Prognostic Relevance of Thymidine Kinase Isozymes in Adult Non-Hodgkin’s Lymphoma

By Peter H. Ellims, T. Eng Gan, Gabriele Medley, and Martin B. Van Der Weyden

To determine whether thymidine kinase (TK) isozyme status adds clinically useful information in adult non-Hodgkin’s lymphoma (NHL), we have analyzed peripheral blood plasma and lymphocytes of 44 patients with NHL for either TK1 or TK2 isozyme activity. On the basis of isozyme status, patients could be divided into two groups that did not differ significantly with respect to known determinants for survival. The median survival of patients exhibiting peripheral blood TK1 thymidine kinase activity was 40 wk and that of individuals with TK2 activity was in excess of 200 wk. These data suggest that peripheral blood TK1 isozyme is a useful independent biochemical marker for a subgroup of NHL who respond poorly to current therapy and thus require new therapeutic approaches.

Despite the clinical utility of the Rappaport classification of non-Hodgkin’s lymphoma (NHL), there exists considerable heterogeneity for disease progression within the subgroups of this classification.1-5 This uncertainty has resulted in the definition of other prognostic parameters, such as tumor bulkiness, specific organ involvement, extent of disease dissemination, and immunologic characteristics of the neoplastic cell.6-13 Another approach for predicting aggressive clinical tumor behavior in the individual patient is exploitation of changing cellular biochemical properties such as isozyme type with transition of the cell from the dormant to the dividing phase.14 Such an isozyme is thymidine kinase, which catalyzes the phosphorylation of thymidine to thymidine monophosphate, an essential precursor for DNA-thymine. In human tissue this enzyme occurs as two distinct forms, designated TK1, which parallels changes in cellular DNA synthesis, and TK2, which remains relatively constant during the cell cycle.15-17 Recently we have demonstrated that in involved solid lymphoid tissue of patients with NHL, there occurs a stepwise increase in TK1 isozyme activity with progressive degrees of morphological dedifferentiation.18

In the present study thymidine kinase isozyme activities in peripheral blood lymphocytes and plasma of individuals with NHL have been examined in an attempt to assess the utility of these biochemical markers in defining clinical behavior and thus potentially determining appropriate therapy. Our finding suggests that presence of the TK1 isozyme identifies many patients who respond poorly to current therapy.

Materials and Methods

Forty-four patients with non-Hodgkin’s lymphoma were entered into the study. Peripheral blood plasma and/or lymphocyte thymidine kinase activity were determined at initial presentation. Plasma thymidine kinase was measured in 41 patients and peripheral lymphocyte thymidine kinase in 38. Initial lymph node or other tissue biopsy material were reviewed and classified according to the modified Rappaport classification.19 Patients were assigned to a clinical stage following the Ann Arbor scheme,20 but staging laparotomy was limited to those patients in whom this procedure was required for tissue diagnosis. Patients received combination chemotherapy of cyclophosphamide, vincristine, and prednisone,21 or these agents together with adriamycin.22 Peripheral blood plasma and lymphocyte thymidine kinase activities were also determined in 32 control individuals; these were comprised of 15 normal laboratory workers and 17 hospitalized patients with non-neoplastic disorders.

Cell Preparation and Enzyme Assay

Lymphocytes were separated from 10 ml of heparinized peripheral venous blood by Ficoll-Hypaque gradient centrifugation and processed as described previously.23 The lymphocytes were suspended at 10⁷ cells/ml of 50 mM Tris-HCl and lysed by rapid freeze-thawing in liquid nitrogen. Cell extracts were centrifuged at 10,000 g at 4°C for 15 min and the supernatant assayed for thymidine kinase activity as described previously using [3H]-thymidine as the radiolabeled substrate.24 Enzyme activity is expressed as nmole/hr/mg protein. Protein was determined according to the method of Lowry et al.25

Plasma thymidine kinase activity was determined in a total volume of 0.1 ml. The reaction mixture contained 100 mM Tris-HCl, pH 7.4, 5 mM adenosine triphosphate, 10 mM magnesium chloride, 10 mM sodium fluoride, 5 μM 6-3H-thymidine (5 Ci/mM), and 0.05 ml of plasma. After incubation for 30 min at 37°C, the reaction was terminated by boiling in a water bath for 1 min. Following centrifugation at 10,000 g for 10 min, aliquots (50 μl) were spotted on Whatman DE-81 paper squares and processed as described previously.26 Under these conditions the enzyme reaction was linear for up to 45 min and over a fivefold range of protein. There was less than 10% variation between duplicate assays. Enzyme activity is expressed as nmole/hr/ml.

TK1 and TK2 isozyme activities were identified on the basis of comparative biochemical properties known to distinguish between the two.13,16,18,23 The TK1 isozyme has a specificity for adenosine...
triphosphate (ATP) as the phosphate donor, while TK2 also uses cytidine triphosphate (CTP), with activity reaching 70%-80% of that obtained with ATP. Deoxy-CTP produces only 15%-20% inhibition of TK1 ATP-mediated activity, but TK2 activity decreases by 70%-80%. Thymidine triphosphate (TTP) markedly inhibits (80%-90%) the activity of both isozymes. At pH 5.0, TK1 activity decreases by 60%-70% of that measured at pH 7.4, while TK2 activity falls only by 15%-20%. The ability of these biochemical properties to distinguish between the TK1 and TK2 isozymes derived from normal or neoplastic lymphocytes has been previously confirmed for the isozymes purified by thymidine-Sepharose affinity chromatography.18

In these studies, equimolar CTP was substituted for ATP, or equimolar dCTP or TTP added to the reaction mixture containing ATP. Reactions carried out at pH 5.0 were performed by substituting 50 mM acetate buffer for Tris-HCl, pH 7.4. The ratio of catalytic activity with CTP as the phosphate donor compared with that observed with ATP was employed as a discriminant between TK1 or TK2 isozyme activity, with ratios of less than 0.4 considered to indicate predominance of TK1 activity.14,21 Patients with TK1 activity in either plasma or lymphocytes or both were classified for analysis into the TK1 subgroup, whereas those exhibiting the presence of TK2 activity in both plasma or lymphocytes were classified into the TK2 subgroup.

Statistical Methods

Clinical differences between groups of patients were compared by the Student's t test and chi-square test for percentage differences. Patient survival curves were constructed from life table calculated by standard methods and the significance of differences between survival curves were determined by a log-rank for life table analysis.26

RESULTS

Control plasma were found to exhibit TK2 activity (mean CTP/ATP 0.75, range 0.65-0.90) with the mean value 0.01 nmole/hr/ml (range 0.005-0.02). Similarly, TK2 isozyme activity (mean CTP/ATP 0.73, range 0.63-0.85) predominated in control lymphocytes, with the mean value 0.18 nmole/hr/mg protein (range 0.09-0.25).

Table 1 shows the histologic types of the patients with NHL grouped according to peripheral blood thymidine kinase isozyme findings. Twenty-six individuals exhibited TK1 isozyme status. Seven patients had nodular lymphoma; poorly differentiated lymphocytic (NDPL) in 5, mixed in 1 (NM), and histiocytic (NH) in 1. The remaining 19 patients had diffuse histology; intermediate differentiated lymphocytic (DIL) in 1, poorly differentiated lymphocytic (DPDL) in 9, and histiocytic (DH) in 9. Of 18 patients with TK2 isozyme findings, 6 had NDPL, while the remaining 12 patients had diffuse lymphoma: well differentiated lymphocytic (DWDL) in 2, DIL in 2, DPDL in 4, and DH in 4. In both groups, the majority of patients had either stage III or IV disease (Table 1).

The individual peripheral blood plasma and lymphocyte thymidine kinase activities as well as mean values for the TK1 and TK2 groups of patients are shown in Fig. 1. Both groups showed higher mean plasma enzyme and lymphocyte enzyme activities when compared with controls, and those for the TK1 group were approximately twofold the corresponding TK2 values. Comparison between the two groups of the ranges of plasma and lymphocyte thymidine kinase isozyme activities showed considerable overlap (Fig. 1), indicating that the level of thymidine kinase activity is not of value in discriminating between TK1 and TK2 isozymes. Furthermore, in both groups there is considerable variation in enzyme activity within the histologic categories.

The clinicopathologic characteristics of the 44 patients with NHL grouped according to peripheral blood thymidine kinase isozyme findings are summarized in Table 2. There was no significant difference observed between the two groups for age, sex, nodular versus diffuse histologic findings, clinical stage, peripheral blood lymphocyte levels, and bone marrow involvement. However, patients with TK1 status showed an increased incidence of systemic symptoms (p < 0.05).

Comparison of the survival of the two groups of patients revealed a striking difference. As shown in Fig. 2, the median survival for patients with peripheral blood TK1 status was found to be 40 wk, while that for the group with TK2 activity is in excess of 200 wk (p < 0.006).

DISCUSSION

Current approaches to the classification of NHL rely on morphological features together with neoplastic cell immunologic surface marker characteristics10-13 or enzyme markers such as terminal deoxynucleotidyl transferase,27 and specific phosphatases.13 Although this approach has yielded considerable information on tumor behavior, the clinical hetero-

### Table 1. Histologic Classification, Peripheral Blood Thymidine Kinase Isozyme Status, and Stage

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<tr>
<th>Histologic Classification</th>
<th>Thymidine Kinase Isozyme</th>
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*Figures in parentheses indicate numbers of patients.
†According to the Ann Arbor staging system.
Peripheral blood thymidine kinase isozyme status appears to be such a parameter. In this study, TK1 or TK2 isozyme findings separated patients with NHL into two groups that did not differ significantly in factors known to influence survival: diffuse or nodular histology, clinical stage, marrow involvement, or peripheral blood absolute lymphocyte levels; only systemic symptoms, the significance of which is uncertain in NHL, predominated in the TK1 isozyme group. Despite this, the independent prognostic capability of thymidine kinase isozyme status is demonstrated by the highly significant difference in median survival of 40 wk versus > 200 wk for individuals with respective TK1 or TK2 peripheral blood isozyme findings.

The reason for this adverse survival associated with peripheral blood TK1 activity is not entirely clear. A recent analysis of thymidine kinase isozymes of involved lymphoid tissue from patients with NHL has disclosed a step-wise increase in mean TK1 isozyme activity with cellular dedifferentiation from intermediate differentiated lymphocytic lymphoma through poorly differentiated lymphocyte to histiocytic lymphoma. Within each subcategory, considerable variation of enzyme activity occurred, suggesting that this probe for clinical aggressiveness may apply not only for whole histologic subgroups but also for the individual patients within these groups. The present findings
extend this concept, as plasma or peripheral lymphocyte TK1 isozyme activity could reflect a spill over of this isozyme from aggressively proliferating tumor or blood dissemination of neoplastic cells. Precedence for this has been the finding of elevated plasma fucosyltransferases28 or lactic acid dehydrogenase levels29 in individuals with progressive NHL and the immunologic demonstration of blood dissemination of tumor-derived cells. Similarly, our findings of TK1 activity in peripheral blood lymphocytes of patients with lymphosarcoma cell leukemia, the disseminated form of NHL, and clinically aggressive chronic lymphocytic leukemia are all compatible with this concept.

Whatever the mechanism, TK isozyme status determination has identified a subgroup of NHL from whom currently used therapeutic approaches appear inadequate. The median survival of these patients approximates that currently found with adult acute leukemia, suggesting that more aggressive therapy may be applicable in this group of patients with NHL.

ACKNOWLEDGMENT

The authors wish to thank Drs. Graeme Brodie, Ivan Epstein, Barry Firkin, Maxwell Whiteside, and Max Schwarz for referring the patients included in this study.

REFERENCES


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