Terminal Deoxynucleotidyl Transferase in Normal and Neoplastic Hematopoietic Cells

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Introduction

In 1973 we first reported our finding of terminal deoxynucleotidyl transferase (TdT) in the circulating leukemic cells of a child with acute lymphoblastic leukemia (1). Until that time this unique DNA synthetic enzyme had been thought to be a special biochemical property of cells undergoing maturation in the thymus (2). Its presence in circulating lymphoblastic leukemia cells suggested that the thymus might play a role in the pathogenesis of this disease in man, analogous to the central position it plays in the pathogenesis of lymphoblastic leukemia in the AKR mouse (3, 4, 5). In addition, it appeared to have potential clinical utility as a new biochemical leukemia cell marker (1, 6).

We have now accumulated extensive data on the expression of this unusual enzyme in leukemia cells from a large number of patients. We have, in addition, been able to identify TdT in both human and murine bone marrow, and have partially characterized the TdT-positive normal marrow cells. Several new facts have emerged from this work which allow for new insight into the cellular origins of leukemia cells in various clinical entities.

Enzyme Extraction and Identification

The preparation of cells and tissues for TdT assays is as previously described (1, 6, 7, 8) with some recent modifications. Immediatley before homogenization phenylmethylsulfonylfluoride, a serine protease inhibitor, and ethanol are added to a concentration of 20 mM and 5 % respectively, in order to inhibit proteolytic degradation of TdT when samples containing cells rich in proteolytic activity (e. g. phagocytic cells in normal bone marrow, or chronic myelogenous leukemia) are studied. After detergent treatment, the crude homogenate is extracted with high

salt which results in more efficient enzyme solubilization (8). TdT activity is then identified following phosphocellulose chromatography of the crude homogenate.

The assay involves providing the enzyme source with a radio-labeled deoxynucleotide triphosphate (usually ^{3}H-dGTP) and a pre-formed DNA primer molecule (usually a short polymer of deoxyadenylic acid, oligo$(dA)_{14}$) onto whose 3'-OH terminus deoxynucleotide monophosphates can be polymerized from deoxynucleotide triphosphate substrates (7, 8).

TdT in leukemia cells

Leukemic cells from almost every patient with acute lymphoblastic leukemia (ALL) contain TdT as part of their biochemical phenotype. The phosphocellulose elution pattern of TdT from the circulating blast cells of a typical patient with ALL is shown in Figure 1. This phosphocellulose elution profile shows two discrete peaks of TdT activity which (Fig. 1B and 1C) maintain their separateness after

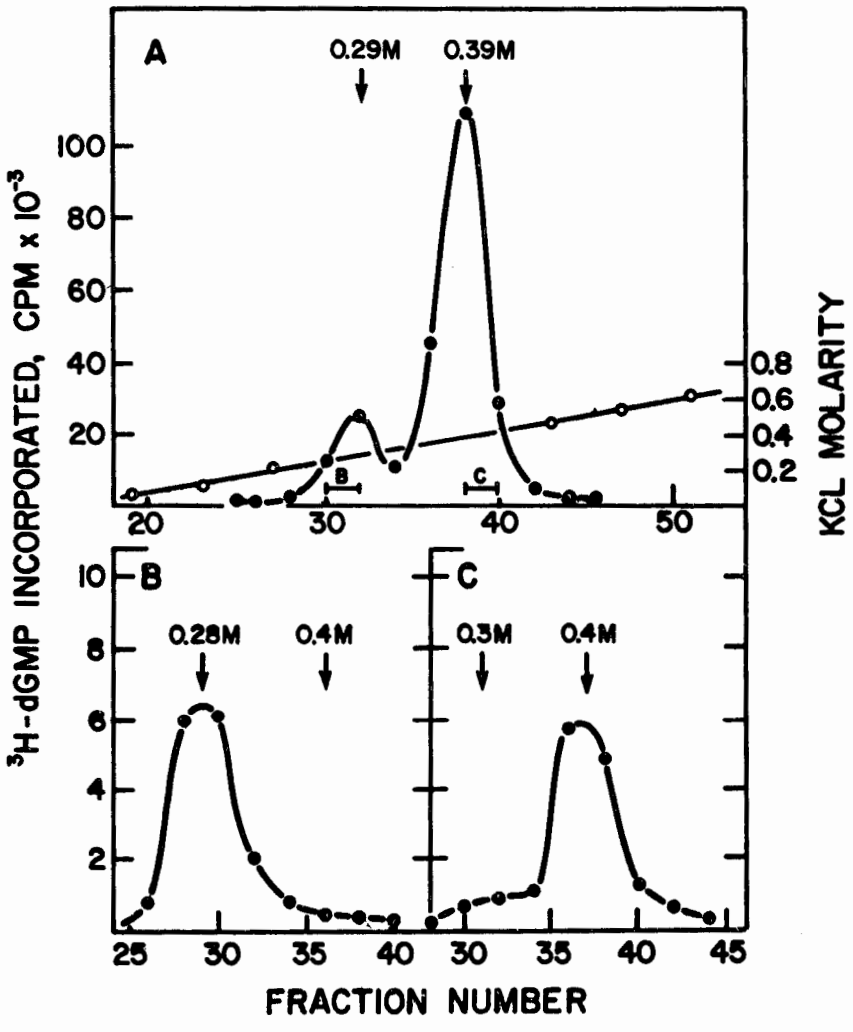

Fig. 1: TdT activity from peripheral blood of a patient with acute lymphoblastic leukemia. Panel A: Activity recovered on initial phosphocellulose chromatography. Panels B and C: activities recovered after rechromatography on phosphocellulose of pooled fractions from initial column (indicated as "B" and "C" in panel A), after dialysis and passage through a DEAE cellulose column.

chromatography on DEAE, followed by rechromatography on a second phosphocellulose column. The relative ratios shown on initial phosphocellulose chromatography of the early eluting (peak I) to late eluting (peak II) peak are fairly constant. In a few patients virtually all the TdT activity was confined to the peak II region, with the peak I region reduced to a "shoulder."

The phosphocellulose pattern shown in Figure 1 is typical of newly diagnosed patients with ALL using leukemic cells from either peripheral blood or bone marrow. However, in 4 (out of 4 studied) bone marrow samples taken from ALL patients with acute, unexpected bone marrow relapse the pattern shown in Figure 2 was seen. Here peak I predominates, with peak II reduced to a shoulder.

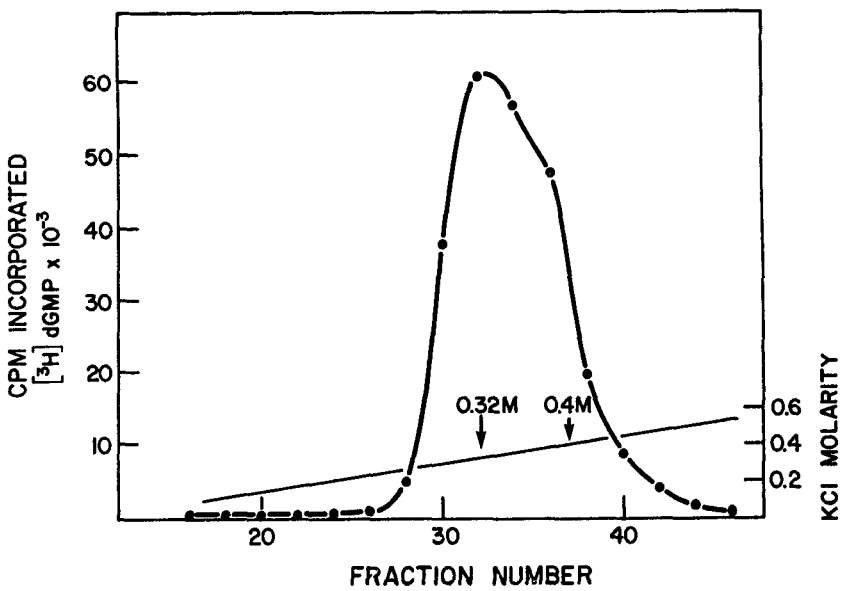

Fig. 2: TdT activity from bone marrow of a patient with acute unexpected bone marrow relapse.

The significance of these patterns has not been established. It is not known whether these peaks exist in separate cell types, whose ratio varies in different stages of the disease, or whether their relative ratios represent molecular phenomena within a single cell population.

We have identified TdT activity in leukemic cells from 32 of 36 patients clinically considered to have ALL. A wide spectrum of patients was sampled, both children and adults, representing both T-cell and null-cell disease. However, as shown in Table 1 it is now evident that TdT is not confined to leukemic cells from patients who are considered to have ALL by the usuall clinical and morphological criteria. As shown in this table, 8 of 22 patients with blast crisis chronic myelogenous leukemia had TdT positive cells. Only 3 of these 8 enzyme-positive samples were felt to have lymphoblastic morphology by their physicians. Some patients with undifferentiated leukemic cell morphology and monomyelocytic leukemia also had TdT positive cells. The TdT activities observed in these non-ALL samples were similar, in terms of chromatographic pattern, to the ALL samples.

In a later section we will propose that this commonality of TdT activity in cells from diverse clinical syndromes implies a biologic relatedness in terms of the

cellular origin of these diseases (the papers by Greaves et al. and Sarin et al. in this volume should also be consulted in this regard).

Terminal transferase in human leukemia cells

Cells Positive		
Acute lymphoblastic leukemia	32 of 36	patients
Blast crisis chronic myelogenous leukemia	8 of 22	„
Acute undifferentiated leukemia	3 of 4	„
Acute monomyeloblastic leukemia	1 of 2	„
Cells Negative		
Acute myeloblastic leukemia	9 of 9	patients
Stable phase chronic myelogenous leukemia	6 of 6	„
Chronic lymphatic leukemia	6 of 6	„
Sezary syndrome	2 of 2	„
Lymphosarcoma cell leukemia	3 of 3	„

TdT in normal cells

THYMOCYTES

The phosphocellulose elution pattern of TdT from human thymocytes is quite similar to leukemia cell TdT. (Compare Figure 1 and Figure 3 below). When thymocytes are further separated on a discontinuous bovine serum albumin (BSA) gradient and assayed for TdT (7), the enzyme activity is maximally expressed in cells from gradient layers 3, 4 and 5 (Fig. 4). When thymocytes from such a BSA gradient are studied for other characteristics, only cells from layers 2 and 3 are found to respond to phytohemagglutinin and allogeneic lymphocytes (9). TdT is therefore maximally expressed in thymocytes which are distinct from those thymocytes which are capable of mature T-lymphocyte immunologic reactivity. In the mouse thymus, TdT is also maximally expressed in cells of medium density as determined by BSA gradient analysis (8).

The ratio of peak I to peak II TdT acitivity varies from layer to layer in BSA gradients of both human and murine thymocytes. Medium density thymocytes contain predominantly peak II, whereas peak I predominates in low density thymocytes. In the murine thymus, during the repopulation period which follows cortisone-induced involution, the ratio of peak I to peak II changes dramatically (Fig. 5). In the first few days following cortisone shock, peak I containing cells predominate. The pre-cortisone ratios are gradually reachieved around day 6.

This fluctuation in peak I/peak II ratios is reminiscent of the variant patterns described above for relapse versus well established disease in ALL. One possible interpretation of these results is that peak I may exist in a more primitive (precursor type) cell, which accumulates during acute "explosive" events, such as thymic repopulation following cortisone shock, or acute leukemia relapse.

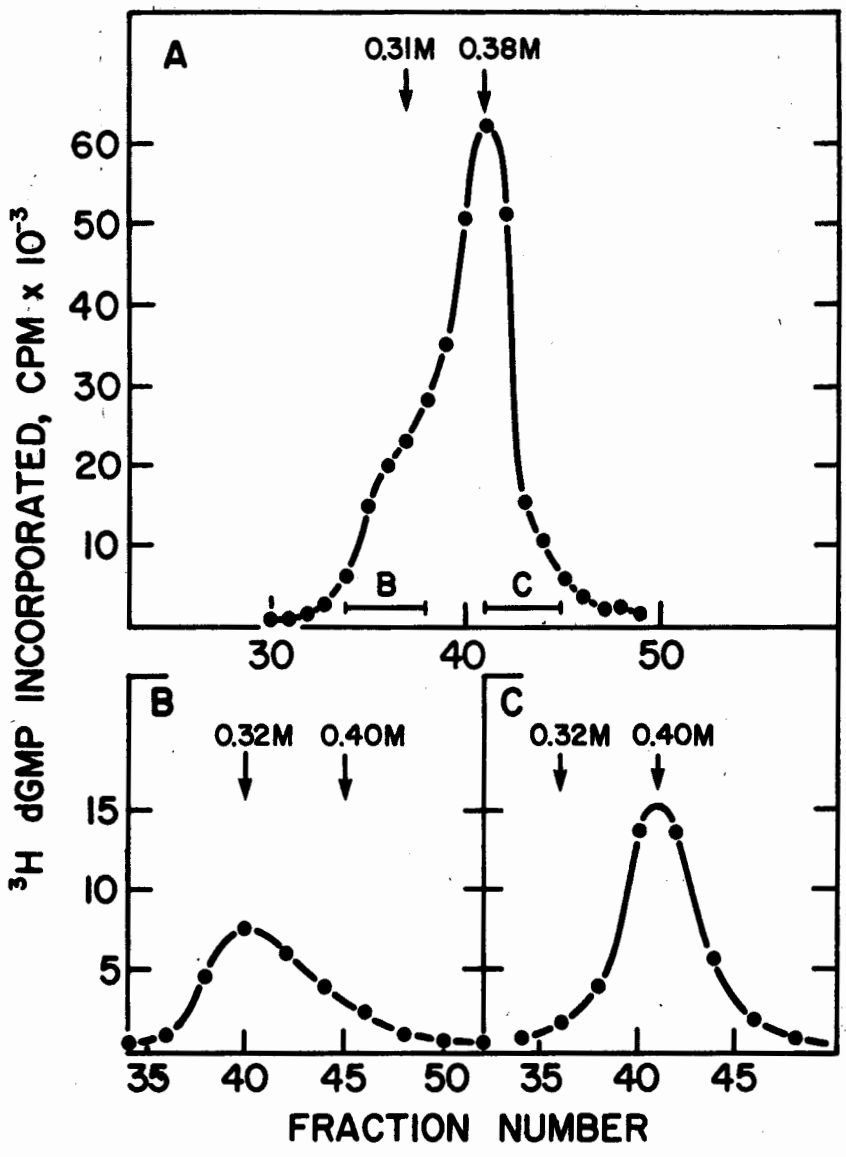

Fig. 3: TdT acitivity from normal human thymocytes.

Panel A: Activity recovered on initial phosphocellulose chromatography. Panels B and C: activities recovered after rechromatography on phosphocellulose of pooled fractions from initial column (indicated as "B" and "C" in pael A), after dialysis and passage through a DEAE cellulose column.

BONE MARROW

It is now clear that there exists in normal bone marrow a population of cells which expresses TdT activity (7, 8, 10). In man, the phosphocellulose chromatographic pattern is a broad peak, eluting between peak I and II positions (Fig. 6). It is similar to the pattern observed when bone marrow cells from ALL patients with acute bone marrow relapse are analyzed (Fig. 6). In the mouse, the normal bone marrow enzyme chromatographs in the thymocyte peak I position, with a small peak in the peak II position, similar to that seen in low density thymocytes. Biochemically the marrow activity from either species is indistinguishable from the thymocyte or leukemic cell activity of the same species.

Some information is available on the nature of the normal marrow cell population(s) which contains TdT. TdT-positive cells can be eliminated from normal

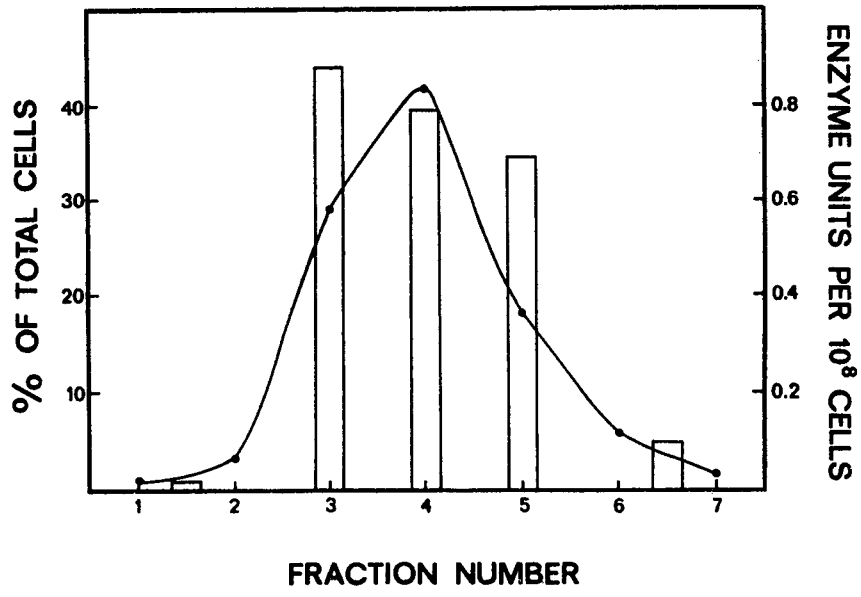

Fig. 4: TdT activity in normal thymocytes fractionated on a 17 to 33 per cent discontinuous bovine serum albumin gradient.

Fractions are numbered from the top of the gradient (17 per cent BSA). The number of cells at each interface, expressed as a per cent of the total number of cells recovered, is shown as closed circles. Enzyme units per 10^8 cells are represented by vertical bars. Bars between fraction numbers refer to assays on pooled cells.

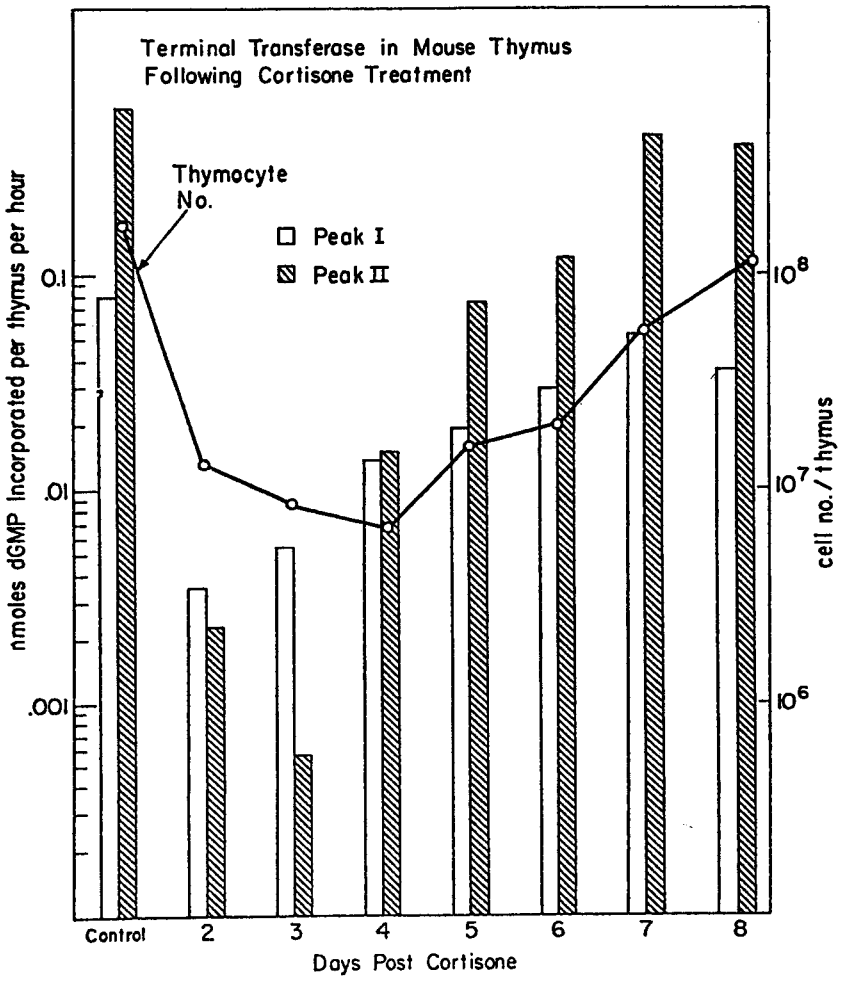

Fig. 5: Effect of cortisone treatment on number of cells and TdT activity in murine thymus.

A single 150 mg/kg dose of cortisone acetate was given to C57Bl/6J mice on day 0. The number of cells per thymus and TdT activity per thymus were determined sequentially thereafter.

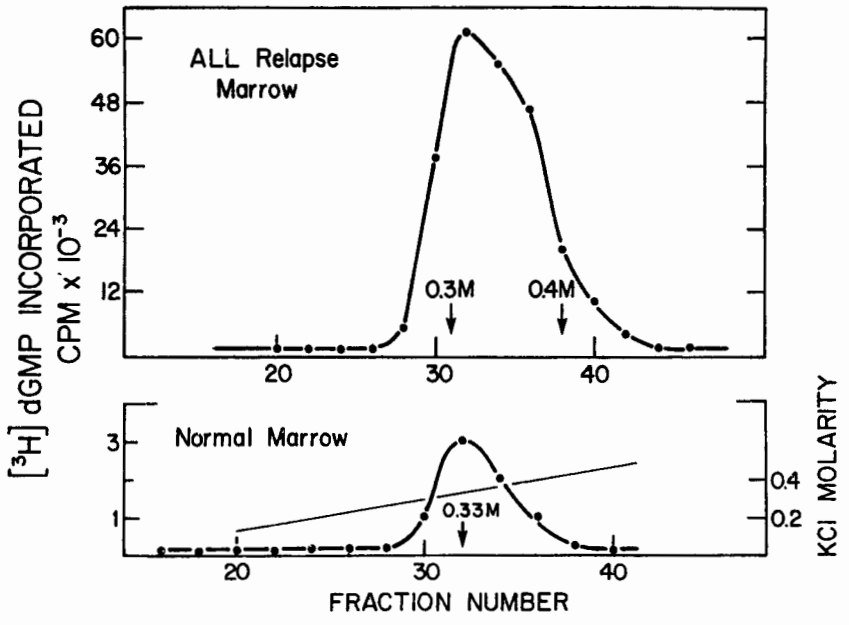

Fig. 6: Chromatographic profiles of TdT activity in acute unexpected bone marrow relapse and normal bone marrow.

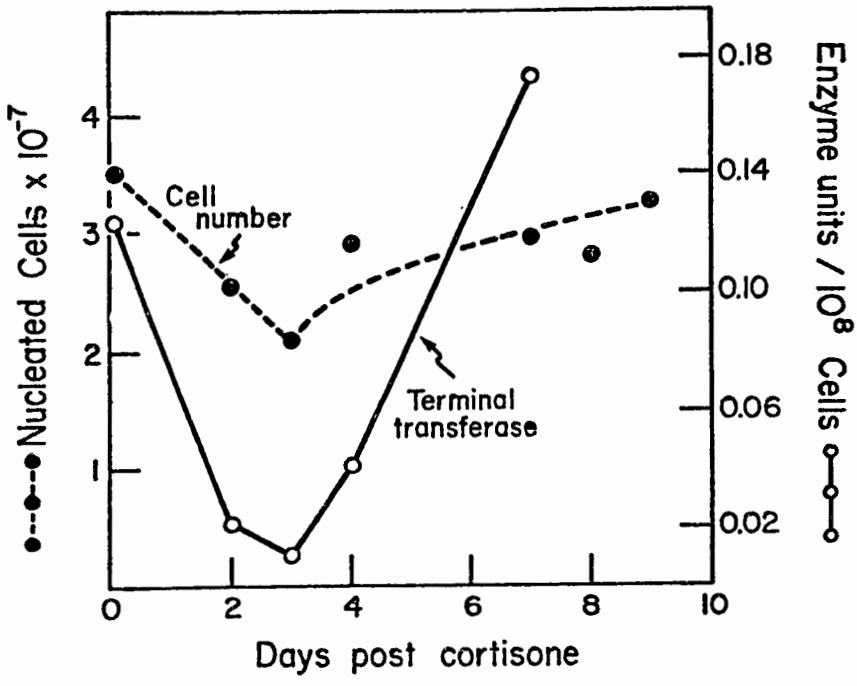

Fig. 7: Effect of cortisone treatment on number of cells and TdT activity in murine bone marrow.

TdT and cell number in the femur and tibia bone marrow were determined as a function of time after a single 150 mg/kg dose of cortisone acetate was given to C57Bl/6J mice on day 0.

mouse marrow by cortisone administration (Fig. 7). Cell losses from the marrow compartment following such perturbation are due to a combination of *in situ* destruction, and redistribution to other sites (thymus, spleen, nodes, peripheral blood) and involve predominately marrow lymphocytes (11, 12). The almost total loss of marrow TdT following cortisone administration suggests a lymphoid nature of the TdT-positive cells.

This impression is supported by independent studies with the thymic hormone thymopoietin (13). When normal murine marrow cell suspensions are incubated with thymopoietin, a certain fraction of marrow lymphocytes can be induced to express thymocyte surface markers. These marrow thymopoietin-responsive cells are believed to be prothymocytes (14). In preliminary studies (Silverstone, Cantor, Boyse, Goldstein and Scheid) the induction of Thy 1 antigen in murine marrow cells, followed by the specific elimination of such cells by cytotoxic treatment with anti-Thy 1 antibody, has resulted in the loss of 50–70 % of marrow TdT activity. This suggests that most of the marrow TdT activity in the mouse resides in marrow prothymocytes.

The precise identification of the enzyme-positive marrow population(s) will be of major interest.

Significance of TdT in leukemia cells

The finding of a normal marker on neoplastic cells is generally taken to mean that the tumor cells are related, in a derivative sense, to the cell line which normally expresses the marker in question. Thus, lymphoid malignancies are frequently classified on this basis, using T-lymphocyte and B-lymphocyte surface properties as markers (15). Although there are limitations to the use of such cell phenotypes to construct the ontogeny of neoplastic differentiation (16), it can provide a substrate for useful working hypotheses.

We have analyzed and interpreted the observation of TdT in leukemic cells in the light of its restricted distribution in normal animals (2, 7, 8, 10, 17). We have taken its presence in leukemic cells to mean that such cells are of the same lineage as those normal marrow or thymus cells which also express TdT. In the thymus it is maximally expressed in thymocytes which can be considered "primitive" in that they lack the functional properties associated with mature T-lymphocytes (7, 8, 9). In the marrow, the majority of the TdT-positive cells are cortisone-sensitive and thymopoietin-responsive and may be thymic precursor cells (prothymocytes). In our view, leukemic cells with this enzyme would derive from either TdT-positive thymic lymphocytes or TdT-positive marrow cells and therefore be lymphoid in nature.

We are aware that this sort of biochemical re-classification of leukemic cells does violence to traditional clinical and morphological concepts. The established categories into which leukemic patients are now classified implies a homogeneity among members of specific categories which does not, in fact, exist. The papers in this volume by Moloney, DeSimone, Henderson, and Wiernik should be consulted in this regard. There is obvious diversity in clinical behavior, and response to therapy, among patients who are classified within a single category. There can be little argument about the need for a re-classification which results in more homogeneous grouping. Such attempts at reclassification are being made by Moore et al. (18) and by Dicke et al. (19) using in vitro growth patterns of leukemic cells, and by Greaves et al. (summarized in this volume) using cell surface markers, to categorize patients into meaningful groups.

The significance and utility in terms of clinical management, of a leukemia cell classification based on TdT positivity or negativity remains to be established. How-

ever, our postulation that TdT-positive leukemic cells are lymphoblastic in nature, irrespective of their conventional morphological classification, is testable clinically. The combination of the drugs vincristine and prednisone results in high remission rates in ALL, but low rates in non-lymphoblastic leukemia (20). Prospective studies are underway to determine whether the presence of TdT in leukemic cells from diverse clinical syndromes augurs for responsiveness to classic anti-ALL therapy.

Our inability at this time to distinguish, either biochemically or chromatographically, between the enzyme activity observed in normal bone marrow and that present in samples from patients with early bone marrow relapse prevents TdT from being a useful tool in monitoring remission or predicting relapse. However, further enzyme purification and/or the development of a fluorescent antibody assay may discriminate between normal marrow and leukemia-associated TdT, and permit such a use.

On a biological level, the isolation and characterization of the normal bone marrow and thymus cell populations which express TdT should provide important defined cell populations for a variety of fundamental studies. Comparisons between these normal TdT-positive cells, and TdT-positive leukemia cells should be helpful in determining the specificity of particular leukemia cell characteristics. TdT-positive normal cells should also be fruitful populations for studies involving leukemogenic agents or events, particularly if they can be propagated in *in vitro* culture systems.

Acknowledgements

We are indebted to Drs. William C. Moloney, David S. Rosenthal, Joel M. Rappeport, John Truman, Norman Jaffe, Demetrius Traggis, Bruce Camitta, Audrey Evans, Jonathan Glass, Robert McAllister, W. H. Churchill, Willy Piessens, Gerard Price, Dane Boggs, George Sartiano, Edwin Foreman, Bayard Clarkson and Mortimer Greenberg for providing us with some of the clinical samples used in this study, to the late Dr. Frederick Stohlman, Jr., for both clinical samples and much stimulating discussion of this work in progress and to Drs. David G. Nathan and Fred S. Rosen for support and encouragement. This work was supported by grants AM 05581, CA 13472, CA 14051, CA 18662, AI05877 and FR-128 from the National Institutes of Health and a contract with the Virus Cancer Program of the National Cancer Institute. Dr. McCaffrey is a recipient of Research Career Development Award no. CA 00099. Dr. Kung is a postdoctoral fellow of the Jane Coffin Childs Memorial Fund for Medical Research. Dr. Parkman is a scholar of the Leukemia Society of America, Inc. Dr. Silverstone is a postdoctoral fellow of the American Cancer Society, Massachusetts Division. Dr. Baltimore is an American Cancer Society professor of microbiology.

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