Structure, Metabolism, and Functions of Peripheral Blood Lymphocytes during Long-Term Persistence of Epstein--Barr Virus

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Study of surface architectonics, blast transformation potential, and cytochemical activity of peripheral blood lymphocytes from children infected with Epstein—Barr virus revealed imbalance between structural, metabolic, and functional state of lymphocytes. This imbalance persists in delayed period after infection and determines long-term viral persistence in the macroorganism.

Key Words: *Epstein—Barr virus; lymphocytes; persistence; scanning electron microscopy; lymphocyte blast transformation; cytochemistry*

Sharp increase in the prevalence of persistent viral infections in recent years is an urgent problem of modern medical and biological science [10]. Viral persistence in the macroorganism is determined by the ability of viruses to survive and replicate in cell populations. This is realized via integration of viral genes into chromosomes of host cells or modulation of immune inductor and effector systems [6].

Potentially oncogenic Epstein—Barr virus (EBV) belongs to the family *Herpesviridae* and is characterized by rapid replication and long-term persistence in the human organism [8,12]. Lifelong persistence is associated with tropism of EBV to immune cells (*e.g.*, lymphocytes) [9]. Invasion of EPS into cell stimulates innate and adaptive immune reactions. However, the ability of EBV to escape immune recognition counteracts elimination of the infectious agent [13].

EBV persists for a long time in differentiated immune cells and suppresses production of virus-specific antibodies. On the one hand, this virus regulates expression of viral antigens in infected cells. On the other hand, EBV stimulates production of immunosuppressors and immunomodulators that block the immune response in macroorganisms [6,12]. EBV inhibits the immune response via blockade of this process, recognition of antigens in the host cell, and mimicry of antigenic determinants. This virus can suppress realization of the immune response. High viral load is accompanied by exhaustion and impairment of T cell immunity that plays a key role in antiviral defense and determine the outcome of primary infection and incidence and severity of recurrent diseases. EBV modulates the ratio between growth and apoptotic factors in infected cells and activates apoptosis in lymphocytes [11].

The virus—cell system is characterized by a dynamic equilibrium. The interaction of EBV with immunocompetent cells results in changes in structural, metabolic, and functional properties of lymphocytes, which contributes to an incomplete initiation and realization of the immune response, leads to the development of secondary immunodeficiency, and promote viral persistence in the macroorganism. This important problem received little attention. Here we studied surface architectonics and functional and metabolic state of peripheral blood lymphocytes from children infected with EBV.

MATERIALS AND METHODS

We examined 30 children (7-14 years) with EBV-induced infection (acute clinical and hematological

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symptoms, *n*=11; clinical convalescence, 1 month postinfection, *n*=7; delayed period, 16-18 months postinfection, *n*=12). The diagnosis was made by clinical, hematologic, serologic (enzyme immunoassay), and molecular genetic studies (polymerase chain reaction). The patients with acute EBV-induced infection had general toxic symptoms, inflammation in the nasopharynx and stomatopharynx, lymphadenopathy of tonsillar, cervical, occipital, axillary, and inguinal lymph nodes, hepatomegaly, and splenomegaly. We observed typical changes in quantitative (leukocytosis, lymphocytosis, and monocytosis) and biochemical blood parameters (increased alanine transaminase and aspartate transaminase activities). Lymphadenopathy of tonsillar and cervical lymph nodes and changes in hemogram were observed in the delayed postinfection period. The control group included 25 conventionally healthy children of the same sex and age. Studies were performed with heparinized blood (25 U/ml) taken in the morning before meal.

Surface architectonics of peripheral blood lymphocytes was assayed by scanning electron microscopy. Lymphocytes were isolated from the blood [5], fixed with 2.5% glutaraldehyde, postfixed with 1% OsO_4, and dehydrated with aqueous solutions of ethyl alcohol in increasing concentrations. Defatted aluminum plates were coated with a thin layer of dehydrated lymphocytes. Preparations were sprayed with copper on a JEE-4B vacuum device (JEOL) and examined under a JEM-100CX II electron microscope equipped with an ASID-4D scanning device (JEOL). The measurements were performed at an accelerating potential and slope angle of 20 kV and 30°, respectively [3]. Depending on characteristics of surface microrelief described by G. I. Kozinets and Yu. A. Simovart [3], lymphocytes were divided into smooth cells and cells with microvilli, lamellar structures (folds and ruffles), spherical bubbles, and surface invaginations. We revealed lymphocytes with a transitional type of the surface membrane. The spherical part had microvilli, and the elongated part was organized in folds.

The culture suspension for lymphocyte blast transformation was prepared as described elsewhere [2]. Phytohemagglutinin (PHA, Difco, 0.01 mg/ml), *Escherichia coli* lipopolysaccharide (LPS, 0.01 mg/ml), and tuberculin (0.05 ml/ml) were added to the culture. The results were expressed in percents of unchanged, transitional, and blast lymphocytes [2].

Peripheral blood smears were prepared by the method of leucoconcentration with Trilon B [5]. Glycogen content in lymphocytes was measured in the periodic acid Shick reaction of McManus. The amount of lipids was determined by the method of Sheehan and Storey. Acid phosphatase activity in lymphocytes was estimated by the method of Goldberg and Barka (azocoupling). Nonspecific esterase activity was evaluated by the method of Hayhoe and Oaglino. Activities of succinate dehydrogenase (SDG) and α-glycerophosphate dehydrogenase in lymphocytes were determined by incubation of cells with the corresponding substrate in the presence of *p*-nitrotetrazolium violet. For quantitative analysis of the results the mean cytochemical index was calculated [7].

The results were analyzed by Mann—Whitney test [1].

RESULTS

EBV is ubiquitous and can be detected in 90% people. Clinical and hematologic symptoms of primary viral infection were previously considered as the leukemoid reaction. At present, pathomorphological changes in hemopoietic organs represent the nonspecific immune response to EBV [8,9,12,13].

Structural changes in the plasma membrane, the most susceptible to exogenous factors, are determined by pathological influence of viruses and serve as the major cause of functional insufficiency in lymphocytes [9]. The study of surface architectonics of peripheral blood lymphocytes from EBV-infected children showed that the count of cells with surface lamellar structures considerably increases at the stage of clinical manifestation and delayed postinfection period (Table 1). The percentage of lymphocytes with surface invaginations and spherical structures markedly increased during convalescence. By contrast, the number of lymphocytes with ciliated surface microrelief decreased throughtout observation. Lymphocytes with intermediate (transitional) type of surface not observed in healthy donors were found in children infected with EBV (Table 1). Morphological changes in lymphocytes can be related to an increase in the number of peripheral blood B cells. They are characterized by a ciliated type of surface architectonics and act as a major reservoir for EBV. The appearance of outgrowths on the cell membrane results from the increase in functional activity of infected immunocompetent cells. However, activity of these cells is insufficient for elimination of the virus [3]. Blood lymphocytes with a transitional type of surface microrelief can be classified as atypical mononuclear lymphocytes transformed by viral antigen [9].

Functional insufficiency of immune cells during long-term persistence of EBV can be associated with the direct influence of viral reproduction on cell activity (decrease of complete loss) and inhibitory effect of soluble viral or cellular factors released from abnormal cells [5,7,13]. The reaction of blast transformation during mitogenic (PHA and LPS) and antigenic stimulation (tuberculin) is an optimal approach to studying

TABLE 1. Surface Architectonics of Peripheral Blood Lymphocytes from Children with EBV-Induced Infection ($X \pm m$)

Parameter	Control	EBV-infected children		
		acute stage	clinical convalescence	delayed period
Surface microrelief, %				
smooth	36.48±3.24	29.97±8.95	32.20±3.24	33.65±3.18
lamellar	11.65±1.73	20.18±1.60*	22.50±3.17*	19.78±2.85*
ciliated	46.04±3.44	41.78±11.00	31.95±3.94**	37.41±3.75**
with bubbles	3.95±0.72	3.76±0.57	6.63±0.77**	5.22±1.05
with invaginations	1.88±0.63	1.46±0.65	5.41±1.77**	1.40±0.37
transitional	—	2.85±0.20	1.31±0.41	2.54±0.59
Blast transformation, %				
PHA	77.39±2.29	59.23±6.27**	78.25±4.33°	70.25±6.30
LPS	71.79±4.50	83.75±1.90	84.00±4.66	61.75±8.83
tuberculin	66.36±4.70	50.55±7.90	58.50±6.77	66.67±0.84
Metabolic state of lymphocytes				
lipid content	0.07±0.01	0.12±0.02**	0.16±0.04**	0.04±0.01+
glycogen content	0.22±0.02	0.23±0.02	0.24±0.05	0.13±0.02*
acid phosphatase activity	0.56±0.04	0.48±0.04	0.41±0.09	0.51±0.07
nonspecific esterase activity	0.24±0.02	0.27±0.02	0.26±0.04	0.19±0.02
SDG activity	2.94±0.30	2.49±0.45	9.15±1.91*	8.40±1.31*
glycerophosphate dehydrogenase activity	2.45±0.32	1.81±0.35	7.37±1.63*°	2.56±0.17°

Note. *$p<0.01$ and **$p<0.05$ compared to the control; °$p<0.05$ compared to children with acute clinical and hematological symptoms of the disease; +$p<0.05$ compared to clinical convalescence.

functional activity of lymphocytes [2]. The intensity of PHA-induced lymphocyte proliferation in children decreased in the acute stage of infection with EBV, but returned to normal 1 month postinfection (Table 1). Low proliferative activity of lymphocytes in response to PHA is probably related to the ability of EBV to suppress selectively functional activity of T cells regulating B cell proliferation [9,14].

Metabolic changes in immunocompetent cells during various stages of the infectious process play an important role in the mechanisms of chronic EBV persistence. A cytochemical study of peripheral blood lymphocytes from children with EBV-induced infection revealed a considerable increase in the content of lipids in the acute stage and during clinical convalescence (compared to healthy donors, Table 1). Activation of immune cells after the interaction with viral antigens intensifies intracellular metabolism [9,12]. The amount of lipids and glycogen in lymphocytes markedly decreased 16-18 months after infection with EBV, which reflects exhaustion of energy reserves. Dehydrogenase activity in lymphocytes increased during convalescence and remained high in the delayed postinfection period (Table 1). These data indicate that immunocompetent cells infected with EBV are characterized by high-intensity respiration and glycolysis processes [12,14].

Our results illustrate the imbalance between structural, metabolic, and functional state of lymphocytes in children with EBV-induced infection. Changes in the surface architectonics, proliferation, and cytochemical activity of immunocompetent cells reflect the inability of the immune system to eliminate the infectious agent and counteract viral aggression, which determines long-term persistence of EBV in the macroorganism.

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