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Pyrimidine and Purine Activities in non-Hodgkin's Lymphoma. Correlation with Histological Status and Survival*

T. ENG GAN,† PETER D. FINCH,‡ JANE L. BRUMLEY,† LYNNE J. HALLAM† and MARTIN B. VAN DER WEYDEN†§

†*Department of Medicine, Monash University Medical School, Alfred Hospital, Commercial Road, 3181, Victoria, Australia and* ‡*Department of Mathematics, Monash University, Clayton, 3168, Victoria, Australia*

Abstract—*The levels of the purine catabolic enzymes, adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP), together with the pyrimidine activities, thymidine phosphorylase (TP) and thymidine kinase isozymes (TK) have been determined for cells obtained from solid lymphoid tissue of 38 patients with non-Hodgkin's lymphoma (NHL) and 14 individuals exhibiting benign reactive lymphoid hyperplasia. Within each NHL histological group subtyped according to the Rappaport classification, and in the reactive hyperplasia group, there was considerable variation in these activities. However, higher levels of TK and TP activities occurred in cells of the histologically unfavourable prognostic NHL groups compared with those of favourable histology or reactive hyperplasia. There was an inverse relationship between survival and elevated TK isozyme 1 and TP levels, which was independent of histological classification and clinical staging. These results indicate that, in addition to morphology, estimations of TK and TP of involved lymphoma cells in NHL is of clinical relevance.*

INTRODUCTION

THE NON-HODGKIN'S lymphomas (NHL) constitute a group of neoplastic disorders of considerable variability in clinical behaviour [1, 2] or responsiveness to conventional therapies [3, 4]. Indeed, this heterogeneity has been the stimulus for their extensive characterization with either histological [5–8] or other biological criteria [9]. In general the various histological classifications of NHL such as the modified Rappaport scheme [10] have broad predictive clinical relevance, but within the various subgroups of such schemata marked individual clinical variations are evident. Other tumour features such as immunological or other biological characteristics are of prognostic clinical value only in their intrinsic potentiality for defining tumour behaviour or therapeutic guidelines. One such parameter appears to be the proliferative capacity of tumour cells. Although earlier studies relating clinical behaviour and tumour kinetic characteristics were inconclusive [11, 12], more recent reports have suggested a correlation between cellular proliferative status, histological subtype, response to therapy and length of first remissions [13–16].

In this study we have examined the distribution of purine and pyrimidine activities in cells of solid involved tissue of NHL in order to define their usefulness for predicting clinical aggressiveness and to ascertain whether individual enzyme patterns exist that may be exploitable by selective chemotherapy. The measured activities include thymidine kinase (TK) or phosphorylase (TP), adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP). Thymidine kinase catalyses the phosphorylation of thymidine to thymidine monophosphate and in human tissue occurs as two distinct isozymes, TK1, the activity of which parallels cellular proliferation, and TK2, the level of which remains relatively constant during the cell cycle [17, 18]. The expression of these isozymes in peripheral blood lymphocytes or serum has recently been demon-

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§To whom requests for reprints should be addressed.

strated to have relevance for survival of patients with NHL [19]. Thymidine phosphorylase promotes the catabolism of thymine to thymidine and an inverse relationship exists between this activity and cellular proliferative capacity or cytotoxicity with thymidine [20–22]. Adenosine deaminase and purine nucleoside phosphorylase catalyse the sequential degradation of adenosine through inosine to hypoxanthine. Controversy still exists whether ADA levels are predictive for cellular proliferative status, but higher ADA levels occur in peripheral cells of acute lymphoblastic leukaemia (ALL), particularly T-ALL, and decreased levels typify chronic lymphocytic leukaemia cells, while PNP levels are less discriminatory [23, 24]. In addition, the level of these purine activities may be critical determinants for tumour cytolysis with ADA inhibitors such as deoxycoformycin or specific purine nucleosides [25–27].

MATERIALS AND METHODS

Patients

Lymphoid tissue obtained by node biopsy from 52 patients over a 3-year period (1980–1982) form the basis of this study. The histological findings were benign reactive hyperplasia in 14 and NHL in 38 patients. In the latter group there were 16 females and 22 males, ranging in age from 19 to 83 yr. Patients with NHL were histologically classified according to the modified Rappaport scheme [10] and clinically staged according to Ann Arbor guidelines [28], employing bone marrow biopsy, bipedal lymphangiography and computerized tomography. Twenty patients were broadly classified into the favourable prognostic histological group: eight diffuse well-differentiated lymphocytic (DWDL); nine nodular poorly differentiated lymphocytic (NPDL); and three nodular mixed lymphoma (NM). Eighteen patients formed the unfavourable prognostic histological group: seven diffuse poorly differentiated lymphocytic (DPDL); and 11 with diffuse histiocytic lymphoma (DHL). Thirteen patients had previous chemotherapy and/or radiotherapy prior to a further lymph node biopsy and entry into the study, and the remaining 25 were studied on presentation. Therapy was determined at the discretion of the attending physician, but patients in the unfavourable prognostic histological group received cyclic cyclophosphamide, adriamycin, vincristine and prednisolone (CHOP) and those with favourable histology, cyclic cyclophosphamide, vincristine and prednisolone (CVP), chlorambucil and prednisolone (CP) or no active intervention [3, 4].

Enzyme assays

All chemicals were obtained from Sigma (St. Louis, MO, U.S.A.). [^{14}C]-Thymine (56 mCi/mM), [^{14}C]-thymidine (56 mCi/mM), [^{14}C]-adenosine (51 mCi/mM) and [^{14}C]-inosine (61 mCi/mM) were obtained from the Radiochemical Centre (Amersham, U.K.). Lymph nodes obtained at biopsy were teased in RPMI 1640 medium and cells were washed twice with 0.15 M sodium chloride. Contaminating red cells were lysed with ammonium chloride. More than 60% of cells so-obtained were neoplastic as determined by May–Grünwald–Giemsa staining and immunological typing employing the following surface markers: rosette formation with sheep and mouse erythrocytes; surface Fc and C3 receptors; and immunoglobulins. Isolated cells were suspended at a density of 4–10 $\times$ 10^7 cells/ml in 0.01 M Tris–HCl buffer, pH 7.4, disrupted by three rapid freeze–thaw cycles in liquid nitrogen and centrifuged at 10,000 *g* at 4°C for 15 min. The supernatants so obtained were assayed for the various enzyme activities as described below. Protein was determined by the method of Lowry *et al.* [29] using bovine serum albumin as standard.

Thymidine kinase was assayed as described previously using a final concentration of [^{14}C] thymidine of 25 μM [19]. Enzyme activity is expressed as units of nmol of product formed/hr/mg protein. Thymidine kinase isozymes were determined on the basis of comparative biochemical properties known to distinguish between the two [30, 31]. 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. Deoxycytidine triphosphate produces only 15–20% inhibition of TK1 ATP-mediated activity but TK2 activity decreases by 70–80%. At pH 5.0 TK1 activity decreases 60–70% of that measured at pH 7.4, while TK2 activity falls only 15–20%. The ability of these biochemical properties to distinguish between TK1 and TK2 isozymes derived from neoplastic lymphocytes has been previously confirmed for the isozymes purified by thymidine –Sepharose affinity chromatography [30].

Thymidine phosphorylase was assayed as described previously using [^{14}C]-thymine as the radiolabelled substrate [20]. Enzyme activity is expressed as units of μmol/hr/mg protein.

Adenosine deaminase and purine nucleoside phosphorylase were assayed as described previously using respectively [^{14}C]-adenosine and [^{14}C]-inosine as the radiolabelled substrate [32]. Enzyme activity is expressed as units of μmol/hr/mg protein.

RESULTS

Immunological typing of NHL lymphoid tissue showed findings compatible with a monoclonal B cell derivation for tumour cells in 36 patients and null cells in the remaining two, while the cells of individuals with benign reactive hyperplasia exhibited no evidence for a monoclonal B cell proliferation.

The distribution of the TK isozyme and TP activities for the various groups studied are shown in Fig. 1 and that for ADA and PNP in Fig. 2. Thymidine kinase activity was that of TK2 isozyme in four patients with benign hyperplasia and in five with favourable histological findings: three with DWDL and two with NPDL. In patients with unfavourable histological findings thymidine kinase activity was that of TK1 in all instances (Fig. 1A). The two individuals with the highest TK1 activities showed histological transition from NPDL to DHL.

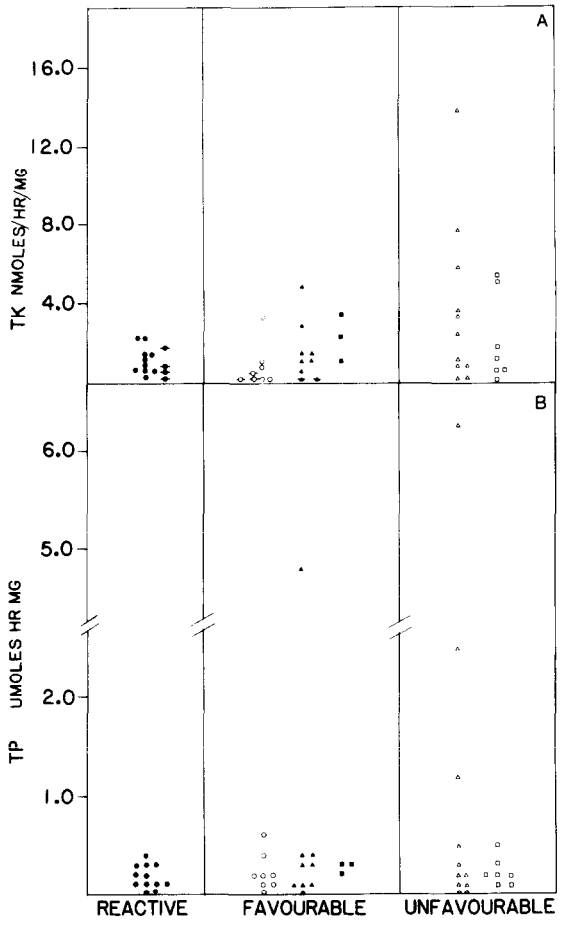

Fig. 1. The distribution of thymidine kinase isozyme (A) and thymidine phosphorylase (B) activities of lymphoid cells of patients with reactive lymphoid hyperplasia and prognostic favourable and unfavourable histological groups of the Rappaport classification of NHL. DWDL (○), NPDL (△), NM (■), DPDL (□), DH (▲). TK2 is indicated by the crossed symbol.

Statistical analysis

The data presented in Figs 1 and 2 suggest that increase in TK and TP activities is associated with progression from reactive through prognostic favourable to unfavourable histological findings. However, this suggestion is difficult to assess because a feature of the activity distribution patterns is the scatter and skewness for individual values of the two groups of patients with NHL. Because of this, non-parametric statistical analyses were used to determine relationship between survivorship and the appropriate enzymic activity. Of the 38 with NHL three were excluded from analysis: two (DHL, DPDL) were lost to follow-up and one (DWDL) died from post-splenectomy complications 3 days after entry in the study. The pertinent data for the remaining patients are presented in Table 1.

Relationship between enzyme activity and survivorship

By survivorship function (St) of a group of patients is meant the proportion that live at least t days. To allow for the censoring of lifetimes by

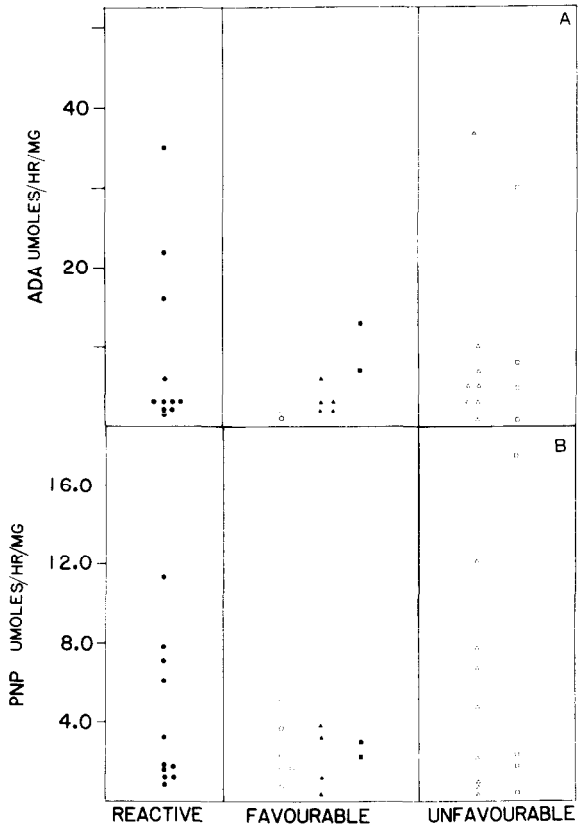

Fig. 2. The distribution of adenosine deaminase (A) and purine nucleoside phosphorylase (B) activities of lymphoid cells of patients with reactive lymphoid hyperplasia and prognostic favourable and unfavourable histological groups of the Rappaport classification of NHL. DWDL (○), NPDL (△), NM (■), DPDL (□), DH (▲).

termination of the study, survivorship was calculated by the actuarial method equivalent here to the Kaplan–Meier product limit method because of the lack of grouping [33].

Thymidine kinase isozyme 1 activity

The four patients with TK2 activity (Nos 19, 20, 24, 28, Table 1) were excluded from analysis. The median TK1 activity for the remaining 31 patients was 1.14 units and the fifteen patients exhibiting TK1 activity in excess of this value were classified as in the TK1 upper medial (UM) group. The survivorship functions of the TK1 LM and UM groups are presented in Fig. 3. Seven of the eight disease-related deaths occurred in the TK1-UM group, the longest survival time being 574 days (No. 7), so the UM survivorship function is indeterminate beyond 575 days. In this group the longest survival time was 574 days (No. 7), so both survivorship functions are indeterminate beyond 575 days. The one death in the LM group (No. 3) occurred after 564 days.

Figure 3 suggests that survivorship is poorer in the UM group, namely those with higher TK1 activities. This suggestion is confirmed by log rank analysis of the data. The total number of days at risk for the TK1-UM group is 8243, with seven deaths, whereas that of the lower-medial (LM) group is 9337, with one death. The corresponding relative risk of the TK1-UM group is $(7/8243) \times 9337 = 7.9$. Log rank resting to compare survival in the TK1-UM and LM groups leads to the results shown in Table 2. The estimated relative risk of the UM group is 7.8. At 3.70, the value of the log rank statistic falls short of 3.84, the 5% significance point on χ^2 with one degree of freedom. However, the numbers involved are too small for the usual χ^2 approximation to hold. Direct computation of the probability of obtaining at most one death in

Table 1. Clinical details and enzymic activities of involved tissue in patients with non-Hodgkin's lymphoma

Case	Histology	Sex/age	Clinical stage	Status	Exposure days	Days to death	TK1	TK2	TP	PNP	ADA
1*	DHL	F/45	IV	PR	838	–	3.40	–	0.14	0.87	2.60
2	DHL	F/70	II	CR	886	–	1.08	–	0.19	4.66	3.15
3	DHL	M/55	IV	Dead	628	546	0.90	–	0.12	2.15	5.05
4	DHL	F/63	IV	PR	605	–	0.14	–	0.34	0.96	1.44
5	DHL	F/59	IV	Dead	476	376	3.50	–	0.51	–	–
6	DHL	M/55	IV	Dead	525	385	13.90	–	2.50	0.67	4.53
7*	DHL	F/36	IV	Dead	884	574	2.50	–	0.26	–	–
8	DHL	F/19	III	Dead	809	292	5.98	–	6.25	6.89	22.33
9	DHL	F/83	III	PR	237	–	0.25	–	1.15	0.99	7.26
10	DHL	M/35	IV	CR	308	–	1.00	–	0.012	–	–
11	DPDL	M/45	I	CR	275	–	0.64	–	0.38	17.56	30.04
12*	DPDL	M/67	IV	PR	956	–	1.99	–	0.04	0.04	8.41
13*	DPDL	M/55	IV	PR	924	–	0.25	–	0.13	–	–
14	DPDL	F/45	IV	CR	332	–	5.30	–	0.15	1.92	46.12
15	DPDL	F/47	IV	Dead	899	376	5.00	–	0.31	2.31	1.16
16*	DPDL	M/49	IV	PR	954	–	0.63	–	0.16	–	–
17	DWDL	F/43	IV	PR	927	–	3.27	–	0.15	2.37	1.58
18	DWDL	M/60	IV	Dead†	885	–	0.90	–	0.09	17.33	0.94
19	DWDL	M/68	IV	PR	1089	–	–	0.18	0.38	5.07	1.52
20	DWDL	F/56	IV	PR	273	–	–	0.47	0.015	3.85	3.48
21*	DWDL	F/68	IV	PR	866	–	0.80	–	0.07	–	–
22	DWDL	M/71	IV	PR	152	–	1.13	–	0.182	1.68	8.79
23*	DWDL	M/73	IV	Dead	757	377	1.42	–	0.20	5.08	2.83
24	NPDL	M/62	IV	PR	1067	–	–	0.15	0.04	0.37	2.49
25	NPDL	M/42	IV	PR	922	–	2.99	–	4.80	6.28	2.01
26*	NPDL	M/52	IV	PR	559	–	0.70	–	0.14	3.84	6.29
27	NPDL	F/54	III	PR	953	–	3.93	–	0.32	–	–
28	NPDL	M/69	III	PR	852	–	–	0.03	0.07	–	–
29*	NPDL	M/57	IV	PR	559	–	1.14	–	0.14	3.12	2.63
30	NPDL	M/52	III	PR	308	–	1.40	–	0.95	1.14	2.08
31	NPDL	M/48	III	PR	443	–	0.77	–	0.95	1.14	2.08
32*	NPDL	M/58	III	PR	1.43	–	1.13	–	0.16	–	–
33	NM	M/71	III	Dead	535	72	2.30	–	0.34	2.38	6.90
34	NM	M/43	IV	PR	546	–	3.40	–	0.26	3.17	13.31
35	NM	F/30	I	PR	988	–	1.13	–	0.18	–	–

*Previous therapy prior to entry to study.

†Death from unrelated cause; PR, partial remission; CR, complete remission.

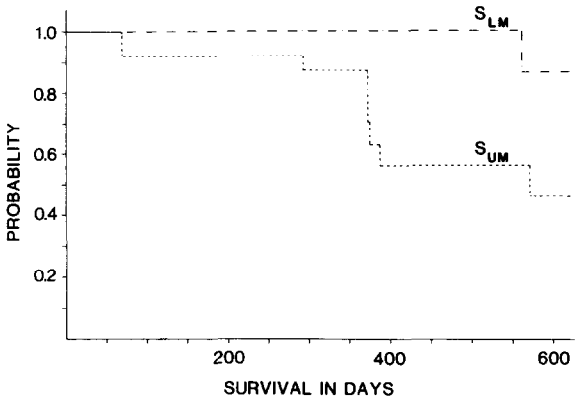

Fig. 3. Survival of patients with non-Hodgkin's lymphoma with elevated thymidine kinase isozyme 1, S_{UM} (----), compared with that of patients with low TK1 activity S_{LM} (- - - -).

the LM group under the null hypothesis of no difference shows that it is 0.024, and so a more appropriate significance level for the comparison in Table 2 is about 2%.

TP activity

Similar results hold for the relationship between survivorship and TP activity for the 35 patients evaluated (Table 1). The median TP activity is 0.182, and there are 17 patients in the TP-UM group. Seven of the eight deaths belong to this group. The number of days at risk in the TP-UM group is 8681, whereas that of the remaining 18 cases is 12,171. The corresponding relative risk of the TP-MU group is 9.8. Comparison of survivorship in the TP-UM and TP-UM groups shows that, as with TK1 activity, survival is poorer among those with higher TP activity.

ADA and PNP activities

ADA and PNP activities were measured in 22 of the 35 patients analysed and are shown in Table 1. The median PNP activity is about 2.37–2.38, the lower value occurring in patient No. 17 and the higher in patient No. 33, a non-survivor. Of the remaining 20 patients, ten, including three non-survivors, had PNP activity below 2.37 and ten, including two non-survivors, had PNP activity above 2.38. In the LM-PNP group the number of days at risk is 5578 and for the UM group 5681 days; since both groups have three deaths, they

Table 2. Log rank comparison of survival in TK1, LM and UM groups

	No. at risk		Deaths			Extent of exposure	
Time	LM	UM	LM	UM	Total	LM	UM
72	16	15	0	1	1	0.516	0.484
292	12	14	0	1	1	0.462	0.538
376	11	11	0	2	2	1.000	1,000
377	11	9	0	1	1	0.550	0.450
385	11	8	0	1	1	0.579	0.421
564	8	6	1	0	1	0.571	0.429
574	7	6	0	1	1	0.538	0.462
			1	7	8	4.217	3.783

$\chi^2 = 3.70$. Estimated relative risk = (7/3.783) × 4.217 = 7.8.

have about the same risk. There does not seem to be any relationship between survivorship and PNP activity. The ADA-LM group comprises 11 patients, including two non-survivors, with ADA activity 3.15 or less, whereas the ADA-UM group consists of 11 patients with four non-survivors, having ADA activity 4.53 or more. The days at risk in the LM group is 7126 and for the UM group 4370. The relative risk of the ADA-UM group is 3.3 and comparison of survivorship in the ADA-UM group with that of the whole ADA group shows that the former is about 76% of the latter.

While these calculations suggest that TK1, TP and ADA activities are of use in predicting for clinical aggressiveness, the possibility that this effect arises because higher levels of these activities are associated with the unfavourable histology subgroups DHL and DPDL exists. That this is not the case is clear from the four-component 2 × 2 tables of Table 3. With the possible exception of the PNP histology two-way classification, which has little interest in this context because PNP activity seems unrelated to survivorship, none of the 2 × 2 tables is indicative of association between histology and level of enzyme activity.

Table 4 gives the rank correlation coefficients, Kendall's τ, for pairs of enzyme activities. Each coefficient was calculated from the cases with measurements on both the activities then in question; that between TK1 and TP involved the 31 cases in the TK1 group whereas that between ADA and TP used only the 22 cases in the ADA group. There is a slight indication of correlation

Table 3. Non-association of medial enzyme activity with histology

	Enzyme activity group							
Histology	TK1-LM	Tk1-UM	TP-LM	TP-UM	PNP-LM	PNP-UM	ADA-LM	ADA-UM
Favourable	8	8	8	9	3	8	7	4
Unfavourable	8	7	11	7	8	3	4	7
Totals	16	15	19	16	11	11	11	11

Table 4. Rank correlation coefficients between enzyme activities

	TK1	TP	PNP	ADA
TK1	-	+0.21	-0.04	+0.06
TP		-	+0.06	+0.12
PNP			-	+0.07

between TK1 and TP activities, their coefficient of rank correlation just about reaching a value significantly different from zero at the 10% level. None of the other coefficients is significantly different from zero and the overall impression is that the four enzyme activities are uncorrelated.

DISCUSSION

The findings of this study suggest that TK1 and TP levels of neoplastic tissue of NHL are potentially useful indicators for clinical behaviour. The current interest in expression of TK isozymes stems from the observations that TK1 activity in peripheral blood lymphocytes or plasma of patients with NHL is predictive for poor survival [19] and a positive correlation exists between cellular TK1 activity and spontaneous tritiated thymidine uptake of isolated leukaemic lymphoid cells [23]. Although the variation in TK1 activity was more pronounced with cells derived from patients with unfavourable prognostic histological findings, considerable overlap of this index occurred between the favourable and unfavourable prognostic histological groups. That not all NHL tissue of low-grade histological malignancy had low TK1 activity is further highlighted when patients are segregated not according to histological findings but to survival. In this instance the distribution of disease extent or histological findings for the two groups are similar, with the differential determinant for survival being variation in TK1 activities. These conclusions are in close agreement with reports which employ as a marker for proliferative index either the cellular thymidine labelling index or flow cytometry for determination of the proportion of NHL cells in the S phase of the cell cycle. With both modalities the percentage of cells in the S phase does not correlate with disease extent and the duration of complete remission or survival is inversely related to the proliferative characteristics of the individual tumour and not the histological findings [14, 15]. The advantage of TK isozyme determination as reported here would appear to be its technical simplicity.

Thymidine phosphorylase activity is virtually absent in peripheral blood lymphoblasts of patients with T cell acute lymphoblastic leukaemia (ALL), whereas in non-T non-B ALL or B lymphoblastoid cells considerable heterogeneity of activity is evident [20, 21, 23]. The findings of this study suggest that for neoplastic NHL B cells a parallel relationship exists between elevated TK1, thymidine phosphorylase activity and unfavourable survival. This high catabolic thymidine phosphorylase activity in NHL may explain the refractoriness of NHL to *in vivo* cytotoxic thymidine therapy [22]. However, the considerable number of patients with both favourable and unfavourable prognostic histological findings and low thymidine phosphorylase activity suggests that selection of patients for thymidine therapy on this basis should be pursued before this therapy modality is abandoned in the treatment of NHL.

Interest in ADA and PNP levels in NHL has centered around the ability of these activities to act as biochemical immunological markers, with higher ADA occurring in T cell lymphoproliferative disorders, while levels of PNP are less discriminatory [23, 24]. In this study among the lymph node samples of patients with NHL there was a wide range of both ADA and PNP activities, with the higher levels found in the unfavourable prognostic histological group, comparable to those exhibited by T-ALL cells [23]. From a practical viewpoint the utility of these activities in B cell NHL remains dubious. A possible exception is that the delineation of specific purine catabolic enzyme patterns may be predictive for individual tumour responsiveness with either ADA or PNP inhibitors such as deoxycoformycin or 8-aminoguanosine. This, together with the development of more clinically orientated histological classifications, will lead to more appropriate treatment regimes for patients with this varied group of diseases.

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