Medical practice, No. 1 (55), 2007

NATURE OF CHANGES OF HEALTH IN CHILDREN OF TENDER AGE, THE CONDITION OF THEIR IMMUNE AND CYTOKINE STATUS AGAINST THE BACKGROUND OF VARIOUS COURSE OF INFECTION INDUCED BY EPSTEIN-BARR VIRUS

Chernysheva O.E., Yulish E.I., Ivanova L.A.

M. Gorky State Medical University of Donetsk

The role of Epstein-Barr virus in the development of immunodeficient diseases in children was studied. Contamination with Epstein-Barr virus was detected in 60% of children. The increase of concentration of pro-inflammatory cytokines, disbalance of B- and T-cellular components of immune system was noted.

Key words: Epstein-Barr virus, immunological status, children.

The characteristic feature of the modern pathology consists in the increase of chronic infectious inflammatory diseases, which are being often formed against the background of persistent infection process induced by herpesviruses (herpes simplex virus, cytomegalovirus, Epstein-Barr virus) [1, 2, 3]. Epstein-Barr virus is characterized by affinity towards lymphoid cells (T- and B-lymphocytes), in which it is protractedly persisting and while transforming them can induce formation of malignant tumors — lymphomas, sarcomas. Its properties are differing from other herpesviruses known with their intracellular aggressiveness [4, 5]. The activators of cytomegaly, herpes zoster, genital and labial herpes nearly always destroy cells, which they penetrate in. Epstein-Barr virus, about which is less known among herpesviruses, behaves more delicately: it parasitizes in the cells of immune system depriving them of the opportunity to perform their predestined functions, and rarely demonstrates the pronounced cytopathic effect on them [11]. However, it even strengthens suspicion towards it, since such property of virus under certain circumstances can induce uncontrolled degenerative changes in the body causing progression of deficiency of its functions [6, 7, 8]. Accordingly, Epstein-Barr virus causes such various diseases as infectious mononucleosis, malignant tumors, autoimmune diseases, secondary immunodeficiency [9, 10].

The main target cells for Epstein-Barr virus are B-lymphocytes. The contaminated B-cells gain the capacity of unrestricted proliferation and synthesis of a large number of antibodies [11, 12]. At the Epstein-Barr viral infection not only changes on the part of B-cellular component of immune system occur, but also violations of cell-mediated response and factors of the innate resistance (macrophages, natural killers, neutrophils, interferon system). The result of induction of immunodeficient condition and ability of Epstein-Barr virus to integrate in the genome of infected cells is its life-long persistence [13, 14].

The objective of our work consisted in determining of role of Epstein-Barr virus (EBV) in the development of immunodeficient diseases influencing on the rate and duration of respiratory diseases in children.

The analysis was conducted of findings of clinical and laboratory examination of 30 children of the group of frequently and chronically ill (FCIC) children in the age from 8 to 14 months. Specific antibodies to EBV— M and G class immunoglobulins (IgM and IgG) to early antigen (EA), capsid antigen (VGA) and nuclear antigen (EBNA) by enzyme-linked immunosorbent assay method (ELISA) were being detected in blood serum using the diagnostical immunoenzymometric system by Vektor-Best (Russia). The indices of cellular and humoral components of immune system were determined by the method of immunophenotyping of membrane antigens of peripheral blood

lymphocyte cluster differentiation: CD3 (general of T-lymphocytes), CD4 (T-helpers), CD8 (T-suppressors), CD16 (NK-cells), CD20 (B-lymphocytes). The level of cytokines — IL-2, IL-6, IL-8, tumour necrosis factor (TNF) were detected by means of monoclonal antibodies by the flow cytofluorometry method. Concentrations of IgA, IgM, IgG immunoglobulins in blood serum were detected by radial immunodiffusion technique in gel according to Mancini. The condition of phagocytic system was estimated by phagocytosis reaction with determination of phagocytic number, phagocytosis percentage and its completeness. Circulating immune complexes (CIC) were detected by means of polyethyleneglycol-6000. The immune profiles of infected children were studied against the background of clinical remission. The immune indicators of children under supervision were compared with the data of 15 apparently healthy peers. The statistical processing was carried out using the STADIA dialogue statistical system.

The nature of EBV infection course was based not only on clinical observations data, but also, which was a leading criterion of EBV diagnostics, on determination of the presence and levels of *DNA* virus, IgM and IgG (Table 1). EBV contamination was detected in 60% of cases (18 children). Among them 4 subjects (22.3%) were diagnosed with the acute infection course (group 1), in 14 (77.7%) — with chronic course (group 2). The observational data are represented in Table 2.

The analysis of clinical data revealed that children under supervision were reported with a high rate of recurrent diseases of respiratory ways of viral etiology. During the period of observation all children endured ARVI (7.4±0.62 per year), which were passing protractedly and with complications. The duration of disease made 12.6±0.27 days. In most cases the impairments of respiratory system parts were observed: obstructive bronchitis was encountered in 14 (77.7%) children, pneumonia — in 7 (38.8%).

When studying antenatal medical history, it was detected that in all children under observation a high risk of intrauterine infection was noted. Feto-maternal disease and obstructed labour was detected in 7 cases (38.9%). According to gestational age, 12 children (66.7%) were born carried to full term, in half of them antenatal hypotrophy was observed, 6 children (33.3%) were born premature.

In children with an acute EBV infection course precursory disease symptoms were characterized with adynamia, detention of body weight gain, temperature increase predominantly up to the subfebrile figures, by inflammation of upper respiratory tracts and not infrequently by bronchial obstruction. When studying peripheral blood, we detected anemia, neutropenia, relative lymphocytosis, monocytosis. Atypical mononuclear cells were not detected.

In children with acute Epstein-Barr viral infection course a great variability of clinical manifestations was noted. Hypotrophy of various degrees and delays in neuro-physical development by 1.5-2 epicrisis terms were diagnosed. According to data of ultrasound and X-ray examinations, thymus gland hyperplasia was detected in 35.7% of children, lymphoadenopathy — in 72.2% of cases, long-term Banti syndrome — in 14.2% of cases. Atopic dermatitis manifestations were observed in one third of children. Against the background of acute ARVI manifestations the development of bronchial obstruction syndrome was noted in 14 (77.7%) children (predominantly relapsing course).

During immunological observations of FCIC EBV infected children, the changes were detected both in specific and non-specific components of immune system (Table 3).

Table 1

Criteria of EBV infection course activity

Parameters	**Acute course**	**Chronic course**		**Symptomless course**
		relapse	remission	
DNA	+	+	-	-
IgM	+	+	-	-

IgG	Increasing over time	Increasing over time	+	+

Table 2

Course of infection induced by Epstein-Barr virus, in frequently and chronic ailing children

Parameters	**EBV-infected children from FCIC group, n=18**			
	Acute course, n=4		**Chronic course, n=14**	
	IgM VCA	**IgM VCA and IgG EA**	**IgG EA**	**IgG EA IgG and EBNA**
Number of children	2 (11.1%)	2 (11.1%)	13 (72.2%)	1 (5.6%)

For example, when studying humoral immunity values in children with acute EBV infection course, against the background of decreasing number of B lymphocytes, the average Ig A, M, G values were almost twofold exceeding the age norms. In group of children with chronic EBV infection course the total number of B-cells was slightly higher. Hyper-production of immunoglobulins in EBV-infected children was considered as a reaction to the long-term viral immunization which determined the inflammatory process chronization.

The state of T-cellular component of immunity system was characterized by a significant disbalance in children with acute EBV-infection course. Total number of T-lymphocytes and their subpopulations exceeded the age norms at the twofold decrease of natural killers' production. CD4/CD8 correlation index was decreased and made 1.29±0.19 in the first examined group at 1.75±0.29 in healthy children ($p<0.05$). At the chronic EBV infection course the condition of T-system was more compensated; however, the number of killers was reduced yet, albeit unreliably. Notwithstanding the activity of cellular component of immunity in EBV-infected children, we detected reduction of markers of activation, proliferation and T-lymphocytes differentiation — IL-2, which was especially pronounced at the chronic infection course.

When estimating functional state of phagocytic system of neutrophils, it was observed that the percentage of phagocytising cells in both groups of infected children was significantly below the healthy peers' values. At the same time, in chronic course the activity and absorbing capacity of granulocytes far exceeded the values in children with an acute disease course. Disbalance in percentage ratio between phagocytising cells and phagocytic number attests to phagocytosis system tension at the still sufficient compensation abilities of these functions of neutrophils. Overcuring ability of phagocytising cells, regardless an infection process stage, was decreasing in both groups of children. For example, in first group children the index of phagocytosis completeness made 0.64±0.036, in children with chronic EBV infection course — 0.63±0.06, which was significantly lower than in healthy peers. Incompleteness of phagocytosis, decreasing number of phagocytising cells in the examined children confirms a suspicion on the participation of Epstein-Barr virus in heterophilic leukocytes' apoptosis. Conspicuous is a disbalance of synthesis of pro-inflammatory cytokines, in particular, IL-6, IL-8, TNF — the basic control factors of specific reactions of cellular component of immunity system. The obtained results attest to the prevailing influence of pro-inflammatory cytokines in FCIC EBV-infected children. For example, we established that in major children of first group (75.0%) the levels of cytokines under study were significantly high. At chronic infection process course interleukins production increased by 1.5-2.0 times as compared with the parameters of the first group and control group children.

Table 3

Parameters of immunological status in children infected with Epstein-Barr virus

Immunity	Measurement	Control group,	EBV infected,	EBV infected,

parameters	units	healthy peers, n=15	acute course, n=4	chronic course, n=14
CD3 (T-lymphocytes)	%	55.7±2.32	70.64±3.22*	73.79±5.34*
	109/l	2.55±1.19	2.51±1.17	3.05±0.95
CD4 (T-helpers)	%	29.3±2.72	34.0±4.16*	38.7±5.83*
	109/l	1.31±1.05	1.3±1.27	1.8±1.04
CD8 (1-suppressors)	%	18.4±2.64	29.2±3.61*	22.2±1.84*
	109/l	0.83±0.07	0.98±0.06	1.0±0.10
CD16 (NK-cells)	%	11.81±1.3	7.12±0.49*	10.48±1.34^{x}
	109/l	0.52±0.02	0.18±0.06	0.45±0.05
CD4/CD8		1.75±0.29	1.29±0.19	1.9±0.82
CD20 (B-lymphocytes)	%	17.7±0.89	14.0±1.64*	15.8±0.76*^{x}
	109/l	0.6±0.08	0.4±0.03	0.5±0.06
Ig-A	mg/ml	0.59±0.14	1.25±0.87*	1.06±0.19*
Ig-M	mg/ml	0.74±0.12	1.71±0.24*	1.47±0.34*
Ig-G	mg/ml	6.85±1.19	11.7±0.36*	12.8±0.28*
% of phagocytising cells	%	50.16±3.9	36.0±4.29*	38.9±3.71*
phagocytic number	units	5.25±0.72	5.01±0.57	4.62±0.61
phagocytosis completeness		0.76±0.09	0.64±0.036*	0.63±0.06*
IL-2	pg/ml	14.6±1.5	12.4±0.71	10.9±1.11
IL-6	pg/ml	3.67±1.72	30.32±5.64*	43.87±8.12*
IL-8	pg/ml	5.94±2.46	17.6±3.07*	59.2±5.41*^{x}
TNF	pg/ml	4.28±1.83	41.6±4.94*	60.7±7.23*^{x}
CIC	absorbance units	55.2±4.28	187.6±26.73*	126.8±22.14*^{x}

Note.

*Data are statistically differing from the control group ($p<0.05$)

^{x}Data are statistically differing from the first group ($p<0.05$)

The particularly high and statistically valid parameter of incoordination of the immune system functions was a multiple increase in CIC level. For example, in the first group CIC concentration made 187.6±26.73 of absorbance units (at the age norm of 55.2±4.28). In children with chronic infection course CIC level was somewhat lower, however exceeding normal parameters (126.8±22.14). It was deemed as progression of pathological process due to sedimentation of immune complexes on certain tissues. The development of such an inflammatory reaction in EBV-infected FCIC children presumably stipulates further formation of the cases of chronic diseases, including autoimmune and allergic.

That said, at the early stage of contamination with EBV in children of the first years of life against the background of antigen load the system of immune adaptation gets activated manifesting in the increase of anti-inflammatory cytokines concentration. The long-term persistence of Epstein-Barr virus in a child's body results in the immune breakdown: the pronounced synthesis of anti-inflammatory cytokines being essential in formation of chronic systemic inflammation.

In this regard, a complex of treatment and rehabilitation actions in FCIC children, in particular those who are EBV-infected, should take into account not only correction of the immune conditions of ill children, but also the balance of production of pro- and anti-inflammatory cytokines.

It should be emphasized that no antiviral or other drug among those which are known to date is able to completely eliminate herpesviral infection from the body. In many respects it is conditioned by

the predominance of intracellular existence, impossibility of sufficiently complete penetration of chemotherapeutical agents and difficulty of their neutralization and removal associated therewith. Therefore, therapeutic approach to herpesviral infection should be largely determined by the frequency, degree of exacerbations severity, diseases complications, as well as by a risk of infection transmission. And the objective of treatment actions is a prophylaxis and therapy of infection process exacerbations and complications development.

The method of treatment of children with active infection course induced by Epstein-Barr virus consists in that for children with a detected active course of infection induced by Epstein-Barr virus (acute or exacerbated chronic) with the pronounced clinical manifestations and organs impairments the combined use of non-cyclic nucleosides (Acyclovir, Valtrex), recombinant α-interferon, which enabled improving the condition of immunological reactivity of body at the same time having no impact on the level of interferon participating in the development of autoimmune processes, was indicated.

For the purpose of a long-term viral suppression we use Flavozid — herbal drug developed in Ukraine, which possesses a pronounced antiviral activity (blocking of virus replication), ability to induction of endogenic interferons (α and γ), stimulating action on the functions of tissular macrophages and antioxidant properties.

Methodology of using Flavozid in the complex of EBV infection treatment: children at the age before one year — 0.5 ml 2 times a day, 2-4 weeks; children at the age of 1-2 years — 1 ml 2 times a day, 2-4 weeks; children at the age of 2-4 years — 1.5 ml 2 times a day from the first to third day, 3 ml 2 times a day starting from the fourth day, children at the age of 4-6 years — 3 ml 2 times a day from the first to third day, 4 ml 2 times a day starting from the fourth day; children at the age of 6-9 years — 4 ml 2 times a day from the first to third day, 5 ml 2 times a day starting from the fourth day; children at the age of 9-12 years — 5 ml 2 times a day from the first to third day, 6 ml 2 times a day starting from the fourth day. Duration of treatment — 1 month.

At relapsing course of infection Flavozid therapy courses should be carried out 3-4 times per year, at symptomless — in FCIC group children — two times per year.

Against the background of treatment undertaken, we could reduce acute infection phenomena in 67% of children, shift relapsing course of chronic herpesviral infection in symptomless course — in 64%.

Reference list

1. Nedilko V.P. Ukrainian children health issues. Healthy child: growth, evolution and problems of norm under modern circumstances.// Materials of international scientific-practical conference, Tchernivtsi, 2002, — Pp. 6-7

2. Marushko Yu.V., Moshchych P.S., Sonkin V.M. Frequently ailing children — a challenging issue of pediatrics // Institute of pediatry, obstetrics and gynaecology, 1999. — No. 4. — Pp. 69-70

3. Pochynok T.V., Omelchenko A.I., Chernyshov V.P. Towards formation of secondary immunodeficient diseases in children with infectious syndromes, who frequently endure ARVI.// Institute of pediatry, obstetrics and gynaecology, 1999. — No. 4. — P. 22

4. Andrushchuk A.O., Tiazhka O.V. Towards etiological and pathogenetic factors of the development and course or recurrent respiratory diseases in children.// Institute of pediatry, obstetrics and gynaecology, 1999. — No. 4. — P. 69

5. Chertok T.Ya., Nibsh G. State of health and medical examination of children of tender age. — M.: Medicine, 1987. — 256 p.

6. Sidorova I.S., Cherniyenko I.N. Fetal infections: chlamydiosis, mononucleosis, herpes, cytomegaly.// Russian bulletin of perinatology and pediatrics, 1998. — No. 3. — Pp. 7-13

7. Khakhalin L.N. Human herpesviral diseases (etiology, pathogenesis, principles of diagnostics and ethiopathogenetic therapy). // Russian bulletin of perinatology and pediatrics, 1997. — No. 3. — Pp. 5-36

8. Zheleznikova G.F., Ivanova V.V., Aksenov O.A. The variants of immune response at acute respiratory viral infections in children.// Virology issues, 1999. — No. 6. — Pp. 249-253

9. Infections induced by Epstein-Barr virus, including infectious mononucleosis.// Schooley R. Internal diseases. In 10 books. Book 4. Translation from English/Edited by E. Braunvald et al. — M.: Medicine, 1994. — Pp. 101-109

10. Polyakov V.E., Lyalina V.N., Vorobiova M.L. Infectious mononucleosis in children and adults.// Epidemiology and infectious diseases, 1998. — No. 6. — Pp. 50-55

11. Kovalchuk L.V., Cheredev A.N. New immunopathogenetic views: apoptotic immunodeficiencies.// Immunology, 1998. — No. 6, — Pp. 17-18

12. Zheleznyakova G.F., Vasyakina L.I., Monakhova N.E. Apoptosis and immune response in children with acute infectious mononucleosis.// Immunopathology, allergology, infectology, 2000. — No. 4. — P. 87

13. Zborovskaya A.A., Aleinikova O.V., Kolomiets N.D. Efficacy of recombinant interferon in therapy of chronic EBV infection in children with oncopathology.// Achievements of medical science of Belarus, 1999. — No. 3. — Pp. 12-14

14. Koresev A.V., Artsimovich N.G. Chronic fatigue immune dysfunction syndrome.// Doctor in charge, 1998. — No. 3. — Pp. 18-22