ORIGINAL RESEARCHES

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PROGNOSTIC VALUE OF THE DYNAMICS OF THE CYTOKINES IN EPSTEIN-BARR VIRUS INFECTIOUS MONONUCLEOSIS DEPENDING ON THE AGE OF THE CHILD

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ABSTRACT

Aim of the study is to reveal prognostic value of dynamics of cytokines in children of different age groups with Epstein-Barr viral infectious mononucleosis.

Material and methods: we conducted a study of 98 children with Epstein-Barr viral infectious mononucleosis, including a comprehensive immunological examination. Statistical data processing was carried out in the «Statistica 10».

Results: in children 3-6 years old was recorded the predominance of Th2 immune response, in children 7-11 years old the type of response was mixed - T1/T2, and in the group of 12-17 years old there was a full launch of Th1 response.

Conclusion: changes in the cytokine profile in the children 3-6 years old can be considered as factors explaining the frequent reactivation of EBV. Complex immunological reaction in the children 12-17 years old can be considered the most favorable response, contributing to the elimination of EBV. The immune response of the children 7-11 years old occupied an intermediate position.

Keywords. Epstein-Barr virus, infectious mononucleosis, children, cytokines, lymphocytes, Th1 immune response, Th2 immune response.

Introduction. The reasons for the active study of Epstein-Barr virus infection (EBVI) are the widespread distribution of the virus, a high percentage of infection in the population, the tropism of EBV to immunocompetent cells, a variety of clinical manifestations, a tendency to chronize the infectious process, the possibility of forming of secondary immunodeficiency or malignant tumors [3, 12, 14]. According to some authors, the factor that determines the failure of the immune response in EBVI is the deficiency of synthesis of proinflammatory cytokines TNF-α, IL-8, IFN-γ against the increased production of IL-4 [6]. Mast cells and basophils produce IL-4, which causes differentiation of CD4+Th-0 in Th-2 [4, 8]. IL-4 and IL-10 play an important role in shifting the immune response towards humoral response. It depresses the production of IFN-γ and the activity of IL-2 and leads to inhibition of cell-mediated immune response [2, 13]. Such cytokine shifts cause a restriction of the intensity and prevalence of the inflammatory process in various organs, but violate the processes of sanogenesis. It leads to the development of recurrent, chronic forms of EBVI, WEB-associated lymphoproliferative diseases and autoimmune pathology [1, 7, 10]. Opposite, the favorable course of EBVI is accompanied by an increase the level of α-IFN, γ-IFN, IL-1β and TNF-α, and consequently a shift in the immune response towards Th1 [5, 9, 11]. Thus, it was found that the relation of cytokines in the blood of a child with EBV is an informative criterion for the severity of the course and prognosis of the outcome of the disease. However, comprehensive information concerning the age features of immunological shifts as criteria of prognosis absents in the literature.

Aim of the study is to reveal prognostic value of dynamics of cytokines in children of different age groups with Epstein-Barr viral infectious mononucleosis.

Materials and methods of research. A dynamic prospective cohort study of 98 children with laboratory-confirmed diagnosis of infectious mononucleosis due to primary Epstein-Barr viral infections was conducted. We formed 3 age groups: I group - 3-6 years (n=29), II group - 7-11 years (n=25) and III group - 12-18 years old (n-45). The criteria for exclusion from the study were the absence of markers of active EBV infection: detection of markers of the activity of other herpes viruses; the presence of symptoms of exacerbation of comorbidity; refusal of legal representatives to participate in the study. The control group included 20 immunologically healthy children. Immunological examination of patients was carried out at the 1st and 3rd week of the disease and consisted of determining the concentration of cytokines (IL-1, IL-2, IL-4, IL-6, IL-10, IFNɣ, TNFa). We performed statistical data processing using the application package “Microsoft Office” and “Statistica10”. According to the results of the Shapiro-Wilk criterion, we found that the distribution of some indicators was different from normal. As a result of it, we expressed the values of indicators like the median, 25th and 75th percentiles (Me (C25-C75)). We tested the hypothesis about the equality of two Me using the Wilcoxon test (for dependent samples) and the Man-Whitney test (for independent samples). The critical level of statistical significance (p) was 0.05.

Results and Discussion. Assessment of age-related peculiarities of cytokine profile at the 1st week of the disease revealed that the content of IL-1, the main proinflammatory cytokine, decreased in children 3-6 years and increased in older age groups (PII=0,034). According to some authors, the absence of pronounced stimulation of synthesis IL-1 in patients with IM can be associated with the inhibitory effect of IL-1RA. The EBV induces the production of IL-1RA in neutrophils. It leads to the insufficiency of IL-1 dependent mechanisms of cellular immunity [8]. A similar trend was evidence of delayed reaction of the immune system of children of preschool age on the replication of EBV (Table 1).

The level of the IFN-γ, inducer of the cellular immune response, was higher in all children in the initial period of the disease than the control group. This increase was statistically significant in children aged 3-6 and 7-11 years (pI=0.000; pII=0.02). The level of IL-2, which plays a role in the early proliferation and differentiation of lymphocytes, significantly decreased in children 3-6 years and increased in children 12-17 years (pI=0,000; pIII=0,004). A similar trend was observed for TNF-α, another mediator synthesized by CD4+ LF type 1 (Th-1) (pI=0.000; pIII=0.000; pI-III=0.03)

(table 1).

Consequently, in children under 12 years on the 1st week of IM developed a deficiency of Th1 immune response. It consisted of a significant reduction of Th1 cytokines (IL-2, TNF-a) and increase in the production of cytokine, triggering the processes of differentiation of Th0 B-lymphocytes in Th1 (IFN-ɣ). A full start of Th1 immune response, manifested by an increase in the level of all Th1 cytokines, occurred in children over 12 years (Table 1).

On the state of the Th2 immune response we tried according to the contents of its major cytokines. The level of IL-4 increased in children under 11 years (pI=0.000; pII-III=0.026). IL-6 increased many times in children of all age groups. Maximum level of IL-6 recorded in children older than 12 years (pI=0,000; pII=0,000, pIII=0,007). The content of another important mediator of Th-2 immune response (IL-10) was significantly high in comparison with the control group (pI=0.000; pII=0.000; pIII=0.000) and inversely proportional to the child's age (PI-III=0.042) (table1).

Thus, the maximum level of Th2 cytokines at the 1st week of IM was observed in children 3-6 years. Such shift in the immune response is a risk factor for difficulty eliminating EBV in the acute period of the IM (Table 1).

In the retest of cytokine profile in children 3-6 years was significantly higher content of IL-1 (pI-II=0,020), compared with older age groups. This indicated the absence of subsiding immune inflammation in the body of young children (Table 2).

The level of production of IFN-ɣ, an inducer of Th-1 immune response, was inversely to the age of children (pI=0.037). The content of IL-2 in all groups was significantly smaller compared to the control index (pI=0.000, pII=0.000, pIII=0.000). However, in the dynamics of the disease production of the cytokine increased in children under 12 years old and was significantly reduced in adolescents. The content of TNF-ɣ in different groups changed similarly (pII=0.002; pIII=0.015; pI-III=0.004) (Table 2).

Consequently, at the 3rd week of IM in children 12-17 years old there was a decrease in the activity of mediators of Th1 immune response. Children of 3-6 years old showed features of disbalance in cell-mediated immune response.

The content of IL-4, the key cytokine of Th2 immune response, remained repeatedly elevated in children of all age groups (pI=0,016, pII=0,000). The level of IL-6 decreased in dynamics. At the same time, in children of 7-11 years old this change was statistically significant in comparison with the control group (pI=0.016, pII=0.000). Production of IL-10 decreased inversely to the age of the child. However, the level of this cytokine at the 3rd week of IM was still significantly high (pI=0.000, pII=0.000; pIII=0.008) (Table 2).

As a result, the level and ratio of cytokines on the 3rd week of IM testified to the dominance of Th2 immune response in all groups. At the same time, the continued elevated level of IL-1 in children 3-6 years old indicated the absence of subsiding immune inflammation.

Based on the data obtained, we can conclude that the optimal response of the immune system to EBV infection develops in older children. It provides activation in the early stages of Th1, fast switching to Th2, its dominance at a later term and a gradual subsiding. Insufficiency of the cellular component, excessive activation of the humoral and increased the level of proinflammatory cytokine IL1 during remitting clinical symptoms were detected in children of preschool age. These factors may cause frequent re-activation of EBV in this age group. The type of immune response in children 7-11 years old had an intermediate character.

Conclusion. In children 3-6 years old were revealed a long-term persisting production of proinflammatory cytokines, predominance of Th2 immune response in the early stages of the IM and its excessive activation in the subsiding symptoms period. These changes can be seen as factors that explain the frequent reactivation of EBV in preschool children.

In the group of 12-17 years old the dynamics of cytokine profile consisted of the activation of Th1 immune response, followed by switching to Th2. This response, in our opinion, is the most favorable response of the macroorganism, contributing to the elimination of EBV.

In children 7-11 years old cytokine profile in the initial period of IM characterized by an active production of Th1 and Th2 cytokines and earlier switching to the humoral response. Apparently, such reaction of the immune system is sufficient to deter the active replication of EBV in the period of long-term convalescence.

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Table 1

Cytokine profile of children at the 1st week of EBV IM

Cytokine	Группа							
	control group		I group 3-6 years old		II group 7-11 years old		III group 12-17 years old	
	Me	25% 75%	Me	25% 75%	Me	25% 75%	Me	25% 75%
IL-1	0,29	0,10 0,53	0,0	0,0 0,90	0,87**	0,15 1,59	0,74	0,38 1,72
IFNɣ	6,70	4,20 14,80	10,90***	10,90 20,50	16,60*	3,60 19,80	11,90	0,0 23,80
IL-2	11,85	8,65 15,0	0,0***	0,0 0,0	0,0	0,0 0,0	57,26**	1,50 60,80
TNFα	17,61	3,65 13,53	1,70***	1,10 5,60	*0,40*	0,30 0,90	*61,70***	47,30 72,89
IL-4	0,008	0,0 0,01	0,20***	0,10 0,20	*0,12*	0,02 0,30	*0,0*	0,0 0,20
IL-6	0,004	0,001 0,005	4,60***	4,60 5,30	8,60***	3,85 9,93	14,89**	5,80 18,90
IL-10	1,65	1,23 1,84	31,30***	31,30 36,70	12,41***	10,2 26,96	13,20***	10,44 18,30

Note: * - statistically significant differences with the control group (p≤0.05; p≤0.01; p≤0.001); 1.70 –between I and III groups (p≤0.05); 0.40 - between II and III groups (p≤0.05).

Table 2

Table 2 Cytokine profile of children at the 3rd week of EBV IM

Cytokine	Группа							
	control group		I group 3-6 years old		II group 7-11 years old		III group 12-17 years old	
	Me	25% 75%	Me	25% 75%	Me	25% 75%	Me	25% 75%
IL-1	0,29	0,10 0,53	1,20	0,25 2,05	0,0	0,0 0,0	0,0	0,0 1,20
IFNγ	6,70	4,20 14,80	16,35**	6,40 32,0	10,60	0,0 16,60	5,90	0,0 18,70
IL-2	11,85	8,65 15,0	0,30***	0,0 1,25	1,0***	0,0 2,10	0,0***	0,0 0,30
TNFα	17,61	3,65 13,53	3,65	1,70 49,35	0,20**	0,0 21,50	0,30*	0,0 2,50
IL-4	0,008	0,0 0,01	0,25*	0,0 0,55	0,30***	0,20 0,70	0,14	0,0 0,10
IL-6	0,004	0,001 0,005	4,50**	0,0 18,45	0,0*	0,0 0,30	5,6	0,0 9,0
IL-10	1,65	1,23 1,84	24,30***	15,50 26,65	8,30***	5,0 11,70	5,60	4,73 12,30

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