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Glutamate dehydrogenase activity in lymphocytes of B-cell chronic lymphocytic leukaemia patients

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Abstract

Objectives: To investigate the pattern of glutamate dehydrogenase (GLDH) activity, *GLUD1* and *GLUD2* expressions in peripheral blood mononuclear cells (PBMC) of untreated B-chronic lymphocytic leukemia (B-CLL) in healthy individuals (HI) and patients with infectious mononucleosis (IM).

Design and methods: GLDH activity was determined in a supernatant obtained from pelleted PBMC. GLUD1 and GLUD2 mRNA expression was determined using a quantitative real-time polymerase chain reaction. $CD19^+$ B cells from PBMC were purified by using positive selection.

Results: The highest GLDH activity was found in PBMC of the B-CLL group followed by the HI group and IM group. The PBMC GLDH activity was higher in 60% of the B-CLL patients according to the established reference interval for our HI (2.17–5.70 μkat/g protein). The greater GLDH activity was also found in the $CD19^+$ cell preparation of the B-CLL patients (two of the three) but not in HI ($n=3$). The median value of *GLUD1* expression was highest in the IM group ($n=11$), followed by the HI ($n=14$) and B-CLL groups ($n=59$) (median 4.69/3.78, $P<0.005$ and 4.69/2.91, $P<0.0005$, respectively). *GLUD2* expression was not significantly different between groups.

Conclusions: The increased GLDH activity is specific for the PBMC of B-CLL patients. The *GLUD1* but not the *GLUD2* gene expression pattern is different between the PBMC of IM and B-CLL patients.

Keywords: Chronic lymphocytic leukaemia; Glutamate dehydrogenase; *GLUD1* and *GLUD2* gene expressions

Introduction

B-cell chronic lymphocytic leukaemia (B-CLL) is characterized by highly variable clinical courses and individual prognoses for the patients [1,2]. The clinical staging systems of Binet and Rai [3,4] remain the basis for assessing prognosis in B-CLL, but accurate identification of prognostic factors is becoming increasingly important in order to define patients requiring aggressive treatments or to predict responses to therapy and its duration [1–4]. Advances in the research of the biology of B-CLL has resulted in a number of biological markers of prognostic relevance, including serum thymidine kinase levels (TK), which correlate with the proliferative activity of CLL cells [5,6], and a new perspective on the malignant clone. Recent data suggest that B-CLL cells are born at a normal to an accelerated rate, with the rate of proliferation varying among patients as was shown by labelling cell DNA *in vivo* with deuterium and by following its disappearance from the leukemic cells with time. An evolving view of the existence of proliferate compartment among B-CLL cells is in contrast to previous accepted belief, considering B-CLL a disease of accumulation due to a presumed defect in programmed cell death [2,7]. The transformations of cells to a cancerous phenotype is often associated with a cognate changes in the transport and metabolism of nutrients such as glucose and glutamine [8–10]. This raises the questions about nutritive needs and metabolism of the B-CLL cells in such circumstances as well as the influence of the still unknown factor(s) that could initiate proliferation or sustain cell viability.

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Glutamate dehydrogenase (GLDH; EC 1.4.1.3.) catalyses the reversible deamination of glutamate to α-ketoglutarate and ammonium ion using NAD(H) or NADP(H) as cofactors. It is thought to play a key role in cellular metabolism and energy homeostasis [11–13]. GLDH is located in mitochondria [14–17] although there is evidence for extra-mitochondrial localization of GLDH [18–22]. The mature GLDH protein is composed of six identical subunits, each of which has a molecular mass of ca. 56 000 and consists of a polypeptide chain around 505 amino acids [23–26]. The enzyme is present in almost all mammalian tissues with the highest levels found in the liver; only erythrocytes do not contain any at all [13,27].

More important is its catabolic function [28,29]. In the metabolism of a cell, GLDH has the key role of acting as a major link between carbohydrate and amino acid metabolism in the vicinity of the citric acid cycle and urea cycle. It functions as a distributor among metabolic pathways together with amino-transferases [13,29,30]. In the pancreatic β cells, GLDH is involved in insulin secretion mechanisms [31], whereas in the nervous system the enzyme may play a role in the metabolism of neurotransmitter glutamate and in neurodegenerative processes [32]. Heat-stabile and heat-labile GLDH enzyme activities can be detected in leucocytes and a partial decrease in leukocyte GLDH activity in patients with some neurodegenerative diseases was observed [33–35].

In humans, GLDH exists in two isoforms encoded with distinct genes [25,36]: the widely-expressed intron-containing *GLUD1* gene [37] linked to chromosome 10 and an X-linked intronless *GLUD2* gene specific for neural and testicular tissues [36,38]. *GLUD1*-derived GLDH is a thermo-stabile enzyme with a marked baseline activity and is inhibited by GTP. *GLUD2* gene-derived GLDH shows a low baseline activity and resistance to GTP inhibition. It is a thermo-labile enzyme that can be fully activated by ADP or L-leucine, much more than *GLUD1*-derived GLDH [17,25,32,36–40].

Many authors have agreed that metabolic processes in leucocytes function in the same way as in other tissues. However, there are few relevant data about the exact purpose of GLDH in leucocytes [10,41]. There is an old published observation that the glutamate dehydrogenase activities were much higher (approximately 200%) in lymphocytes than in granulocytes [42]. Our preliminary data obtained by measuring the leukocyte's GLDH activity in patients with myeloproliferative diseases, such as chronic myeloid leukaemia in the chronic phase, essential thrombocythemia and polycythemia vera, and in the B-CLL patients, showed a frequent and marked increase of the leukocyte's GLDH activity in the B-CLL but not in the examined myeloid pathology.

Therefore, we continued the study, measuring the GLDH activity, *GLUD1* and *GLUD2* gene expression in the peripheral blood mononuclear cells (PBMC) of untreated B-CLL patients. By comparing the results to those of healthy individuals (HI) and patients with infectious mononucleosis (IM) (a disease with notably activated lymphocytes), we wanted to investigate the pattern of GLDH activity, *GLUD1* and *GLUD2* expressions and the potential of the PBMC GLDH activity as additional biological markers of B-CLL.

Patients and methods

Patients and control subjects

The study populations consisted of HI, B-CLL and IM patients. Their basic characteristics are presented in Table 1. All the participants were Caucasians and were included in the study after informed consent was obtained.

In total, 62 B-CLL patients (22 females, 40 males), median age 65 (range from 37 to 88 years) in various Binet stages (52 Binet A, 6 Binet B, 4 Binet C) diagnosed at the Division of Internal Medicine, Department of Haematology, at the University Medical Centre Ljubljana were included in the study. All patients had a sustained lymphocytosis, greater than 5×10^9/L, and immunophenotype ($CD19^+/CD5^+/CD23^+$) as the B-cell clone characteristic. All patients were untreated at the time of

Table 1
Basic characteristics for healthy individuals, B-chronic lymphocytic leukaemia (B-CLL) and infectious mononucleosis patients.

Variables	HI (n=60)	B-CLL (n=62)	IM (n=21)
Age (years)	45 (21–63)	65 (37–88) [a]***	19 (6–28) [a]***, [b]***
Gender, female (%)	35.0	35.5	52.4
White blood cell count-WBC (10^9/L)	6.0 (3.6–9.3)	31.6 (11.7–169.4) [a]***	11.8 (5.2–17.0) [a]***, [b]***
Differential white cell count			
Neutrophils-Ne (10^9/L)	3.45 (1.5–5.8)	5.13 (0.26–25.6) [a]**	3.85 (1.4–7.2) [a] N.S., [b]*
Lymphocytes-Ly (10^9/L)	1.7 (1.1–2.9)	25.7 (5.72–137.7) [a]***	7.1 (2.8–11.8) [a]***, [b]***
Monocytes-Mo (10^9/L)	0.4 (0.2–0.8)	1.02 (0.1–27.9) [a]***	1.3 (0.1–2.8) [a]***, [b] N.S.
Eosinophils-Eo (10^9/L)	0.1 (0–0.5)	0.12 (0–0.75) [a] N.S.	0.02 (0–0.3) [a]**, [b]***
Bazophils-Ba (10^9/L)	0.034 (0–0.1)	0.08 (0–2.0) [a]***	0.035 (0–0.6) [a] N.S., [b]**
Red blood cell count-RBC (10^{12}/L)	4.62 (3.57v5.86)	4.33 (3.13–5.86) [a]**	4.81 (3.74–5.57) [a] N.S., [b]***
Haemoglobin-Hb (g/L)	142 (123–165)	133.5 (96–171) [a]**	141 (111–164) [a] N.S., [b]*
Platelet count-Plt (10^9/L)	254 (157–339)	189.5 (43–465) [a]***	250 (150–352) [a] N.S., [b]**

Data presented are median (range) or % of patients; HI, healthy individuals; B-CLL, B-cell chronic lymphocytic leukaemia patients; IM, infectious mononucleosis patients.
Peripheral blood counts were performed with automated blood cell counter Beckman Coulter LH 750 (Beckman Coulter, Inc., Fullerton, CA, USA).
*P<0.05; **P<0.01; ***P<0.001; N.S., statistically irrelevant data.
[a] B-CLL patients, IM patients vs. HI.
[b] B-CLL patients vs. IM patients.