Eur J Cancer Clin Oncol, Vol. 21, No. 8, pp. 945-950, 1985.
Printed in Great Britain.

0277-5379/85$3.00+0.00

Relation between Enzymatic Activities and the Degree of Malignancy of Human Lymphomas*†

P. VEZZONI,‡§ R. GIARDINI,|| M. RAINERI,‡ M. R. POZZI,‡ R. LUCCHINI,‡ M. A. VEZZONI,¶ L. CLERICI,** C. BESANA,‡ C. RUGARLI‡ and F. RILKE||

‡*Istituto San Raffaele, Cattedra di Patologia Speciale Medica V, University of Milan,* ||*Department of Pathology, Istituto Nazionale Tumori, Milan,* ¶*Servizio di Oncologia, Fatebenefratelli Hospital, Milan and* **Laboratory of Biochemistry, Biology Group, D.G. XII, Ispra, C.E.C., Joint Research Centre, Ispra (VA), Italy*

Abstract—*The relationship between the intracellular levels of DNA polymerase alpha (DP-alpha), adenosine deaminase (ADA) and lactate dehydrogenase (LDH) and the degree of malignancy of human lymphomas was investigated. Twelve non-neoplastic lymph nodes and 88 malignant lymphomas were examined. For non-Hodgkin's lymphomas (NHL) the low or high grade of malignancy was established according to three classifications: the Rappaport, the Kiel and the Working Formulation for Clinical Usage, with the latter also recognizing an intermediate grade group. Non-neoplastic lymph nodes had significantly lower levels of all the three enzymes than those found in high-grade malignant NHL (the P value ranged from <0.02 to <0.001). Hodgkin's disease, a slowly evolving neoplasia, showed lower levels of DP-alpha ($P < 0.001$) and ADA ($P < 0.001$), but not of LDH, than high-grade NHL. Among NHL, whatever classification was used, the low-grade malignant lymphomas had significantly lower levels than the high-grade ones for all the three enzymes ($P < 0.005$ or $P < 0.001$). The intermediate-grade group of the Working Formulation differed from the high-grade group for DP-alpha ($P < 0.01$) and ADA ($P < 0.02$) but not for LDH. It differed from the low-grade group only for ADA ($P < 0.005$). Lymphoblastic and Burkitt's lymphomas were the groups with the highest levels of the three enzymes. Among low-grade lymphomas very low values were found in the histological entities defined as DLWD in the Rappaport classification, CLL and lymphoplasmacytoid immunocytoma in the Kiel classification and small lymphocytic (group A) in the WF. The levels of all enzymes in these histotypes were always significantly different from the other low-grade histotypes, and from the intermediate-grade ones of the WF. In the Kiel classification polymorphous lymphoplasmacytoid lymphoma, recently recognized as a group with a quite aggressive clinical course, was characterized by high levels of all three enzymes. Moreoever, among centroblastic-centrocytic (Cb-Cc) lymphomas those associated with a better prognosis (Cb-Cc follicular, with small centrocytes) had lower levels of DP-alpha ($P < 0.05$) than those with a mixed cellular population. Taken together, these data suggest that intracellular enzyme values may have a role in better defining the prognosis of NHL.*

Accepted 22 February 1985.
*Supported in part by a Grant from Consiglio Nazionale delle Ricerche, Italy (Progetto Finalizzato Oncologia, 8.1.1.).
§This publication is contribution No. 13 of the Program Biochemical Characterization of Human Lymphomas, 13° Gruppo Operativo (Chairman Prof. C. Rugarli), and partially contribution No. 2178 of Programme of Biology, Radiation Protection and Medical Research, Directorate General XII for Research, Science and Education of the Commission of the European Communities.
§To whom requests for reprints should be addressed at: via Belgirate 18, Milano, Italy.

INTRODUCTION

HUMAN malignant lymphomas are a heterogeneous group of tumors that differ with regard to clinical behavior and survival. Hodgkin's disease (HD) is usually characterized by a slow clinical evolution, while non-Hodgkin's lymphomas (NHL) are more heterogeneous, including histotypes with a good or poor prognosis [1]. Many histological classifications categorize the degree of malignancy of the neoplasia by histological, cytochemical and immunological

criteria, but additional parameters could be useful for a better definition of the aggressiveness of lymphoid tumors. In the present study we investigated three enzymatic activities to determine whether they are related to the grade of malignancy as defined by the histological diagnosis only. DNA polymerase alpha (DP-alpha), the main replicative enzyme [2], adenosine deaminase (ADA), a lymphocyte differentiation enzyme [3], and lactate dehydrogenase (LHD), whose serum concentration is thought to be of prognostic value in NHL [4], were determined in cellular extracts of 12 non-neoplastic lymph nodes and in 88 specimens obtained from patients with malignant lymphomas. The data were analyzed with reference to the diagnosis, and for NHL three classifications were taken into consideration: the Rappaport [5] and the Kiel [6] classifications and the Working Formulation for Clinical Usage (WF) [1].

MATERIALS AND METHODS

Lymph nodes were obtained from patients with non-neoplastic disease of the lymph nodes (12 cases) and from patients with node-based lymphomas (78 cases). Neoplastic cells from the blood of nine patients with lymphoma in leukemic phase or chronic lymphocytic leukemia were also examined. In one case cells were obtained from pleural effusion, giving a total of 100 cases.

Biopsies were obtained from the operating room, and a portion for enzymatic analyses was cut and immediately frozen at -70°C until use. Mononuclear cells were isolated from heparinized blood by centrifugation on Ficoll gradients [7] and stored as cellular pellets. On the day of the test the frozen tissues were thawed, minced and resuspended in 5 vols of 0.25 M potassium phosphate buffer, pH 7.2. Cellular pellets were thawed and dispersed in the same phosphate buffer at a concentration of 2×10^8 cells/ml. The samples were disrupted in a Dounce homogenizer, the homogenates were spun at 100,000 *g* for 60 min and the supernatants were considered as crude extracts. All the procedures were carried out at 4°C.

Hodgkin's disease was classified according to the Rye modification of Lukes and Butler's classification [8]. Non-Hodgkin's lymphomas were classified according to the Rappaport classification [5], including the modification of Nathwani *et al.* [9] for the lymphoblastic lymphoma, the Kiel classification [6] and the Working Formulation for Clinical Usage [1]. Unclassifiable lymphomas were included only when at least the grade of malignancy could be determined histologically.

Diffuse lymphocytic well-differentiated, nodular lymphocytic poorly differentiated and nodular mixed types of the Rappaport classification were considered as low-grade, while all the other types were defined as high-grade malignant lymphomas.

The enzymatic assays were performed on the crude extracts as previously described [10, 11]. The enzymatic activities of DP-alpha and ADA were expressed as Units/mg of protein. The Unit of DNA polymerase alpha is the amount of protein that polymerized 1 nmol of tritiated nucleotide in 1 hr. The Unit of adenosine deaminase is the amount of the enzyme that catalyzed the deamination of 1 nmol/ml of adenosine/min (a_{mol} = 8.65). Intracellular LDH activity was expressed as International mUnits/mg of protein. Protein was measured by the method of Bradford [12] using gamma globulin as a standard.

Radioactive dTTP was purchased from the Radiochemical Centre, Amersham (U.K.). Unlabelled nucleotides and nucleosides were from PL Biochemicals (U.K.). Activated calf thymus DNA was prepared by partial digestion with DNase I according to Fansler and Loeb [13]. All other chemicals and biochemicals were of the purest grade from commercial sources. For statistical analysis Student's *t* test was employed. Only histological types with four or more cases were subjected to statistical analysis.

RESULTS

DNA polymerase alpha

The enzymatic activity of DP-alpha was determined for 12 cases of non-neoplastic lymph nodes, 30 cases of HD and 58 cases of NHL. The results are shown in Tables 1–3.

Both Hodgkin's and non-neoplastic lymph nodes had low contents of the enzyme and there was no difference between their mean levels. However, they differed significantly from the high-grade groups of NHL ($P < 0.001$ in each case), from the low-grade group of the Kiel classification ($P < 0.005$ for HD and $P < 0.05$ for non-neoplastic lymph nodes) and from the intermediate group of the Working Formulation ($P < 0.001$ for HD and $P < 0.02$ for non-neoplastic lymph nodes). Hodgkin's disease tissues, but not non-neoplastic lymph nodes, were also different from the low-grade group of the WF ($P < 0.05$).

Significant differences were also found among NHL. In all three classifications the mean enzymatic values in the low-grade entities were

Table 1. Distribution of enzymatic activities of DNA polymerase alfa (DP-alpha), adenosine deaminase (ADA) and lactate dehydrogenase (LDH) among non-neoplastic lymph nodes, Hodgkin's disease and non-Hodgkin's lymphomas classified according to Rappaport

	DP-alpha		ADA		LDH	
Histological diagnosis	No. of cases	Mean (range)	No. of cases	Mean (range)	No. of cases	Mean (range)
Low-grade NHL (tot)	18	1.60(0.49/2.44)	18	51 (14/105)	14	1515(1086/2295)
DLWD + CLL	10	1.36(0.49/2.33)	10	34 (14/78)	8	1291(1086/1758)
NLPD	8	1.88(1.01/2.44)	8	73 (26/105)	6	1811(1465/2295)
High-grade NHL (tot)	40	3.16(0.78/5.80)	40	263 (14/990)	20	2354(1179/4237)
DLPD	8	2.38(1.12/4.36)	8	79 (18/142)	4	1908(1179/2284)
DM	4	2.90(1.69/4.25)	4	135 (97/188)	3	2017(1418/2345)
NH	1	0.78 —	1	96 —	—	— —
DH	6	2.41(1.50/4.29)	6	150 (62/333)	2	2709(2526/2893)
LL + ALL	16	3.92(2.11/5.80)	16	442 (56/990)	7	2131(1327/2942)
BL	4	3.87(2.17/5.27)	4	43 (14/ 58)	3	3350(2256/4237)
UL	1	3.00 —	1	26 —	1	3000
Hodgkin's disease	30	1.39(0.15/2.93)	30	78 (19/241)	14	2395(1591/3276)
Non-neoplastic lymph nodes	12	1.40(0.44/2.62	12	61 (18/113)	6	1728(930/2399)

*Table 2. Distribution of enzymatic activities of DP-alpha, ADA and LDH among non-Hodgkin's lymphomas classified according to the Kiel classification**

	DP-alpha		ADA		LDH	
Histological diagnosis	No. of cases	Mean (range)	No. of cases	Mean (range)	No. of cases	Mean (range)
Low-grade NHL (tot)	31	2.04(0.49/4.36)	31	75 (14/229)	22	1709(1086/2345)
CLL	7	1.38(0.76/1.79)	7	26 (14/ 48)	6	1616(1433/1758)
Lymphoplasmacytoid	2	0.78(0.49/1.08)	2	41 (18/ 64)	2	1137(1086/1188)
Polymorphous lymphoplasmacytoid	4	2.79(1.69/4.25)	4	121 (97/138)	4	2084(1418/2345)
Centrocytic	5	2.34(1.12/4.36)	5	73 (18/142)	2	1285(1179/1392)
Centroblastic-centrocytic	13	2.16(1.01/3.30)	13	93 (26/229)	8	1880(1465/2295)
High-grade NHL (tot)	27	3.47(0.78/5.80	27	301 (14/990)	13	2568(1327/4237)
Centroblastic	3	2.19(0.78/4.29)	3	111 (94/144)	1	2526 —
Lymphoblastic + ALL	16	3.92(2.11/5.80)	16	442 (56/990)	7	2131(1327/2942)
Burkitt's	4	3.87(2.17/5.27)	4	43 (14/ 58)	3	3350(2556/4237)
Immunoblastic	3	1.93(1.39/2.55)	3	171 (62/333)	1	2893 —
Unclassifiable	1	3.00 —	1	26 —	1	3000 —

*For Hodgkin's and non-neoplastic lymph node values see Table 1.

significantly lower than those present in the high-grade groups ($P < 0.001$). In the WF the intermediate-grade was statistically different from the high-grade ($P < 0.01$) but not from the low-grade group. Among the various histological types of NHL, lymphoblastic lymphoma (LL) and Burkitt's lymphoma (BL) were those with the highest DP-alpha contents, with no difference between them. Among the low-grade malignant lymphomas there was a subset characterized by a very low content of DP-alpha. This subset was equivalent to the DLWD of the Rappaport classification, to the CLL and lymphoplasmacytoid immunocytes of the Kiel classification and to the small lymphocytic (A) of the WF. There was always a significantly statistical difference between: DLWL vs NLPD ($P < 0.05$) in the Rappaport classification; CLL + lymphoplasmacytoid immunocytoma vs polymorphous lymphoplasmacytoid immunocytoma ($P < 0.005$), vs centrocytic ($P < 0.05$) and vs centroblastic-centrocytic ($P < 0.05$) in the Kiel classification; and group A vs B + C ($P < 0.005$) in the WF. It is noteworthy that group C did not differ from groups D and E of the WF.

Moreover, we found that among Cb-Cc, the values in the four cases composed of small lymphocytic cells (group B of the WF) differed from those present in the six cases composed of both large and small cells (group C) ($P < 0.05$).

Adenosine deaminase

Adenosine deaminase activity was tested on the same cases investigated for DP alpha.

*Table 3. Distribution of enzymatic activities of DP-alpha, ADA and LDH among non-Hodgkin's lymphomas according to the Working Formulation for Clinical Usage**

Histological diagnosis	DP-alpha No. of cases	DP-alpha Mean (range)	ADA No. of cases	ADA Mean (range)	LDH No. of cases	LDH Mean (range)
Low-grade NHL (tot)	21	1.76(0.49/3.30)	21	57 (14/118)	15	1662(1086/2295)
A + CLL	10	1.40(0.49/2.42	10	36 (14/ 97)	8	1562(1086/2289)
B	4	1.60(1.01/2.12)	4	50 (26/ 88)	1	1476 —
C	7	2.55(2.03/3.30)	7	90 (39/118)	6	1880(1086/2295)
Intermediate-grade NHL (tot)	13	2.44(0.78/4.36)	13	107 (18/229)	7	1984(1179/2526)
D	4	2.14(0.78/4.29)	4	96 (90/105)	2	2082(2062/2102)
E	5	2.34(1.12/4.36)	5	73 (18/142)	2	1285(1179/1392)
F	3	3.32(2.81/4.25)	3	167 (134/229)	2	2314(2284/2345)
G	1	1.50 —	1	144 —	1	2526 —
High-grade NHL (tot)	24	2.98(1.39/5.80)	24	325 (14/990)	12	2572(1327/4237)
H	3	1.93(1.39/2.55)	3	171 (62/333)	1	2893 —
I + ALL	16	3.92(2.11/5.80)	16	442 (56/990)	7	2131(1327/2942)
J	4	3.87(2.17/5.27)	4	43 (14/ 58)	3	3350(2556/4237)
Unclassifiable	1	3.00 —	1	26 —	1	3000 —

*For Hodgkin's and non-neoplastic lymph node values see Table 1.

Hodgkin's and non-neoplastic lymph nodes did not statistically differ and were characterized by a low content of the enzyme. In all three classifications of NHL used the mean values were significantly lower in Hodgkin's and non-neoplastic lymph nodes than in the highly malignant NHL ($P < 0.001$ in all three classifications for HD; $P < 0.02$, $P < 0.01$ and $P < 0.005$ in the Rappaport, Kiel and Working Formulation respectively, for the non-neoplastic lymph nodes).

Among NHL, in all three classifications the values found in the low-grade were significantly lower than those present in the high-grade entities ($P < 0.005$ for the Rappaport classification and $P < 0.001$ for the Kiel classification and the Working Formulation). In the latter the intermediate-grade was significantly different from both the low- ($P < 0.005$) and the high-grade group ($P < 0.02$) in terms of enzyme activities.

Among the low-grade malignant lymphomas, the same subset (DLWD, CLL + immunocytoma, small lymphocytic) with very low levels of DP-alpha also showed low contents of ADA. The statistical differences between: (1) DLWD vs NLPD ($P < 0.05$); (2) CLL + lymphoplasmacytoid immunocytoma vs polymorphous lymphoplasmacytoid immunocytoma ($P < 0.001$); (3) CLL vs Cc ($P < 0.05$); CLL vs Cb-Cc ($P < 0.05$); and group A vs B + C ($P < 0.001$) were always significant. No difference was found between Cb-Cc with small and mixed cellular populations.

Lactate dehydrogenase

Lactate dehydrogenase concentrations were determined for six cases of non-neoplastic lymph nodes, 14 cases of Hodgkin's disease and 34 cases of NHL.

In non-neoplastic lymph nodes LDH values were not different from those of low-grade NHL but were different from those of high-grade NHL ($P < 0.001$ in all the three classifications). HD had higher LDH values than non-neoplastic lymph nodes ($P < 0.025$) and the low grade NHL of all the three classifications (always $P < 0.001$). The values in HD differed neither from any high grade NHL nor from the intermediate group of the WF.

Among the NHL, the values were more elevated in high-grade than in low-grade malignant lymphomas in all three classifications ($P < 0.001$). The intermediate-grade group of the Working Formulation was not different from the low-grade and high-grade ones. As already shown for DP-alpha and ADA, the DLWD/CLL + immunocytoma/small lymphocytic subset was characterized by a very low LDH content that was significantly different from that of the other low-grade histological types: DLWD vs NLPD, $P < 0.05$; CLL vs Cb-Cc, $P < 0.02$; A vs C, $P < 0.02$.

A statistical analysis among subtypes of Cb-Cc was not carried out because of the small number of cases.

DISCUSSION

The present investigation was undertaken to study the relationship between the expression of three enzymatic activities and the degree of malignancy of the lymphoid neoplasms as defined by histological criteria alone.

We previously suggested [11] that, if an association could be demonstrated, these enzymes might be useful as markers of the malignancy of the neoplasia.

In this paper we tested our hypothesis by analyzing low-grade and high-grade malignant NHL according to three widely used histological classifications: the Rappaport, the Kiel and the Working Formulation. In all these pathological classifications the values found in the low-grade entities significantly differed from those present in the high-grade ones for all three enzymatic activities examined, suggesting that these enzymes are actually related to the proliferative activity of the neoplasia. Moreover, all three enzymes were able to identify the most slowly proliferating tumors (DLWD, CLL + immunocytoma, small lymphocytic) because the values in this group were significantly lower than in the other groups associated with a good prognosis.

However, the relative predictive ability of each enzyme was different. We found that the DNA polymerase alpha gave the closest pathological correlation because, in addition to distinguishing between low- and high-grade malignant lymphoma, it showed very low levels in DLWD/CLL/small lymphocytic lymphomas and very high levels in the highly malignant Burkitt's and lymphoblastic lymphomas. Moreover, this enzyme was low in non-neoplastic lymph nodes as well as in Hodgkin's disease, which is associated with a slowly evolving clinical behaviour. Therefore we think that DP-alpha is a strictly proliferation-associated enzyme that might be useful for clinical purposes.

From our data, it can be seen that ADA is also related to the proliferative activity of these neoplasias. However, ADA is also dependent on the stage of differentiation reached by both normal and neoplastic lymphoid cells [3, 11]. Since in LL and BL, ADA expression is related to the maturation stage of the neoplastic cells, they should be excluded when the prognostic value of ADA is evaluated. We demonstrated that ADA levels are higher in high-grade than in low-grade malignant lymphomas, even when LL and BL are excluded from the analysis. Moreover, the low values expressed by non-neoplastic lymph nodes and HD are consistent with a role of ADA in the proliferation of the lymphoid cells.

Serum LDH levels are of prognostic significance in NHL [4]. The intracellular content of LDH is lower in low-grade than in high-grade malignant lymphomas. The high concentrations present in the histological types associated with a poor prognosis as well as the larger tumoral mass in advanced stages are likely to be the best explanation for the prognostic role of serum LDH [10]. We showed here that the intracellular concentration of LDH was also able to identify the group with the best prognosis. However, the high values found in HD suggest that LDH is not regulated exclusively by the proliferative rate of the cellular population.

A group with an intermediate degree of malignancy is recognized only by the WF, even though it may be easily identified also in the Kiel classification, but not in the Rappaport classification. However, which histological subtypes should be included in this intermediate group is debatable. Enzymatic data might be relevant to this problem, because we showed that: (1) the group C of the WF is enzymatically more similar to groups D and E than to group A; (2) polymorphous lymphoplasmacytoid lymphoma, originally thought to be a low-grade malignant lymphoma and later recognized as a quite aggressive type [14, 15], has high contents of all three enzymes; (3) among Cb-Cc lymphomas the four cases composed of small cells had lower DP-alpha levels than the six cases composed of small and large cells ($P < 0.05$). In conclusion, the present work demonstrates that in NHL there is a relationship between the enzymatic contents of the various histotypes and their grade of malignancy. However, more data are needed to firmly establish the clinical usefulness of these enzymatic determinations in predicting the survival of the individual cases.

Acknowledgements—We thank Dr Margaret Merlini for advice and comments, Prof. Bonini for encouragement, Mr Brazzelli for technical assistance and Mrs Daniela Moioli for preparation of the manuscript.

REFERENCES

1. Non-Hodgkin's Lymphoma Pathologic Classification Project. National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas. *Cancer* 1982, **49**, 2112–2135.
2. Bollum FJ. Mammalian DNA polymerases. *Prog Nucleic Acids Res Mol Biol* 1975, **15**, 109–144.
3. Barton R, Martinik KF, Hischhorn R, Goldschneider I. The distribution of adenosine deaminase among lymphocyte populations in the rat. *J Immunol* 1979, **12**, 216–220.
4. Ferraris AM, Giuntini P, Gaetani GF. Serum lactate dehydrogenase as a prognostic tool for non-Hodgkin's lymphomas. *Blood* 1979, **54**, 928–932.

5. Rappaport H. Tumors of the hematopoietic system. In: *Atlas of Tumor Pathology* Washington, DC, U.S. Armed Forces Institute of Pathology, 1966, Sect. 3. Fasc. 8, 9.
6. Gerard Marchant R, Hamlin I, Lennert K, Rilke F, Stansfeld AG, van Unnik JAM. Classification of non-Hodgkin's lymphomas (letter). *Lancet* 1974, **ii**, 406–408.
7. Boyum A. Isolation of mononuclear cells and granulocytes from human blood. *Scand J Clin Lab Invest* 1968, **21**, (Suppl. 97), 57–76.
8. Lukes RJ, Craver LF, Hall TC, Rappaport H, Ruben P. Report of the Nomenclature Committee. *Cancer Res* 1966, **26**, 1311.
9. Nathwani BN, Kim H, Rappaport H. Malignant lymphoma, lymphoblastic. *Cancer* 1976, **38**, 964–983.
10. Vezzoni MA, Lucchini R, Giardini R, Raineri M, Murone M, Vezzoni P. Lactate dehydrogenase levels in cellular extracts of human malignant lymphoma. *Tumori* 1983, **69**, 279–282.
11. Vezzoni P, Giardini R, Lombardi L *et al.* Multienzymatic analyses of human malignant lymphomas. *Cancer* 1984, **54**, 489–499.
12. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein dye binding. *Anal Biochem* 1976, **72**, 248–260.
13. Fansler BS, Loeb MAS. Sea urchin nuclear DNA polymerase. In: Grossman L, Moldave K, eds. *Methods in Enzymology*. New York, Academic Press, 1974, Vol. 29, 53–70.
14. Kruenger GRF, Rojo Medina J, Klein HO *et al.* A new working formulation of non-Hodgkin's lymphomas. *Cancer* 1983, **52**, 833–842.
15. Silvestrini R, Piazza R, Riccardi A, Rilke F. Correlation of cell kinetic findings with morphology of non-Hodgkin's malignant lymphomas. *JNCI* 1977, **58**, 499–504.