Eur J Cancer Clin Oncol, Vol. 19, No. 3, pp. 333–337, 1983.
Printed in Great Britain.

0277-5379/83/030333-05$03.00/0

Altered Intracellular Enzyme Activity of Monocytes and Lymphocytes in Hodgkin's Disease*

P. H. M. DE MULDER, P. D. MIER,† B. E. DE PAUW, H. VAN RENNES,† J. TH. P. JANSSEN, D. J. T. WAGENER and C. HAANEN

Division of Haematology, Department of Internal Medicine and †Department of Dermatology, University Hospital St. Radboud, Geert Grooteplein Zuid 8, 6525 GA Nijmegen, The Netherlands

Abstract—*To evaluate metabolic functionality of monocytes and lymphocytes in Hodgkin's disease (HD) we studied 3 enzymes of the intermediary metabolism, G-6-PDH, PHI, ICDH, and the acid hydrolases, NAG and ACP. These enzymes were measured in purified cell fractions of 9 patients with advanced disease and 11 normal controls. The cells were isolated with cell scatter-monitored counterflow centrifugation. Enzymes were measured in the cell lysates by means of fluorimetric microassays. In the monocytes of HD patients a significantly increased G-6-PDH activity was found ($P < 0.01$), indicating an enhanced activity of the hexose monophosphate shunt. The other enzymes showed no clear differences compared to normal controls. The lymphocytes of HD patients showed a significantly augmented activity of both G-6-PDH ($P < 0.001$) and PHI ($P < 0.01$), pointing to an increased HMPS and glycolytic activity. These findings are in support of an enhanced metabolic activity of both monocytes and lymphocytes in HD.*

INTRODUCTION

ALTHOUGH the impairment of cell-mediated immunity in Hodgkin's disease (HD) is well established [1, 2], data concerning lymphocyte and monocyte function are not in mutual concurrence. For both lymphocytes and monocytes, increased [3–7] and diminished [1, 2, 8–10] activities have been described. In a recent study we could clearly show an enhanced monocyte-mediated antibody-dependent cytotoxicity in HD patients [11], indicating an increased function of these cells.

Since it may be assumed that any functional change will be a reflection of alterations in the underlying metabolic patterns, we measured the activity of 5 key enzymes in monocytes and lymphocytes from 9 patients with HD and 11 normal controls. Monocytes and lymphocytes were isolated with cell-scatter monitored counterflow centrifugation (elutriation) [12] and enzyme measurements made by means of microfluorimetric techniques. The enzymes studied were: glucose-6-phosphate dehydrogenase (G-6-PDH) of the hexose monophosphate shunt (HMPS), the glycolytic enzyme phosphohexose isomerase (PHI), the Krebs cycle enzyme isocitrate dehydrogenase (ICDH) and two lysosomal enzymes β-*N*-acetylglucosamidase (NAG) and acid phosphatase (ACP).

Accepted 21 September 1982.
*This study was supported by the Queen Wilhelmina Foundation, The Netherlands Organisation against Cancer (K.W.F./N.O.K.) and the Maurits and Anna de Kock Foundation.

MATERIALS AND METHODS

Materials

Materials used for the monocyte and lymphocyte isolation were described previously [12]. Glucose-6-phosphate, nicotinamide adenine dinucleotide phosphate (NADP) and NADPH, fructose-6-phosphate (grade I), G-6-PDH (type XV), isocitric acid (type I), L(+)lactic acid (grade 1), 4-methylumbelliferone (4-MU) and linked derivatives (4-MU-P, 4-MU-NAG) were all obtained from the Sigma Chemical Co, St. Louis, MO, U.S.A. The fluorochrome 4′,6-diamidino-2-phenylindole.2HCl (DAPI) was purchased from Boehringer Mannheim and demineralized bovine serum albumin (BSA) from Povite, Amsterdam, The Netherlands. All other materials.

Table 1. Patient population

	Stage of disease				
	I	II	III	IV	*n*
A	1	—	1	1	3
B		1	1	4	6
n	1	1	2	5	

Total = 9 patients: age: 37 ± 14 yr; sex: all male; histology: 4 nodular sclerosis, 4 mixed cellularity and 1 lymphocyte predominance.

as listed in Table 2, were of analytical purity and obtained from Merck (Darmstadt, Germany).

Patients and controls

Eight newly diagnosed biopsy-proven patients with Hodgkin's disease and one patient with a relapse after radiotherapy and chemotherapy were included in this study. The histological material was classified according to the Rye Conference modification of the Lukes-Butler classification [13]. Staging procedures were carried out according to the criteria recommended at the Ann Arbor Conference [14]. None of the patients had concomitant overt infectious disease. The clinical data are summarized in Table 1. A group of 11 healthy volunteers (10 men and 1 woman, age 30 ± 5 yr) served as controls.

Monocyte and lymphocyte isolation

The suspensions were prepared as previously described [12]. Briefly, a mononuclear cell suspension was obtained from 50 ml heparinized venous blood after Ficoll-Isopaque (1.077 g/ml) buoyant density centrifugation. After one wash-step the sample was introduced into the elutriator (Beckman J2-21 C centrifuge equipped with a JE-6 elutriator rotor with standard separation chamber). By gradually diminishing the centrifugal speed at a constant counterflow rate, cells emerge from the separation chamber mainly according to their size. Continuous cell-scatter monitoring enables optimal separation for each sample. Four fractions were gathered, a lymphocyte fraction containing approximately 90% of all elutriated lymphocytes with a purity of 99%, a mixed lymphocyte and monocyte fraction, a monocyte fraction with about 80% of the elutriated monocytes with an average purity of 90% and, after flushing the tubing system, a rest fraction. After washing, the lymphocyte and monocyte fractions were further analyzed. Differential counting of May-Grünwald-Giemsa and non-specific esterase [15]-stained cytocentrifuge preparations was performed on 400 cells.

Biochemical assays

The three enzymes of intermediary metabolism (G-6-PDH, PHI and ICDH) were measured by

Table 2. Assay systems for the individual enzymes

	Reaction conditions					Fluorescence	
Enzyme	Composition	Volume (μl)	pH	Time (min)	Stopped by:	λex	λem
G-6-PDH	Tris, 5 μmol $MgCl_2$, 1 μmol EDTA, 0.5 μmol G-6-P, 20 nmol NADP, 10 nmol	120	7.6	30	0.2 M Na_2CO_3, pH 10.5, 0.5 ml	342	455
PHI	Tris, 5 μmol $MgCl_2$, 1 μmol EDTA, 0.5 μmol F-6-P, 20 nmol NADP, 10 nmol G-6-PDH, 0.1 IU	120	7.6	30	0.2 M Na_2CO_3, pH 10.5, 0.5 ml	342	455
ICDH	Tris, 5 μmol $MgCl_2$, 1 μmol $MnCl_2$, 1 μmol EDTA, 0.5 μmol Isocitrate, 20 nmol NADP, 10 nmol	120	7.6	60	H_2O, 0.5 ml	342	455
NAG	Lactate, 4 nmol 4 MU-NAG, 20 nmol	40	4.3	60	0.2 M Na_2CO_3, pH 10.5, 1.0 ml	362	450
ACP	Lactate, 2 μmol 4 MU-P, 4 nmol	40	3.6	60	0.2 M Na_2CO_3, pH 10.5, 1.0 ml	362	450

fluorimetric determination of NADPH generated in the presence of appropriate substrates using either direct or coupled systems. The acid hydrolases (NAG and ACP) were assayed by fluorimetric determination of 4-MU released from the corresponding 4-MU-linked derivatives. The individual assay methods are detailed in Table 2; in all cases, preliminary experiments were carried out to verify the linearity of the system with respect to incubation time and enzyme activity. Cells (about 10^6) were lysed in 500 μl of aqueous bovine serum albumin solution (1 mg/ml; BSA) by one cycle of freeze–thawing followed by 2×10 sec ultrasonication at 0°C. The lysate was centrifuged (5 min, 3000 *g*) and appropriate dilutions of the supernatant made in BSA. Duplicate 20-μl aliquots of the diluted lysate were added to either 100 μl (G-6-PDH, PHI and ICDH) or 20 μl (NAG or ACP) of the pre-mixed reagent. After incubation at 37°C, the reaction was stopped by cooling in ice and dilution and the fluorescence determined using a 'Fluorispec' fluorimeter (Baird Europe B.V., The Hague, The Netherlands). After subtraction of appropriate blanks (BSA in place of lysate), activity was calculated as nmol/min. μgDNA by reference to NADPH or 4-MU standards. Preliminary experiments revealed that lysates showed no change in enzyme activities for at least 3 months at −80°C; all samples were therefore assayed within this period after isolation of the cells. The DNA content of the lysate was determined by fluorescence of the complex with DAPI [16] using a modification described by Mier *et al.* [17].

Statistics

The Wilcoxon two-sample test was used to compare the results.

RESULTS

After elutriation the mean purities (± standard deviations) of lymphocytes for the HD group and normal controls were 97 ± 3.7 and 99 ± 0.6% respectively. For the monocyte suspensions the corresponding purities were 87.0 ± 5.8 and 88.2 ± 3.3%.

The results of the enzyme measurements are presented in Figs 1 and 2. The G-6-PDH activity of the monocytes from patients with HD (1.65 ± 0.29 nmol/min. μg DNA) was significantly increased ($P < 0.01$) in comparison to normal control monocytes (1.34 ± 0.19). No differences were found in PHI, ICDH, NAG and ACP activity.

In the lymphocytes of HD patients both G-6-PDH and PHI activity were significantly increased: G-6-PDH for HD was 0.67 ± 0.13, and for the normal controls 0.45 ± 0.01 ($P < 0.01$). PHI activity in HD and normals was respectively 5.1 ± 1.1 and 3.9 ± 0.7 ($P < 0.01$). Although ICDH and the lysosomal enzymes NAG and ACP in HD lymphocytes tended to be higher, the differences did not reach $P = 0.01$.

DISCUSSION

The increased intracellular level of G-6-PDH in purified lymphocytes and monocytes of 9 patients with HD indicates an enhanced hexose monophosphate shunt (HMPS) activity, reflecting

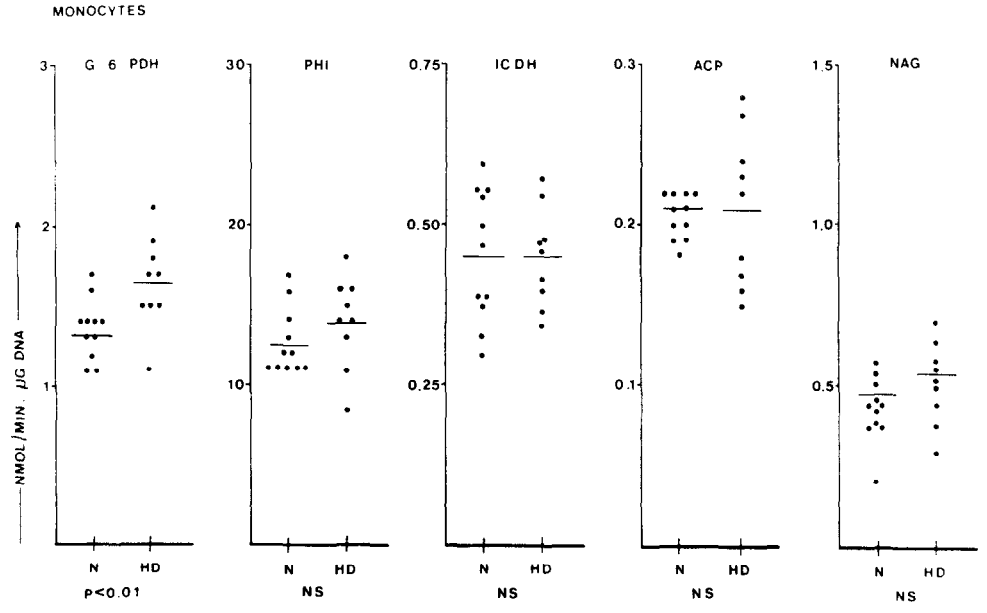

Fig. 1. Activity of G-6-PDH, PHI, ICDH, ACP and NAG in monocytes from patients with HD and normal controls.

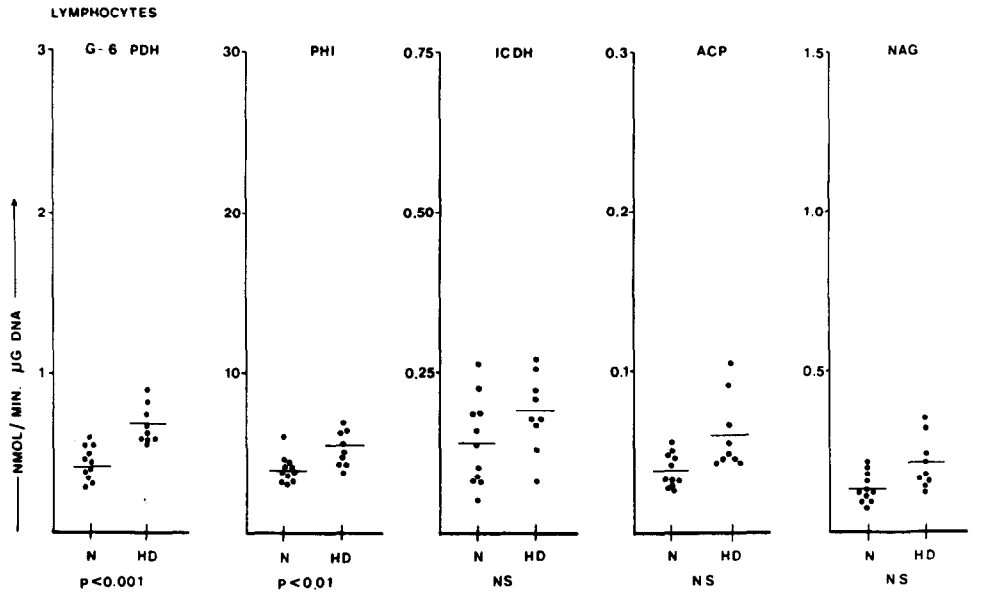

Fig. 2. Activity of G-6-PDH, PHI, ICDH, ACP and NAG in lymphocytes from patients with HD and normal controls.

ribose and NADPH production, essential for DNA, RNA and protein synthesis. In view of studies pointing to an augmented HMPS activity in lymphokine-activated monocytes [18] and in PHA-stimulated lymphocytes [19], these findings are in support of an increased lymphocyte and monocyte activity in HD. No correlation between age and G-6-PDH activity in monocytes, as described by King *et al.* [20], was observed in our study. Using radiolabeled glucose, King *et al.* [21] registered an increased HMPS activity in adherent monocytes from male patients with malignant lymphoma, and they also noted an augmented glycolytic and Krebs cycle activity. However, the results in our study concerning the activity of PHI of the glycolytic pathway and IDCH of the Krebs cycle did not confirm this observation for monocytes from patients with HD.

In the lymphocytes, next to the increased HMPS activity, the observed level of PHI is in agreement with an increased function of the glycolytic pathway, which is supposed [22] to be involved in lymphoblastic transformation. The occurrence of increased spontaneous lymphocyte transformation in patients with HD [4] seems to corroborate the validity of this observation. No alteration in the Krebs cycle enzyme ICDH was found in HD lymphocytes.

There is, to our knowledge, no information available concerning NAG and ACP activity of monocytes of HD patients. These enzymes are important for the hydrolytic degradation of glycoproteins, mucopolysaccharides and glycolipids. The results of this study reveal a comparable level for monocytes of both HD patients and controls.

The activity of several lymphocytic lysosomal enzymes are shown to be both increased [23, 24] and decreased in lymphoproliferative diseases [25]. Woessner *et al.* [26], studying the lysosomal enzyme β-glucuronidase in lymphocytes from patients with HD, found values within or above the normal level. Our figures for NAG and ACP activity tended to be increased in HD lymphocytes without reaching a statistically significant level ($P > 0.01$).

In conclusion, our findings are in support of an increased function of monocytes and lymphocytes in HD.

Acknowledgements—We are indebted to E. van de Ven for his technical assistance and G. Hoogendoorn, M.D. (Deventer), J. Steenbergen, M.D. (Amersfoort) and M. Scheerder, M.D. (Venlo) for referral of their patients for this study.

REFERENCES

1. AISENBERG AC. Studies on delayed hypersensitivity in Hodgkin's disease. *J Clin Invest* 1962, **41**, 1964–1970.
2. LEVY R, KAPLAN HS. Impaired lymphocyte function in untreated Hodgkin's disease. *N Engl J Med* 1974, **290**, 181–186.
3. CROWTHER D, FAIRLEY GH, SEWELL RL. Significance of the changes in the circulating lymphoid cells in Hodgkin's disease. *Br Med J* 1969, **2**, 473–477.

4. DE PAUW BE, WAGENER DJTH, SMEULDERS JBJH, GEESTMAN EJM, WESSELS JMC, HAANEN C. Lymphocyte density distribution profile and spontaneous transformation related to the stage of Hodgkin's disease. *Br J Haematol* 1980, **44**, 359–364.
5. STEIGBIGEL RT, LAMBERT LH, REMINGTON JS. Polymorph-nuclear leukocyte, monocyte and macrophage bactericidal function in patients with Hodgkin's disease. *J Lab Clin Med* 1976, **88**, 54–62.
6. GOODWIN JS, MESSNER RP, BANKHURST AD, PEAKE GT, SAIKI JH, WILLIAMS RC. Prostaglandin-producing suppressor cells in Hodgkin's disease. *N Engl J Med* 1977, **297**, 963–968.
7. PEHAMBERGER H, LUDWIG H, PÖTZI P, KNAPP W. Increased monocyte-mediated antibody-dependent cellular cytotoxicity (ADCC) in Hodgkin's disease. *Br J Cancer* 1980, **41**, 778–781.
8. BLAESE RM, OPPENHEIM JJ, SEEGER RC, WALDMANN TH. Lymphocyte-macrophage interaction in antigen induced *in vitro* lymphocyte transformation in patients with Wiskott–Aldrich syndrome and other diseases with anergy. *Cell Immunol* 1972, **4**, 228–242.
9. URBANITZ D, FECHNER I, GROSS R. Reduced monocyte phagocytosis in patients with advanced Hodgkin's disease and lymphosarcoma. *Klin Wochenschr* 1975, **53**, 437–440.
10. KOHL S, PICKERING LK, SULLIVAN MP, WALTERS DL. Impaired monocyte–macrophage cytotoxicity in patients with Hodgkin's disease. *Clin Immunol Immunopathol* 1980, **15**, 577–585.
11. DE MULDER PHM, DE PAUW BE, PENNINGS A, WAGENER DJTH, HAANEN C. Increased antibody-dependent cytotoxicity mediated by purified monocytes in Hodgkin's disease. *Clin Immunol Immunopathol* In press.
12. DE MULDER PHM, WESSELS JMC, ROSENBRAND DA, SMEULDERS JBJM, WAGENER DJTH, HAANEN C. Monocyte purification with counterflow centrifugation monitored by continuous flow-cytometry. *J Immunol Methods* 1981, **47**, 31–38.
13. LUKES RJ, CRAVER LF, HALL TC, RAPPAPORT H, RUBIN P. Report of the nomenclature committee. *Cancer Res* 1966, **26**, 1911.
14. CARBONE PP, KAPLAN HS, MUSSHOFF K, SMITHERS DW, TUBIANA H. Report of the committee on Hodgkin's disease staging classification. *Cancer Res* 1971, **31**, 1860–1861.
15. LAWRENCE C. GROSSMANN R. Simple butyrate esterase stain for monocytes. *Stain Technol* 1980, **54**, 321–323.
16. KAPUSCINSKY J, SKOCZYLAS B. Simple and rapid fluorometric method for DNA microassay. *Anal Biochem* 1977, **83**, 252.
17. MIER PD, VAN RENNES H, VAN ERP PEJ, ROELFZEMA H. Cutaneous sialidase. *J Invest Dermatol* 1982, **78**, 267–269.
18. ROCKLIN RE, WINSTON CT, DAVID JR. Activation of human blood monocytes by products of sensitized lymphocytes. *J Clin Invest* 1974, **53**, 559–564.
19. SAGONE AL, LOBUGLIO AF, BALCERZAK SP. Alterations in hexose monophosphate shunt during lymphoblastic transformation. *Cell Immunol* 1974, **14**, 443–452.
20. KING GW, PARA MF, LOBUGLIO AF, SAGONE AL. Human monocyte metabolism: male and female. *J Reticuloendothel Soc* 1974, **17**, 282–289.
21. KING GW, LOBUGLIO AF, SAGONE AL. Human monocyte glucose metabolism in lymphoma. *J Lab Clin Med* 1977, **89**, 316–321.
22. ROOS D, LOOS JA, Effect of phytohaemagglutinin on the carbohydrate metabolism of human blood lymphocytes after inhibition of the oxidative phosphorylation. *Exp Cell Res* 1973, **77**, 121–126.
23. SIPPEL WG, ANTONOWICZ I, LAZARUS H, SCHACHMAN H. Lysosomal and mitochondrial enzyme activities in human lymphoid cell lines obtained from children with acute lymphoblastic leukaemia and controls. *Exp Cell Res* 1975, **91**, 152–158.
24. FLANDRIN G, DANIEL MT. β-Glucuronidase activity in Sézary cells. *Scand J Haematol* 1974, **12**, 23–31.
25. DEMPSEY SI, CROCKARD AD, BRIDGES JM. An estimation of β-glucuronidase and *N*-acetyl-β-D-glucosaminidase activity in normal and chronic lymphocytic leukaemia lymphocytes. *Acta Haematol* 1980, **64**, 141–147.
26. WOESSNER S, MILLÁ F, ROZMAN C. A study of lymphocytic β-glucoronidase in various benign and malignant lymphatic processes. *Acta Haematol* 1974, **51**, 84–90.