VIROLOGY

Enzymatic Status of Blood Lymphocytes in Young Children with Epstein–Barr Virus Infection

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Activities of NAD(PH)-dependent dehydrogenases in peripheral blood lymphocytes were studied in children aged 1-3 years in the dynamics of the disease caused by Epstein–Barr virus. A relationship between changes in activities of the studied enzymes and disease period was revealed. The disorders of blood lymphocyte enzymatic status persisted during convalescence.

Key Words: *enzymes; lymphocytes; Epstein-Barr virus*

Infectious mononucleosis (IM) is one of the most frequent pathology caused by Epstein–Barr virus (EBV) in children. IM is now regarded as an immune disease. Active proliferation of the virus in all organs containing lymphoid tissue leads to changes involving all components of the immune system [1,2].

It is proven that the metabolic reactions of immunocompetent cells (ICC) underlie the functional manifestations of these cells. For example, enzymes of glycolysis and pentose phosphate cycle in lymphocytes are stimulated during the development of blast transformation reaction [6,9,11]. The expression of $CD4^+$ and $CD8^+$ antigens on blood lymphocytes depends on the intracellular concentrations of adenosine and adenosine diphosphoric acid [3]. Immunodeficiency-associated intracellular enzymes were detected [8].

Studies of ICC metabolism in young children are an important problem. The most significant dynamic changes in the cells associated with fulminant growth and differentiation, on the one hand, and with rapid development of metabolic disorders at the cellular level as a result of exposure to pathological factors, on the other, can be expected in this population.

We studied activities of NAD(PH)-dependent dehydrogenases in the blood lymphocytes over the course of EBV infection in young children.

MATERIALS AND METHODS

Children aged 1-3 years with IM caused by EBV with medium severe disease course were observed during the acute phase (68 patients, days 2-5 of disease) and during convalescence (19 children; weeks 3-4). Control group consisted of 54 healthy children of the same age.

The diagnosis of IM was verified by PCR with reagents for detection of EBV DNA in blood lymphocytes (DNA Technologies). Serum IgM VCA, IgG EA-D, and IgG EB NA-1 were evaluated by EIA with commercial test systems (Human). All patients were positive for blood lymphocyte EBV DNA and for serological markers of acute EBV infection (EBV-VCA IgM, EBV-EA-D IgG).

Peripheral blood lymphocytes were isolated in ficoll-Verograffin density gradient [4].

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In order to measure enzyme activities, the suspension of isolated lymphocytes (0.5-1.0×10^6 cells) after single freezing/defrosting cycle was additionally destroyed by osmotic lysis by adding distilled water (1:5 by volume) and 1.0-2.0 mM dithiotreitol. The suspension of destroyed lymphocytes (200 μl) was added to 600 μl incubation mixture containing the appropriate substrate and cofactor.

Importantly that ADP in concentrations of 2.15 and 1.30 mM were added to the incubation mixture for measuring activities of NADIDH and NADPIDH, respectively; NH_4Cl (5.0 mM) was added to the incubation medium for measuring activities of NADHGDH and NADPHGDH, and EDT (0.5 mM) was added for measurements of glutathione reductase.

After incubation at 37°C (30 min for enzymatic reactions with NAD(P) reduction or 5 min for reactions with NAD(P)H oxidation), 200 μl incubation mixture was added to the cuvette of a BLM-8801 bioluminometer containing 50 μl flavine mononucleotide (FMN; 1.5×10^{-5} M), 10 μl NAD(P)H:FMN oxidoreductase-luciferase system, and 50 μl 0.0005% aldehyde C_{14} (all reagents of the bioluminescent system were diluted in 0.1 M K^+,Na^+-phosphate buffer (pH 7.0). The bioluminescence level was measured and the NAD(P)H concentration in the sample was determined using the calibration curve. The NAD(P)H:FMN oxidoreductase-luciferase system has been prepared from *Photobacterium leiognathi* luciferase and *Vibrio fischeri* oxidoreductase, purified by ion exchange chromatography and gel filtration, at Institute of Biophysics, Siberian Division of the Russian Academy of Sciences.

Since the cells have a certain amount of substrates for various metabolic reactions (including those catalyzed by the studied enzymes), the parameters provisionally called "basal substrate level" were evaluated. The measurements were carried out under the same conditions as for the above dehydrogenases, but buffer was added into incubation mixture instead of the appropriate substrate.

In order to plot the calibration curve reflecting the relationship between bioluminescence intensity and NAD(P)H concentration, 200 μl standard NAD(P)H (10^{-9}-10^{-4} M) was put into the bioluminometer cuvettes containing bioluminescent reagents in the above concentrations, after which the bioluminescence intensity was measured. Because of the wide pH range of the buffers used for measurements of dehydrogenase activities and the pH dependence of bioluminescence of the enzymatic system from luminescent bacteria, the calibration curves were plotted for each buffer pH.

Activity of NAD(PH)-dependent dehydrogenases was estimated by the formula:

$$A=(|\Delta C|\times V\times 10^6)/T$$

where $|\Delta C|$ is the difference in the NAD(P)H concentrations in "enzyme" and "enzyme basal level" samples, V is sample volume (ml), and T duration of incubation.

Activity of NAD(PH)-dependent dehydrogenases was expressed in enzyme units per 10^4 cells, where 1 U=1 μmol/min.

The data were statistically processed using Statistica 6.0 software. The significance of differences in the studied parameters in independent samples was analyzed using Mann–Whitney's test. The results of measurements of quantitative parameters in the compared groups were presented as the median (Me) and interquartile interval with LQ of 25 percentiles and UQ 75 percentiles.

RESULTS

Study of activities of NAD(PH)-dependent dehydrogenases in blood lymphocytes during the acute period of EBV infection (Table 1) showned elevation of G6PDH (the key enzyme of pentose phosphate cycle, whose products are utilized in the macromolecular synthesis reactions [5,7]).

Analysis of activities of the studied dehydrogenases of predominantly involved in energy metabolism revealed significant decrease in NADHLDH activity in blood lymphocytes from children during the acute period of EBV infection in comparison with the control (Table 1). On the other hand, activities of NADPIDH, NADGDH, and NADHMDH (the key enzymes of the malate aspartate shunt) in these patients were higher than in the control (Table 1). In addition, NADPMDH activity significantly decreased and NADHGDH and NADPHGDH activities increased during the acute period of EBV infection in comparison with the corresponding parameter in the control group (Table 1). High level of glutathione reductase activity in blood lymphocytes in this group of patients in comparison with the control (Table 1) is worthy of note.

Hence, the enzymatic status of blood lymphocytes in children with EBV infection during its acute period is characterized by hyperproduction of ribose-5-phosphate, intensification of NADPH-dependent macromolecular synthesis reactions, and reduction of anaerobic LDH reaction (which reflects reduced substrate flow in glycolysis), high level of NAD-dependent oxidative deamination of glutamic acid, significant reduction of shunting of the Krebs cycle slow portion via malic enzyme, more intense outflow of intermediate products through NADHGDH and NADPHGDH from the tricarbonic acid cycle to amino acid metabolism reactions. The accessory isothiocitrate dehydrogenase

TABLE 1. Activities of NAD(PH)-Dependent Dehydrogenases (μU) of Blood Lymphocytes in Children with EBV Infection (Me, LQ-UQ)

Parameter	Control group (n=54)		EBV infection				p
	Me	LQ-UQ	acute period (n=68)		convalescence (n=19)		
			Me	LQ-UQ	Me	LQ-UQ	
G6PDH	1.43	0.75-3.06	3.78	1.05-11.07	2.75	1.64-3.54	$p_1<0.05$
G3PDH	0.04	0.01-0.16	0.09	0.01-0.89	0.11	0.02-0.43	
NADLDH	12.75	4.28-26.78	12.16	1.58-38.72	18.84	1.87-27.41	
NADPMDH	8.58	1.93-11.56	0.04	0.01-0.16	0.17	0.06-1.84	$p_1<0.001$
							$p_2<0.001$
							$p_3<0.01$
NADPGDH	0.11	0.01-0.70	0.17	0.01-0.88	0.97	0.46-2.45	$p_2<0.01$
							$p_3<0.05$
NADPIDH	5.08	1.35-9.57	26.33	5.02-61.09	25.98	12.87-41.85	$p_1<0.01$
							$p_2<0.01$
NADMDH	15.93	6.87-23.63	15.17	1.21-41.29	13.87	6.63-23.70	
NADGDH	0.20	0.01-1.31	17.02	0.01-25.34	1.97	0.01-10.48	$p_1<0.001$
							$p_3<0.05$
NADIDH	0.72	0.03-1.65	2.68	0.01-26.10	4.37	0.02-10.17	
NADHLDH	20.31	0.01-41.02	4.23	0.01-50.38	27.68	0.01-59.54	$p_1<0.05$
NADHMDH	35.59	5.78-65.86	187.95	55.25-289.61	152.87	50.48-284.54	$p_1<0.01$
							$p_2<0.01$
GR	0.02	0.01-6.88	13.77	6.70-20.51	8.39	2.15-10.88	$p_1<0.001$
							$p_2<0.05$
							$p_3<0.05$
NADHGDH	21.74	5.16-41.20	48.13	9.36-114.28	90.75	65.70-126.86	$p_1<0.05$
							$p_2<0.001$
NADPHGDH	14.72	9.72-36.84	35.88	19.81-49.09	23.87	15.67-40.69	$p_1<0.05$

Note. Significant differences between the parameters: p_1: during the acute period and in the control; p_2: in convalescents and controls; p_3: during acute period and convalescence.

reaction was stimulated and the role of malate aspartate shuttle in cell energetics increased. In addition, a high level of glutathione reductase was observed (this enzyme is involved in the system of cellular antioxidant defense and modulates, to a certain measure, the lymphocyte proliferative activity [10,12,13]).

High activities of NADPIDH, NADHMDH, and NADHGDH in blood lymphocytes persisted during convalescence. The role of the Krebs cycle slow portions shunting through malic enzyme activation increased in comparison with the acute period of the disease (Table 1). The parameters of NADGDH activity did not differ significantly from the control and are significantly lower than the values during the acute period. The increase of NADPGDH activity during convalescence in comparison with the control is worthy of note. Activity of glutathione reductase remained above the control and below the level observed during the acute period of EBV infection (Table 1).

Hence, the enzymatic status of ICC is changed in young children with EBV infection. The pattern of restructuring of the enzymatic profile in blood lymphocytes depends on the disease period. The most pronounced changes were observed during the acute period of EBV infection. The enzymatic status of blood lymphocytes is not restored during convales-

cence. Hence, it seems that metabolic correction of intracellular metabolic processes in ICC is required in young children with EBV infection.

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