

# The Research of Dynamics of Immune Responsibility Indicators in Patients with Epstein–Barr Virus (EBV) Infections

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## Abstract

The main task of modern infectious immunology is the elucidation of immunopathogenetic mechanisms of the unfavourable course of the disease. The course of the infectious process caused by the viruses and the microorganisms factors. At the heart of the complicated flow or chronicity of the process is an ineffective immune response that is not able to prevent the dissemination of the virus or the complete elimination of the pathogen from the body, which is the cause of the formation of relapses or chronic course of the disease. In the modern literature, there is evidence that in patients who have suffered from infectious mononucleosis (IM), regardless of the severity of the disease, a secondary immunodeficiency develops, which is the cause of possible bacterial complications. After IM is not always observed update of the immune balance and changes in the hemogram staying for a long time.

**Keywords:** research, dynamics, immune, responsibility, indicators, patients, epstein–barr virus, EBV, infections

## Introduction

Immunological status of patients with IM has a number of features. The essence of the general pattern of changes is to increase the number of T- and B-lymphocytes at the MI, and in the subpopulation of T-lymphocytes, an increase in the number of cytotoxic cells, which makes it possible to regard the IM as a lymphoproliferative process. Increasing the level of T lymphocytes with suppressor activity is one of the main regulatory mechanisms for suppressing the early stages of B-lymphocyte expression, both directly acting on them and indirectly, inhibiting the activation of T-helper cells. In turn, the reduction of T-helper cells leads to blockage induction of apoptosis. Consequently, with IM there is a slowdown in apoptosis of "exhausted" effector cells and there is no impediment to their participation in the immune response. Ultimately, with EBV infection, the probability of occurrence of auto-reactive, as well as

malignant cell clones may appear. A number of researchers note from the side of the humoral link immunity increase the amount of IgA and IgM, which is characteristic of severe forms of MI.

In addition, many researchers are actively studying the relationship between the severity of the course and individual indicators of the immune system. At the primary infection, neutralizing antibodies, antibodies of IgM and IgG antibodies to VCA are formed, and later to EA and NA antigens of EBV. It is believed that the mild IM is associated with an effective immunological defence of the T cell line of immunity and a high level of  $\alpha$ -IFN. The severe course of the infection is due to insufficiency of both the cellular and humoral immunity, accompanied by low concentrations of  $\alpha$ -IFN, the reversal of IFN products from  $\alpha$  to  $\gamma$ -type and violation of the elimination of common CIC.

The number of studies devoted to the study of the nature of immune disorders of laboratory manifestations of IM depending on the stage of the infectious process in adult patients in our country is very limited. It also needs to clarify the diagnostic significance of specific methods of ELISA and PCR in different stages of the infectious process.

**The aim of the study.** To investigate the nature and extent of immune status disorders in patients with acute EBV infection.

**Materials and methods.** To accomplish our research objectives, we examined 60 patients with IM.

The diagnosis of IM in patients under our supervision was based on clinical, anamnestic and laboratory data. All patients have undergone a medium-severe form of IM.

The complex of patients' examination included clinical analysis of blood, detection of atypical mononuclear cells, determination of specific Ig to the EBV by solid-phase immunoassay, detection of the DNA of the EBV by polymerase chain reaction (PCR) in blood and saliva in the dynamics of the disease. In order to confirm the diagnosis, in addition to the general analysis of blood, a series of serological and molecular genetic studies were performed. As a screening rapid blood test for the presence of EBV infection, a heterophilic test in the Hoff-Bauer modification was used (Chirekskina, 1973).

The blood lymphocyte phenotype was determined using a flow-through laser cytometry on a FACS-Calibur (USA) apparatus using monoclonal antibodies (Dumbaeva, 2002; Hanunova, 1999). For identification on the cells of CD3 +, CD4 +, CD8 +, CD16 +, CD20 +, CD25 +, CD8 + CD28 +, CD8 + CD28-, appropriate antibodies labelled with FITC were used. The monoclonal antibodies INF $\gamma$ -RS-5, IL-4-PE, TFR $\beta$ -FITC (eVioscience, Beckman) were used to identify the TF lymphocytes INF $\gamma$  (Th1-cells), IL-4 (Th2-cells) TFR $\beta$ 1 (Th3-cells) (eBioscience, Beckman Coulter, R&D System). All stages of preparation of samples for laser cytofluorometry were

conducted in accordance with the protocols of the manufacturer. The method of simple radial immunodiffusion in a gel was used to study the content of Ig classes A, M, G in serum.

The results of the research were processed by the method of variation and correlation statistics using the program "Statistic 10.0 for Windows". For each variation series, the average arithmetic ( $M$ ), mean square deviation ( $\sigma$ ), average error of the arithmetic mean ( $m$ ) was calculated. Methods of parametric and nonparametric statistics were also used. Quantitative and qualitative analysis of intra-system and inter-system correlation connections was carried out using the method of correlation structures and the sequential analysis of Wald.

### **Results of the research**

The complex analysis of the state of the immune response, its nature and intensity, the balance of the subpopulations of the reactive cells, and the production of immunoregulatory molecules is of great importance in the study of pathogenesis and clinics of the EBV infection, which ultimately helps identify the antiviral strategy of the organism.

The subpopulative composition of the main lymphocytes, as well as the indicators reflecting the state of the humoral immune response - the content of CIC and IgA, M, G was studied in the peripheral blood of patients with IM in the dynamics of the disease - in the period of rapid and convalescence (before discharge from the hospital). The results are shown in table 1.

The analysis of the results of the study on the content of relative and absolute indices of the major subpopulations of lymphocytes revealed the heterogeneity of the content of these immunocompetent cells in the period of the onset of the disease. As can be seen from table 1, the subpopulation composition of lymphocytes in the group of patients with IM was characterized by certain qualitative and quantitative differences compared with the control group.

Thus, during the acute period of IM in the peripheral blood of patients due to high content of leucocytes observed significant increase of the relative and absolute number of some subpopulations of lymphocytes compared with the data of the control group patients.

Thus, in patients with IM, the increase in content [CD3<sup>+</sup> - 87.21±3.34%; CD4<sup>+</sup> - 47.16±1.07%; CD8<sup>+</sup> - 44.16±3.78%; CD16<sup>+</sup> - 16.61±0.6; CD20<sup>+</sup> - 18.91±0.9%; CD8<sup>+</sup>CD28<sup>+</sup> - 17.6±1.1%; CD25<sup>+</sup> - 21.4±1.92%], ( $p<0.05$ ) compared with similar parameters of the control group [CD3<sup>+</sup> - 65.85±3.5%; CD4<sup>+</sup> - 42.0±1.31%; CD8<sup>+</sup> - 29.4±1.9%; CD16<sup>+</sup> - 14.52±0.44%; CD20<sup>+</sup> - 13.5±0.5%; CD8<sup>+</sup>CD28<sup>+</sup> - 14.8±0.9%; CD25<sup>+</sup> - 16.0±1.45%] ( $p<0.05$ ) was exceeded. The increase in Th1-cell content was also observed in probability and was 15.2±0.94% versus 11.1±1.1% ( $p<0.05$ ). The values below the control values were CD8<sup>+</sup>CD28<sup>+</sup>, which did not differ from the control

values –  $5.7 \pm 0.3\%$  ( $p > 0.05$ ), and the Th2-cell content tended to decrease:  $10.4 \pm 1.78$  versus  $12.4 \pm 1.43$  ( $p > 0.05$ ), respectively.

Thus, the study of the phenotypic spectrum of lymphocytes showed an increase in the content of mature T-lymphocytes (CD3<sup>+</sup>), cytotoxic T-suppressor cells (CD8<sup>+</sup>), cells expressing the activation marker CD25<sup>+</sup> (receptor IL-2). The imbalance in the ratio Th1/Th2 ( $p < 0.001$ ), due to an increase in the relative content of Th1-cell Th-lymphocytes, confirms that the effective protection and elimination of the pathogen is formed by transforming the T-helper response toward Th1 cells. In addition, an increase in the number of lymphocytes that carry the receptor to IL-2 (CD25<sup>+</sup>) in the acute period is evidently indicative of activation of the immune system and an increase in the number of cells that respond to IL-2.

In the period of convalescence, a probable reduction was observed only with respect to the relative content of lymphocytes from  $57.67 \pm 2.81$  to  $45.65 \pm 2.32\%$  and  $5.74 \pm 0.65$  to  $3.6 \pm 0.38 \times 10^9$  ( $p < 0,05$ ), the index of CD3<sup>+</sup> was  $87.21 \pm 3.34$  to  $79.21 \pm 2.29\%$  ( $p < 0.05$ ).

**Table 1. Subpopulation of lymphocytes of peripheral blood of patients with MI (M ± m)**

Indicator	IM, the acute period (n=60)	IM, the period of convalescence (n=60)	Control (n=20)
Leukocytes, ( $10^9/\text{l}$ )	$12,7 \pm 0,8$ <sup>1,2</sup>	$6,77 \pm 0,36$	$5,37 \pm 0,18$
Lymphocytes, (%)	$57,67 \pm 2,81$ <sup>1,2</sup>	$45,65 \pm 2,32$ <sup>1</sup>	$30,1 \pm 1,75$
Lymphocytes, ( $10^9/\text{l}$ )	$5,74 \pm 0,65$ <sup>1,2</sup>	$3,6 \pm 0,38$ <sup>1</sup>	$2,55 \pm 0,18$
CD3 <sup>+</sup> -cells, %	$87,21 \pm 3,34$ <sup>1,2</sup>	$79,21 \pm 2,29$ <sup>1</sup>	$65,85 \pm 3,5$
CD4 <sup>+</sup> -cells, %	$47,16 \pm 1,07$ <sup>1</sup>	$44,6 \pm 0,98$	$42,0 \pm 1,31$
CD8 <sup>+</sup> -cells, %	$44,16 \pm 3,78$ <sup>1</sup>	$36,6 \pm 2,7$ <sup>1</sup>	$29,4 \pm 1,9$
CD16 <sup>+</sup> -cells, %	$16,61 \pm 0,6$ <sup>1</sup>	$14,8 \pm 0,46$	$14,52 \pm 0,44$
CD20 <sup>+</sup> -cells, %	$18,91 \pm 0,9$ <sup>1</sup>	$16,11 \pm 0,6$ <sup>1</sup>	$13,5 \pm 0,5$
CD8 <sup>+</sup> CD28 <sup>+</sup> -cells, %	$17,6 \pm 1,1$ <sup>1</sup>	$15,5 \pm 0,9$	$14,8 \pm 0,6$
CD8 <sup>+</sup> CD28 <sup>-</sup> -cells, %	$5,7 \pm 0,3$	$5,3 \pm 0,27$	$5,1 \pm 0,3$
CD8 <sup>+</sup> CD28 <sup>+</sup> /CD8 <sup>+</sup> CD28 <sup>-</sup>	$3,08 \pm 0,3$	$2,9 \pm 0,3$	$2,9 \pm 0,4$
CD25 <sup>+</sup> -cells, %	$21,40 \pm 0,92$ <sup>1</sup>	$19,41 \pm 0,86$ <sup>1</sup>	$16,0 \pm 0,65$
Th1 (INFγ), %	$15,2 \pm 0,94$ <sup>1</sup>	$13,7 \pm 0,98$	$11,1 \pm 1,1$
Th2 (IL-4 <sup>+</sup> ), %	$10,4 \pm 1,78$	$11,3 \pm 1,67$	$12,4 \pm 1,43$
Th1/Th2	$1,46 \pm 0,06$ <sup>1</sup>	$1,21 \pm 0,07$	$0,89 \pm 0,09$

Notes: <sup>1</sup> is a probable difference with the control group ( $p < 0.05$ ), <sup>2</sup> is the probable difference with the parameters of the convalescence period ( $p < 0.05$ ).

Other clusters of lymphocytes tended to decrease the content compared to those at the acute period of the IM ( $p > 0.05$ ) and did not differ with probability compared with

the data of the convalescence period ( $p > 0.05$ ). It should be noted that in this period, the probable differences with the control data had a relative content of lymphocytes of  $45.65 \pm 2.32$  versus  $30.1 \pm 1.75\%$  and  $3.6 \pm 0.38$  versus  $2.55 \pm 0.18 \times 10^9$  ( $p < 0.05$ ); CD3<sup>+</sup> levels were  $79.21 \pm 2.29$  versus  $65.85 \pm 3.5\%$  ( $p < 0.05$ ); CD8<sup>+</sup> –  $36.6 \pm 2.7$  versus  $29.4 \pm 1.9\%$  ( $p < 0.05$ ); CD20<sup>+</sup> –  $16.11 \pm 0.6$  versus  $13.5 \pm 0.5\%$  ( $p < 0.05$ ) and CD25<sup>+</sup> –  $19.41 \pm 0.86$  versus  $16.0 \pm 0.65\%$  ( $p < 0.05$ ).

It should be noted that in this period the distribution of the content of the indicators according to the control data was characterized by diversification, which in our opinion confirms a rather slow regression of clinical symptoms in the MI and the existence of an imbalance in both the acute period and the period of convalescence.

## Conclusions

1. In patients with IM in the period of the acute period of the disease, probable violations from the cellular immunity level, characterized by an increase in the number of cells with killer activity: mature T-lymphocytes (CD3<sup>+</sup>), cytotoxic T-suppressor cells (CD8<sup>+</sup>), cells expressing activation marker CD25<sup>+</sup> (receptor IL-2) and a sharp drop in Th1/Th2.
2. In the period of convalescence of the IM in a greater number of patients, the prevalence of the Th2-type immune response was detected, indicating a prolonged period of convalescence and predisposition to chronic disease.

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