology and biological function. The small lymphoid cells or thymocytes concentrate in the mid-portion of the

gradient, whereas large lymphoid cells concentrate near the top. Gradient fractions also differ from one another biochemically. The major class of small lymphoid cells (fractions 4,5,6) contains high levels of terminal deoxynucleotidyl transferase and high molecular weight DNA polymerase, but relatively low levels of thymidine kinase and low rates of incorporation of thymidine into DNA. Since all of the terminal transferase present in thymus is recovered from the gradient, the small lymphoid cell must be its major location. Two minor classes of cells, fraction 1 rich in large lymphoid cells and fraction 7 rich in small lymphoid cells, contain very low levels of terminal transferase and DNA polymerase, but high levels of thymidine kinase and high rates of incorporation of thymidine into DNA. Use of a thymidine pulse as a test for DNA synthesis would clearly pinpoint these minor classes of cells as extremely active in DNA synthesis, whereas the low levels of DNA polymerase suggest that this may not be so. These conflicting results illustrate the difficulties in interpretating data from thymidine pulses without knowledge of the size of intracellular nucleotide pools and rates of transport and phosphorylation of the labeled nucleoside. All of these findings center interest on deoxynucleotide metabolism in cells from thymus as a favorable biochemical method for distinguishing lymphoid cells. Further investigation of this area may aid in understanding the biology of the thymus and its role in the development of immunocompetence.

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