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TYPES OF IMMUNE RESPONSE FOR VARIOUS ESTHTEIN-BARR FORMS OF VIRAL INFECTION

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In 321 patients with different forms of EBV infection in the age range from 19 to 57 years (mean age 33,1 ± 11,7 years) different types of immune response were isolated and studied. All participants in the study were divided into groups of comparable sex and age: patients with infectious mononucleosis (n = 138); patients with various forms of chronic EBV infection (n = 183); clinically healthy volunteers (n = 20). During the study all ethical norms were observed in accordance with international and Ukrainian protocols. Clinical examination of patients and healthy volunteers included examining complaints, an epidemiological history, a history of illness and life, an objective examination, instrumental and laboratory studies in dynamics. Statistical processing of the results of the study was carried out by parametric and nonparametric methods using the program Statistika 6.0, for each variational series, the absolute values (n), the arithmetic mean (M), the mean error of the arithmetic mean (m) were calculated. It was found that patients with different forms of EBV infection have a reliable cytokine imbalance. Four main types of immune response were identified: normoreactive, dissociative, hyporeactive and hyperreactive. The revealed types of immune response testify to inadequate cellular-humoral reactivity of the organism in conditions of prolonged persistence of EBV, which is manifested by a tendency to suppress cell-mediated and enhancing humoral mechanisms of the immune response and is reflected in the clinical and biochemical manifestations of the disease and leads to a protracted undulating course of the disease.

KEY WORDS: Epstein-Barr virus, types of immune response, course of the disease

ТИПИ ІМУННОЇ ВІДПОВІДІ ПРИ РІЗНИХ ФОРМАХ ЕПШТЕЙНА-БАРР ВІРУСНОЇ ІНФЕКЦІЇ

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На 321 пацієнті з різними формами ВЕБ-інфекції у віковому діапазоні від 19 до 57 років (середній вік 33,1 ± 11,7 років) були виділені і вивчені різні типи імунної відповіді. Всі учасники дослідження були розділені на зіставні за статтю та віком групи: пацієнти з інфекційний мононуклеоз (n = 138); пацієнти з різними формами хронічної ВЕБ-інфекції (n = 183); клінічно здорові добровольці (n = 20). В ході роботи були дотримані всі етичні норми згідно з міжнародними і українськими протоколами. Клінічне обстеження пацієнтів і здорових добровольців передбачало вивчення скарг, епідеміологічного анамнезу, анамнезу захворювання і життя, об'єктивний огляд, інструментальні та лабораторні дослідження в динаміці. Статистична обробка результатів дослідження проводилася параметричними і непараметричних методами з використанням програми Statistika 6.0, для кожного варіаційного ряду розраховували абсолютні значення (n), середнє арифметичне (M), середню помилку середнього арифметичного (m). Встановлено, що пацієнти з різними формами ВЕБ-інфекції мають достовірний цитокіновий дисбаланс. Було виділено чотири основних типи імунного реагування: нормореактівний, діссоціативний, гіпореактивний і гіперреактивність. Виявлені типи імунного реагування свідчать про неадекватну клітинно-гуморальної реактивності організму в умовах тривалої персистенції ВЕБ, що проявляється схильністю до пригнічення клітинно-опосередкованих та посилення гуморальних механізмів імунної відповіді і відображається в клініко-біохімічних проявах хвороби і призводить до затяжного хвилеподібний перебіг захворювання.

КЛЮЧОВІ СЛОВА: вірус Епштейна-Барр, типи імунної відповіді, перебіг хвороби

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ТИПЫ ИММУННОГО ОТВЕТА ПРИ РАЗЛИЧНЫХ ФОРМАХ ЭПШТЕЙНА-БАРР ВИРУСНОЙ ИНФЕКЦИИ

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На 321 пациенте с различными формами ВЭБ-инфекции в возрастном диапазоне от 19 до 57 лет (средний возраст 33,1 ± 11,7 лет) были выделены и изучены различные типы иммунного ответа. Все участники исследования были разделены на сопоставимые по полу и возрасту группы: пациенты с инфекционным мононуклеозом (n = 138); пациенты с различными формами хронической ВЭБ-инфекции (n = 183); клинически здоровые добровольцы (n = 20). В ходе работы были соблюдены все этические нормы согласно международным и украинским протоколам. Клиническое обследование пациентов и здоровых добровольцев предусматривало изучение жалоб, эпидемиологического анамнеза, анамнеза заболевания и жизни, объективный осмотр, инструментальные и лабораторные исследования в динамике. Статистическая обработка результатов исследования проводилась параметрическими и непараметрическими методами с использованием программы Statistika 6.0, для каждого вариационного ряда рассчитывали абсолютные значения (n), среднее арифметическое (M), среднюю ошибку среднего арифметического (m). Установлено, что пациенты с различными формами ВЭБ-инфекции имеют достоверный цитокиновый дисбаланс. Было выделено четыре основных типа иммунного реагирования: нормореактивный, диссоциативный, гипореактивный и гиперреактивный. Выявленные типы иммунного реагирования свидетельствуют о неадекватной клеточно-гуморальной реактивности организма в условиях длительной персистенции ВЭБ, что проявляется склонностью к подавлению клеточно-опосредованных и усилением гуморальных механизмов иммунного ответа и отображается в клинико-биохимических проявлениях болезни и приводит к затяжному волнообразному течению заболевания.

КЛЮЧЕВЫЕ СЛОВА: вирус Эпштейна-Барр, типы иммунного ответа, течение болезни

INTRODUCTION

The relevance of the Epstein-Barr virus infection (VEB) is due to a high degree of infection of the population not only in Ukraine but worldwide, since specific antibodies to this virus are detected in almost 95 % of the adult population. Specific tropism of VEB to immunocompetent cells, systemic damage to internal organs, a wide range of clinical forms of the disease, and the absence of specific prevention is the subject of research by many scientists [1–2]. Thus, many clinical forms of VEB (tumor and non-tumor) have been described, in which the virus plays the role of an etiological factor: chronic active EBV infection; X-linked lymphoproliferative disease; nasopharyngeal carcinoma; Burkett's lyphoma; Hodgkin's disease; lymphoproliferative disease [2].

It has been established that VEB has a large set of genes, which allows it to escape to a certain extent from the human immune system. In particular, VEB generates proteins – analogues of a number of human interleukins and their receptors that change the immune response [3–4]. In addition, VEB is highly mutually beneficial, which allows him for a certain time to avoid exposure to specific immunoglobulins and cells of the host's immune system.

The prognosis of the outcomes of VEB infection depends on the presence and severity of immune dysfunction, the genetic predisposition to certain VEB-associated diseases, as well as on the presence of a number of external factors damaging the immune system.

A number of studies have shown that the predominant part of cytokines produced by Th-2 lymphocytes, is associated with long-term viral persistence and chronic process, while activation of the Th-1 type - with spontaneous recovery from acute forms of VEB [5–7]. It is of interest to identify and study the production of basic regulatory cytokines with the establishment of immunological response types in patients with VEB infection.

OBJECTIVE

The aim of the study was to study the dynamics of multidirectional synthesis of cytokines (IL-1β, TNF-α, IL-6, IL-2, IL-4 and IL-10) and to determine the types of immune response for predicting the clinical course of

the disease in patients with various forms of VEB-infection.

MATERIALS AND METHODS

The work was carried out on the basis of the Department of General and Clinical Immunology and Allergology of the Medical Faculty of the V. N. Karazin Kharkiv National University in 2009–2015.

The study involved 321 patients with VEB infection, the average age was 33,1 ± 11,7 years. Based on the purpose of the study, all patients were conditionally divided into the following groups: the first group - patients with infectious mononucleosis (IM, n = 138) with laboratory-proven signs of primary viral infection; the second group includes patients with various forms of chronic VEB infection (n = 183), among them: serous meningitis (n = 8), chronic tonsillitis (n = 32), nonspecific lymphadenopathy (n = 48), prolonged subfebrile condition n = 54), reactive arthritis (n = 16), chronic fatigue syndrome (n = 25). The comparison group consisted of 20 clinically healthy young people with no signs of acute or any chronic pathology, the mean age was 24.1 ± 3.2 years.

During the study, the provisions of the Helsinki Declaration of the World Medical Association, the ethical code of the doctor of Ukraine were observed, in addition, all participants received informed consent.

During the study, complaints, an epidemiological history, a history of the disease and life, an objective examination, instrumental and laboratory methods of investigation, as well as detection of the presence of atypical mononuclear cells, detection of specific antiviral antibodies (VCA-IgM, EA-IgM and EBNA-IgG) in the blood serum by ELISA (IBL, Germany) and Vector-Best (RF), the detection of VEB DNA by polymerase chain reaction (PCR) in the blood and saliva, the activity of aspartic and alanine transferase (AsAT, AlAT), lactate dehydrogenase (LDH) and creatinine phosphate kinase (CKF), fibrinogen in the course of the disease were assessed. To confirm the diagnosis as a screening express blood test for the presence of VEB, a heterophile test was used in the Hoff-Bauer modification (Chireshkina N. M. 1973). In a part of the patients, serological examinations for the herpes simplex virus type 1 + 2 (HSV-1 + 2), cytomegalovirus (CMV), toxoplasma, hepatitis viruses (A, B and C), HIV were performed for differential diagnostics. For this, anti-CMV-IgM, anti-toxo-IgM, anti-HAV-IgM, HBsAg, anti-HBc-total and anti-HIV-1 + 2 total test systems were used.

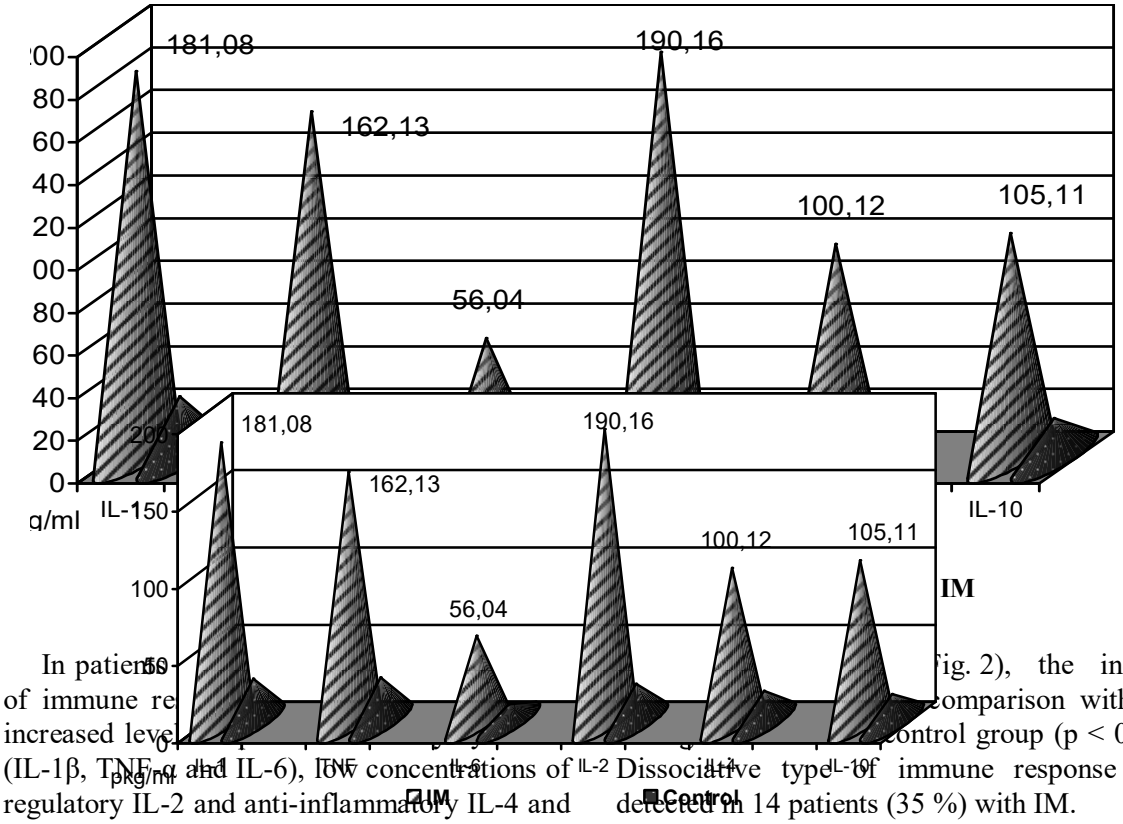
Molecular genetic studies included the determination of VEB replicative activity based on detection of DNA in the blood serum by PCR, in addition serum concentrations of the cytokines studied were determined: IL-1β, IL-6, IL-6, IL-2, IL-4, IL- 10, using the manufacturer's instruction with the use of a ELISA. Technical analysis was carried out in the clinical diagnostic laboratory of the Kharkov Regional Clinical Infectious Diseases Hospital and the Sinevo Medical Laboratory.

The statistical processing of the results of the study was carried out by parametric and nonparametric methods using the Statistika 6.0 for Windows program (Stat Soft Inc, USA) on a PC with a Pentium II Celeron 850 PPGA processor. For each variational series, the absolute values (n), the arithmetic mean (M), the mean error of the arithmetic mean (m) were calculated.

RESULTS AND DISCUSSION

Analysis of the dynamics of the cytokine profile in patients with VEB showed multidirectional changes in the synthesis of the investigated proinflammatory and anti-inflammatory cytokines, which was the basis for the establishment of four types of immune response: I – normoreactive type (significant increase in proinflammatory and anti-inflammatory cytokines), II – dissociative (high rates of pro-inflammatory cytokines background of low values of regulatory IL-2 and anti-inflammatory cytokines), III – hyporeactive (low concentrations of inflammatory and anti-inflammatory cytokines) and IV – hyperreactive (high concentrations of both pro-inflammatory and anti-inflammatory cytokines).

Analysis of proinflammatory (IL-1β, TNFα, IL-6), regulatory (IL-2) and anti-inflammatory cytokines (IL-4, IL-10) production data in patients with IM with normoreactive type of immune response (Fig. 1) revealed reliable increase in all the studied parameters in 5,2–7,7 times ($p < 0.05$) in comparison with the control levels. This type of immune response was detected in 17 patients (42.5 %) with IM.



In patients [illegible] (Fig. 2), the indices of immune re[illegible] comparison with the increased leve[illegible] control group ($p < 0.05$). (IL-1β, TNF-α and IL-6), low concentrations of regulatory IL-2 and anti-inflammatory IL-4 and Dissociative type of immune response was detected in 14 patients (35 %) with IM.

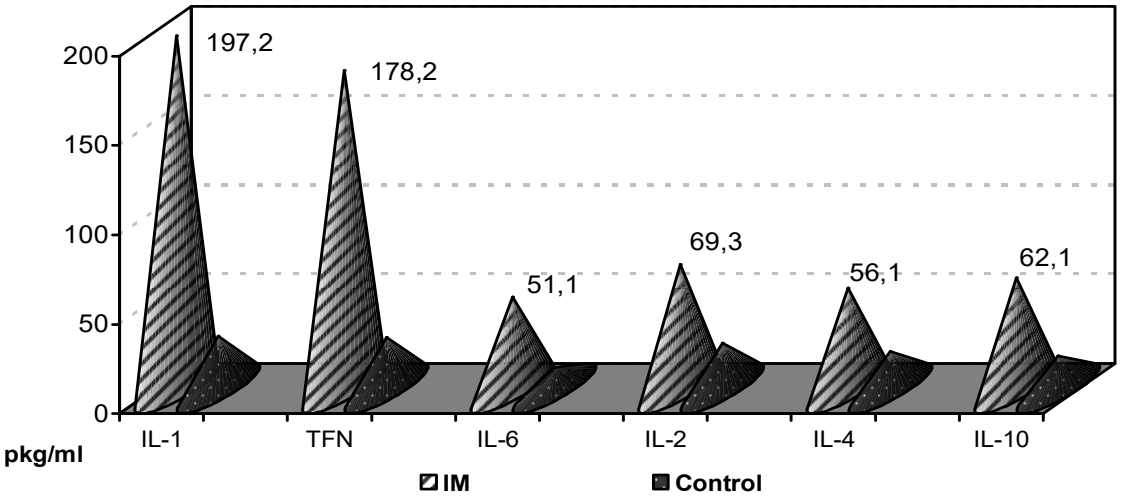


Fig. 2. Dissociative type of immune response in patients with IM

The presented results of the study with a hyperreactive type of immune response in patients with IM (Fig. 3) were characterized by reliably high levels of the studied parameters in comparison with similar parameters in patients with normoreactive type (on average in 1,5–2 times) and control group data (on average in 8–10 times) ($p < 0.05$). This type was detected in 22,5 % (9 patients) with IM.

When comparing the severity and duration of the main clinical and biochemical indices in patients with IM with different types of immune response, we found some differences presented in Table 1.

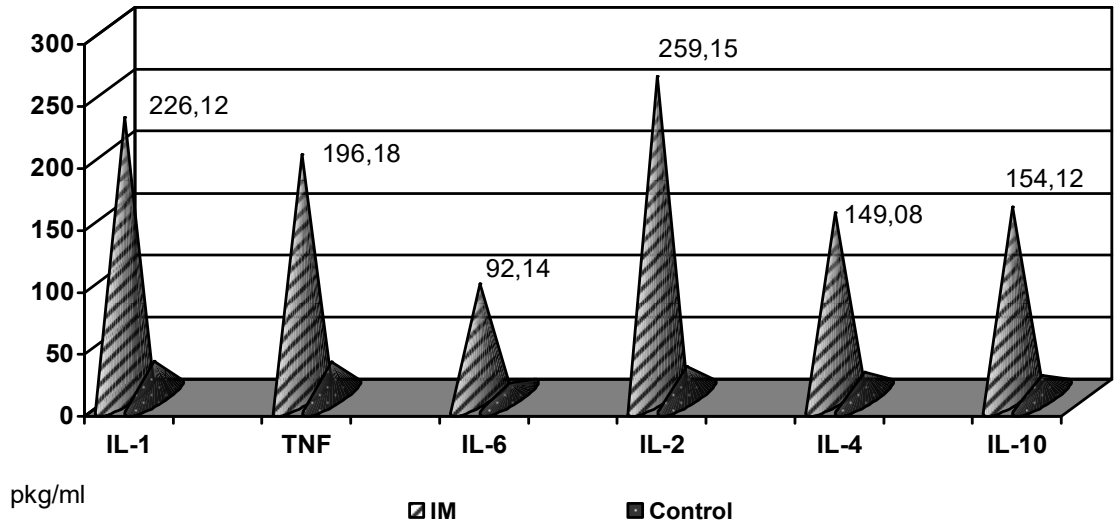


Fig. 3. Hyperreactive type of immune response in patients with IM

Table 1

The duration of individual clinical symptoms, depending on the type of immune response in patients with MI (n = 40)

Clinical symptoms	Duration of symptoms for different types of immune response (M ± m) days		
	Normoreactive type n = 17	Dissociative type n = 14	Hyperreactive type n = 9
General weakness	7,1 ± 1,3	9,9 ± 1,8 [1,2]	8,6 ± 1,2 [3]
Fever	8,4 ± 1,4	13,9 ± 2,1[1, 2]	10,9 ± 1,8 [3]
Headache	5,8 ± 1,7	6,4 ± 1,8	6,2 ± 1,3
Sleep disturbance	6,9 ± 1,4	8,2 ± 2,2	7,4 ± 1,5
Nausea	4,8 ± 1,7	5,7 ± 1,2	5,6 ± 1,3
Pain in the throat	5,4 ± 1,8	6,9 ± 1,7 [1]	6,2 ± 1,5
Lymphadenopathy	10,7 ± 1,2	18,9 ± 1,5 [1,2]	14,8 ± 1,1 [3]
Hepatolienal syndrome	9,5 ± 2,1	12,9 ± 1,8 [1,2]	10,8 ± 2,2
Bed-days	11,9 ± 1,8	15,9 ± 1,4 [1,2]	12,9 ± 1,7

Note: 1 – p < 0.05 between normoreactive and dissociative types of immune response; 2 – p < 0.05 between dissociative and hyperreactive types of immune response; 3 – p < 0,05 between normoreactive and hyperreactive types of immune response.

As can be seen from the presented data, in patients with IM with established normoreactive type of immune response, the main clinical symptoms were characterized by a shorter duration than in the patients with dissociative type and hyperreactive type against the background of antiviral therapy.

Thus, the general weakness in patients with dissociative type of immune response lasted 9,9 ± 1,8 days, whereas in patients with normoreactive and hyperreactive type – 7,1 ± 1,3 and 8,6 ± 1,2 days, respectively ($p < 0.05$). The duration of the fever was also longer in patients with a dissociative type of immune response – 13,9 ± 2,1 compared with the rates of patients with normoreactive and hyperreactive type – 8,4 ± 1,4 and 10,9 ± 1,8 days, respectively ($p < 0.05$). The duration of intoxication symptoms in the form of headache, sleep disturbances and nausea was not statistically significant between groups ($p > 0.05$). The presence of pain in the throat was more prolonged in patients with dissociative type of immune response 6,9 ±

1,7 days ($p < 0.05$) compared with those of patients with normoreactive and hyperreactive type – 5,4 ± 1,8 and 6,2 ± 1,5 days, respectively. Significant differences were also observed in the duration of lymphadenopathy in patients with a dissociative type of immune response of 18,9 ± 1,5 days ($p < 0.05$) compared with the data of the 1st and 3rd groups, with the groups also differing among the groups reliability. The duration of hepatolienal syndrome was significantly higher with a dissociative type of immune response – 12,9 ± 1,8 days ($p < 0.05$), compared with other groups. The length of stay of patients with MI on inpatient treatment also was longer in patients with dissociative type of immune response – 15,9 ± 1,4 days ($p < 0.05$), compared to similar data of patients with normoreactive and hyperreactive type – 11,9 ± 1,8 and 12,9 ± 1,7 days, respectively.

Levels of concentrations of the studied parameters in patients with chronic forms of EBV infection are presented in Fig. 4 and 5.

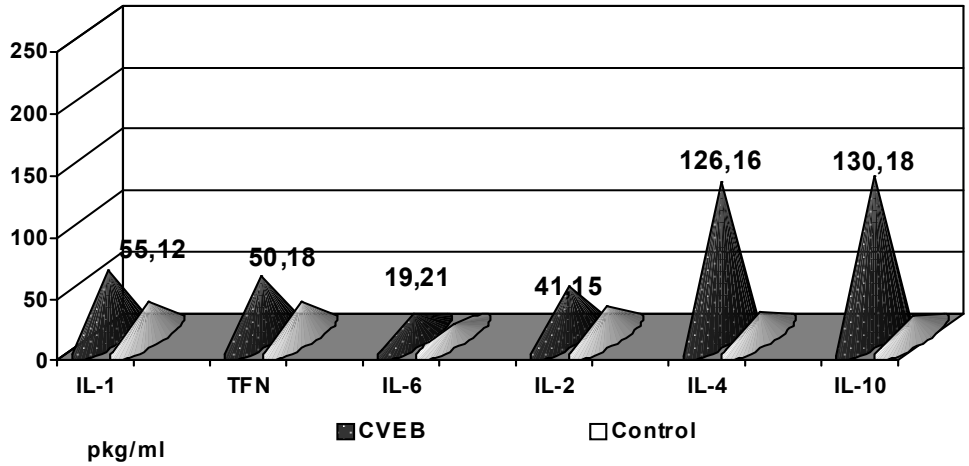


Fig. 4. Dissociative type of immune response in patients with HVEB infection

Thus, in patients with a dissociative type of immune response, low production of pro-inflammatory cytokines and regulatory IL-2 was observed, whereas levels of anti-inflammatory IL-4 and IL-10 significantly increased in accordance with the activity of the process. The level of IL-4 exceeded in 5.7 times the parameters of the control group, and IL-10 was 6 times higher than the mean values of the control group ($p < 0.05$).

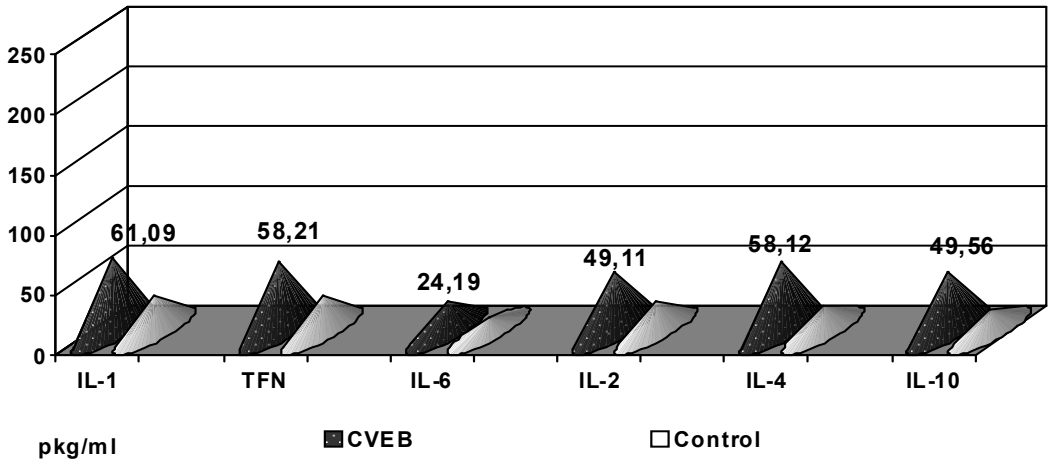


Fig.5. Hypoactive type of immune response in patients with HVEB infection

Among the patients with HVEB with the revealed hyporeactive type of immune response, the synthesis of proinflammatory and anti-inflammatory cytokines was characterized by low concentrations, the indices were practically within the values of the control group and did not differ statistically ($p > 0.05$).

The established types of immune response to the clinical course of HVEB are given in Table. 2.

Table 2

The duration of the main clinical symptoms, depending on the type of immune response in patients with HVEB (n = 80)

Clinical symptoms	Duration of symptoms for different types of immune response (M ± m) days	
	Hyporeactive type n = 33	Dissociative type n = 47
General weakness	5,6 ± 1,2	10,1 ± 1,3*
Arthralgia, myalgia	4,6 ± 0,9	8,9 ± 1,0*
Lymphadenopathy	4,8 ± 1,1	9,7 ± 1,2*
Subfebrile condition	5,9 ± 1,8	10,4 ± 1,4*
Hepatolienal syndrome	18,8 ± 2,2	26,5 ± 2,0*

*Note: * - p <0.05 between the hypotreactive and dissociative type of immune response.*

As can be seen from the presented data, in patients with HVEB with established hyporeactive type of immune response, the main clinical symptoms were characterized by a shorter duration than in the patients with a dissociative type against the background of antiviral therapy.

Thus, the general weakness in patients with a dissociative type of immune response lasted 10,1 ± 1,3 days, whereas in patients with a hyporeactive type it was 5,6 ± 1,2 days, respectively ($p < 0.05$). The phenomena of arthralgia and myalgia were also less pronounced in a group of patients with a hyporeactive type of immune response and were 4,6 ± 0,9 and 8,9 ± 1,0 days, respectively. The duration of peripheral lymphadenopathy was also lower in patients with a hyporeactive type of immune response: 4,8 ± 1,1, compared with data in patients with dissociative type 9,7 ± 1,2 ($p < 0.05$). The subfebrile condition was significantly shorter in patients with a hyporeactive type of immune response (5,9 ± 1,8 days) than in the dissociative type (10,4 ± 1,4 days), ($p < 0.05$). Hepatolienal syndrome was the longest clinical symptom in HVEB patients with both hyporeactive (18,8 ± 2,2 days) and dissociative (26,5 ± 2,0 days) immune response ($p < 0.05$).

Despite the significant achievements of modern medicine, many questions about the nature of the cytokine-producing ability of immunocompetent cells and their immunopathogenetic characteristics in HVEB infection are still unclear, and the literature data do not contain unambiguousness and sufficient justification.

Studies of recent years [5–7] found that the cytokine spectrum in VEB infection depends on the balance of the immune response of the body. Most researchers agree that the predominant participation of cytokines produced by Th-2 lymphocytes is associated with viral persistence and process chronization, and Th-1 with spontaneous recovery and elimination of the pathogen.

We found that in patients with VEB four types of immune response are observed: normoreactive, dissociative, hyporeactive and hyperreactive. These types of immune response testify to inadequate cellular-humoral reactivity of the organism in conditions of long-term persistence of EBV, which is manifested by a tendency to suppress cell-mediated and enhancing humoral mechanisms of the immune response and is reflected in the clinical and biochemical manifestations of the disease and leads to a protracted undulating course of the disease.

CONCLUSIONS

In patients with different forms of EBV infection, there is a significant cytokine imbalance. In patients with VEB, four types

of immune response are observed: I – normoreactive type (significant increase in proinflammatory and anti-inflammatory cytokines); II – dissociative (high proinflammatory cytokines against low values of regulatory IL-2 and anti-inflammatory cytokines); III – hyporeactive (low concentrations both pro-inflammatory and anti-inflammatory cytokines) and IV – hyperreactive (high concentrations of both pro-inflammatory and anti-inflammatory cytokines). The established types of immune response testify to the inadequate cellular-humoral reactivity of the organism under conditions of prolonged persistence of EBV, which is manifested by a tendency to suppress cell-mediated and amplified humoral mechanisms of the immune response and is reflected in the clinical and biochemical manifestations of the disease and leads to a protracted undulating course of the disease.

PROSPECTS FOR FUTURE STUDIES

Interesting and promising are the studies aimed at drug correction of the revealed disorders with established types of immune response in patients with HVEB and studying the influence of the latter on the outcomes of the disease, the development of complications and the activity of the process, which will be the subject for our further study.

REFERENCES

1. Vozianova Zh. I. Infectious mononucleosis as a polyethiological disease / Zh. I. Vozianova, A. I. Glay // Modern infections. – 2004. – No. 2. – P. 37–41.
2. Isakov V. A. Herpesvirus infections of man: a guide for doctors. / V. A. Isakov, E. I. Arkhipova, D. V. Isakov. – St. Petersburg, 2006. – 303 p.
3. Cen O. Latent Membrane Protein 2 (LMP2) / O. Cen, R. Longnecker. // Curr Top Microbiol Immunol. – 2015. – No. 391. – P. 151–180.
4. Fish K. Epstein-Barr virus latent membrane protein 2A enhances MYC-driven cell cycle progression in a mouse model of B lymphoma. / K. Fish, J. Chen, R. Longnecker. // Blood. – 2014. – No. 123. – P. 530–540.
5. Ketlinskiy S. A. Tsitokiny / S. A. Ketlinskiy, A. S. Simbirtsev. – Sankt-Peterburg: OOO «Foliant», 2008. – 552 s.
6. Krasnitskaya A. S. Immunologicheskiye aspekty khronicheskogo tonzillita, assotsiirovannogo s virus Epshteyna-Barr infektsiyey / A. S. Krasnitskaya, N. A. Borovskaya. // Fundamental'nyye issledovaniya. – 2012. – S. 299–305.
7. Uroven' syvorotochnykh tsitokinov pri limfoproliferativnykh zabolevaniyakh / N. P. Domnikova, E. E. Petrusenko, O. V. Reshetnikov, S. L. Ryzhikova, N. A. Varaksin // Novosti «Vektor-Best». – 2010. – No. 2 (56). – S. 4–7.