МЕДИЦИНСКИ УНИВЕРСИТЕТ – СОФИЯ

ЦЕНТРАЛНА МЕДИЦИНСКА БИБЛИОТЕКА

ОБЩА МЕДИЦИНА

GENERAL MEDICINE

Списанието се обработва във:
EBSCO
Scopus
Excerpta Medica
БД Българска медицинска литература

Обща мед.
Obshta med.

ТОМ XXI • 2019 • БРОЙ 6

СЪДЪРЖАНИЕ

CONTENTS

ОБЩА МЕДИЦИНА 6/2019

ISSN 1311-1817 УДК 611

Организационен секретар: *Св. Цветанова*
Стилова редакция *Д. Танчева*
Корекция: *В. Цъклева*
Редакция на англ. резюмета: *В. Колев*
Страниране: *О. Маркова*

Реклама – *В. Герчева – GSM: 0888 282 422, e-mail: vania_gercheva@abv.bg*

Подписана за печат на 29.11.2019 г.

Централна медицинска библиотека
1431 София, ул. "Св. Г. Софийски" № 1

✆ 952 16 45, 952 23 93
e-mail: svetlamu@mail.bg

THE EFFECT OF STREPTOCOCCUS ON THE NASAL AND OROPHARYNGEAL MUCOSA TO THE CYTOKINE RESPONSE OF CHILDREN WITH INFECTIOUS MONONUCLEOSIS

O. Guz, S. Kuznetsov

Kharkiv National Medical University – Kharkiv, Ukraine

ВЛИЯНИЕ НА СТРЕПТОКОКУС НА НАЗАЛНАТА И ОРОФАРИНГЕАЛНАТА ЛИГАВИЦА ВЪРХУ ЦИТОКИНОВИЯ ОТГОВОР ПРИ ДЕЦА С ИНФЕКЦИОЗНА МОНОНУКЛЕОЗА

Е. Гуз, С. Кузнецов

Харковски национален медицински университет – Харков, Украйна

Abstract. *The article presents the results of a comparative reaction of interleukins of blood in children with infectious mononucleosis (IM) in different periods of the disease, with the presence and absence of dissemination of streptococcus on the nasal and oropharyngeal mucosa. To achieve the goal, 60 children aged from three to seven years with infectious mononucleosis, who were treated at the Regional Children's Infectious Clinical Hospital of Kharkiv, Ukraine, were examined. Infectious mononucleosis was confirmed by positive results of disease markers by ELISA (anti-EBV IgM and IgG) and PCR (detection of EBV DNA in the blood). The immune status of patients was assessed by indicators of the levels of interleukins 1β, 4, TNFα. In 30 children (the first group), Streptococcus pyogenes was isolated in concentrations of 10^{-5} and higher during bacteriological examination of the nasal and oropharyngeal mucosa. The second group (30 children) – streptococcus was not detected on the nasal and oropharyngeal mucosa. Analysis of the study results found that the presence in patients of streptococcus on the nasal and oropharyngeal mucosa in the acute period of infectious mononucleosis leads to a more pronounced reaction of pro-inflammatory interleukins in their blood and inhibition of the response of anti-inflammatory interleukin 4 ($P < 0.05$). Later, there was a slow decrease in the levels of interleukin 1, TNF-α and inhibition of the increase in the content of IL-4. We believe that determining the levels of interleukins may allow us to predict the severity and course of the disease in the early stages of the disease. Ct can also serve as a promising direction for improving the treatment of patients and, therefore, reducing adverse outcomes.*

Key words: *children, infectious mononucleosis, immunity, interleukin, streptococcus, symptoms*

Резюме. *В статията са представени резултатите от сравнителна реакция на кръвните интерлевкини при деца с инфекциозна мононуклеоза (ИМ) в различни периоди на заболяването, с наличието и отсъствието на разпространение на стрептококи на носната и орофарингеалната лигавица. За целта са изследвани 60 деца на възраст от 3 до 7 години с инфекциозна мононуклеоза, лекувани в Областна детска инфекциозна клинична болница в Харков, Украйна. Инфекциозната мононуклеоза беше потвърдена от положителни резултати от маркери на заболяването чрез ELISA (анти-EBV IgM и IgG) и PCR (откриване на EBV ДНК в кръвта). Имунният статус на пациентите се оценява чрез показатели за нивата на интерлевкини 1β, 4, TNFα. При 30 деца (първа група) се изолира Streptococcus pyogenes в концентрации от 10^{-5} и по-високи по време на бактериологично изследване на носната и орофарингеалната лигавица. При втората група (30 деца) не се установява стрептококус върху лигавицата на носа и орофаринкса. Анализът на резултатите от проучването показва, че наличието при пациентите на стрептококи на носната и орофарингеалната лигавица през острия период на*

инфекциозна мононуклеоза води до по-изразена реакция на провъзпалителни интерлевкини в кръвта им и до инхибиране на противовъзпалителния отговор на интерлевкин-4 (P < 0.05). По-късно се наблюдава бавно намаляване на нивата на интерлевкин-1, TNF-α и инхибиране на увеличаването на съдържанието на IL-4. Ние смятаме, че определянето на нивата на интерлевкините може да ни позволи да предвидим тежестта и хода на заболяването през неговите ранни стадии. Също така то може да послужи като обещаваща посока за подобряване на лечението на пациентите и следователно за намаляване на нежеланите последствия.

Ключови думи: *деца, инфекциозна мононуклеоза, имунитет, интерлевкин, стрептококи, симптоми*

Introduction

The urgency of the problem of infectious mononucleosis (IM) is determined by the high level of infection of the children's population with the Epstein-Barr virus (EBV), the possibility of developing an unfavorable course of the disease and the formation of prolonged immunosuppression with deficiency of T-cell and phagocytic immunity [1].

The specific tropism of the Epstein-Barr virus (EBV) to immunocompetent cells, systemic damage of internal organs, a wide range of clinical forms of the disease and the absence of specific prevention are the subject of research by many scientists [4]. Today, infectious mononucleosis (IM) is considered a disease of the immune system [7]. Active proliferation of the virus leads to structural changes in many organs and a decrease in the response of the immune system [9].

The main functions of the immune system are the recognition and elimination of foreign macromolecules [13]. When the antigen, including bacteria and viruses, penetrates and affects the human body, the phagocytic and T-cell systems of the immune system are activated and, as a result, the cytokines – interleukins, which determine the severity of the inflammatory process [10]. The cells of the phagocytic system produce IL-1β, IL-6, TNF-α, which are called pro-inflammatory mediators [2]. The cytokines are linked to the receptors of the immune cells; the activation of the genetic system of the cell takes place and the synthesis of new proteins and intracellular reactions begins, which leads to the proliferation and differentiation of the immune cells [5]. Pro-inflammatory mediators have a wide range of activating effects, including a cascade of pathological reactions: effects on the thermoregulatory center, activation of the lymphocytic link, increased activity of neutrophils, stimulation of fibrinolysis, stimulation of pro-coagulation activity, synthesis of acute proteins of the liver cells, stimulation of hematopoiesis, activation of phagocytes [6]. This leads to the activation of oxygen secretion, leukotrienes; the penetration of capillaries, the increased influx of circulating neutrophils and macrophages into the site of infection [11]. TNF-α is synthesized in the focus of acute inflammation of T-LF and B-LF, NK-cells, monocytes/macrophages. It induces the activation of neutrophils and macrophages, as well as their chemotaxis. In macrophages, under the influence of TNF-α, the synthesis of growth factors (colony stimulating factors), interferon-γ, interleukins (IL-1, IL-8), and prostaglandins increases. Together with IL-1, TNF-α induces the synthesis of cells of the acute phase proteins by the cells of the mononuclear phagocytic system of the liver. The described effects of TNF-α have a protective effect, because they promote the phagocytosis of pathogenic microorganisms by activated neutrophils and macrophages. TNF-α induces the activation and proliferation of T-LF, causes the death of tumor and other morphologically altered cells (infected with viruses, bacteria, parasites) [14]. Interleukin-4 (IL-4) is one of the most important anti-inflammatory biological regulators. According to the direction of action, it does not belong to classical pro-inflammatory cytokines and is characterized by a broad spectrum of action on B-cells, T-cells, thymocytes, macrophages, hematopoietic progenitors, eosinophils, neutrophils and epithelial cells [17]. IL-4 contributes to the inhibition of the TNF-α, IL-1β, IL-6, IL-8 production by monocytes/macrophages, leading to a weakening of the excessive effect of inflammatory mediators on the body and to the simultaneous activation of the humoral response [16]. IL-4, like other components of the cytokine network, has an important role in maintaining cellular homeostasis (between blood cells, connective tissue, epithelial and endothelial cells, etc.). It is assumed that the combination of action and the balance between the effects of pro-inflammatory (IL-1β, TNF-α, etc.) and anti-inflammatory cytokines (IL-4, etc.) underlies the development and result of the infectious process, and also determines the severity of course and prognosis. [12].

At the same time, the results of the authors who studied the reaction of blood interleukins in children

with IM are very controversial. Some researchers believe that with this disease, the level of pro-inflammatory interleukins in the debut of the disease remains within the age norm [19]. Others indicate high numbers in their blood [15]. At the same time, they observe a slight increase in their level in the dynamics of the disease, the latter – about maintaining their high content with a mathematically slightly significant tendency to decrease towards the recovery period [20]. Contradictory opinions can be seen also in the analysis of literature data on the content of anti-inflammatory cytokines in children with IM during the course of the disease. M.V. Antonova (2018) demonstrates low rates of the IL-4 reaction at the onset of the disease [3]. At the same time, K. Rostgaard (2014) indicates an increase in the level in the acute period [18].

The above is the argument of the expediency of further studying the reaction of blood interleukins in patients with IM, which can ensure increased knowledge on the pathogenesis of the disease and improve methods for predicting the course of the disease and treatment.

Many authors believe that not only EBV, but also bacteria on the mucosa of the nasopharynx play an important role in the formation of the clinical picture and the immune response [14]. Thanks to studies in recent years, it has been found that 60-80% of children with healthy nasal and oropharyngeal mucosa receive streptococcus [8].

There are few works in the literature that consider the effect of streptococcus on the formation of the cytokine immune response of patients, the clinical picture of the disease, its course and outcomes.

Purpose of the study: to determine the value of streptococcus on the mucous membrane of the nose and oropharynx in the reaction of interleukins in blood in children with mononucleosis.

Materials and methods

The study included 60 children aged three to seven years with mononucleosis, who were treated in the Regional Children's Infectious Clinical Hospital, Kharkiv, Ukraine. The diagnosis of IM was verified based on the positive results of the search for disease markers by ELISA (anti-EBV IgM and IgG) and PCR (detection of EBV DNA in the blood). In 30 children (the first group), Streptococcus pyogenes was isolated in concentrations of 10^{-5} and higher during bacteriological examination of the nasal and oropharyngeal mucosa. In the second group (30 children), streptococcus was not detected on the nasal and oropharyngeal mucosa. Antistreptolysins were not detected in the blood of children of the first group. The immune status of patients was assessed by indicators of the levels of interleukins 1β, 4, and TNFα. Determination of the content of interleukins in the blood of children with mononucleosis in children was carried out in the acute period (1-2 days of the disease) and the period of early convalescence (7-10 days). As a comparison, we took the corresponding indicators of 30 healthy children of the same age and sex. Statistic processing of the obtained results was carried out using computer programs Excel and Statistica 6.0. The reliability of the difference of values was revealed using Student's criterion and Fisher's method. Differences were considered significant at a significance level of $P < 0.05$.

Results and Discussion

All children showed characteristic signs of activation of the mechanisms of interleukin response in the acute period of mononucleosis. The content of pro-inflammatory IL-1β in the serum in the debut of the disease in patients of both groups was significantly higher than in healthy children ($P1 < 0.05$, $P2 < 0.05$). In children with additional infection of the streptococcus of the nasal and oropharyngeal mucosa, the concentration of IL-1β was 18.66 ± 1.19 ng/ml and was significantly higher than the corresponding indicators of the second group – 9.8 ± 0.91 ng/ml ($P3 < 0.05$).

The period of the onset of mononucleosis is accompanied by a significant increase in the blood of patients with TNF-α compared with the content in healthy children ($P1 < 0.05$, $P2 < 0.05$), while the level of pro-inflammatory TNF-α in patients of the first group was significantly higher than similar indicators of children of the second group ($P3 < 0.05$).

When studying the levels of IL-4 in the blood serum of children of the studied groups, an increase in the content was found in comparison with healthy children, however, a significant difference in the content was determined only in terms of the children of the second and control groups ($P2 < 0.05$). And although in patients of the first group there was an increase in the level of IL-4 in the blood, it was less significant than in children of the second group ($P3 < 0.05$).

All of the above indicates that in children with IM with and without the presence of streptococcus on the mucosa of the nose and oropharynx, similar changes in cytokine homeostasis are observed in the form of an increase in the acute period of the disease levels of pro-and anti-inflammatory interleukins. However, the degree of this increase depends on the presence of streptococcus. In children, on whose mucous membrane of nasopharynx is detected streptococcus, the reaction of pro-inflammatory

Table 1. The content of interleukins in the blood of children with IM at the onset of the disease (M ± m)

Interleukins: ng/ml	First group (n = 30)	P1	Second group (n = 30)	P2	Control group (n = 30)	P3
IL-1β	18.66 ± 1.19	< 0.05	9.80 ± 0.91	< 0.05	5.32 ± 1.73	< 0.05
IL-4	3.66 ± 0.24	> 0.05	5.40 ± 0.30	< 0.05	2.06 ± 0.94	< 0.05
TNF-α	11.20 ± 1.43	< 0.05	6.90 ± 1.12	< 0.05	2.69 ± 1.67	< 0.05

*Notes: P1 – the first group relative to the control group; P2 – reliability of indicators of children of the second and control groups; P3 – reliability of indicators of children of the first and second groups

Table 2. The level of interleukins in the blood in children with IMin the period of early reconvalescence (M ± m)

Interleukins: ng/ml	First group (n = 30)	P1	Second group (n = 30)	P2	Control group (n = 30)	P3
IL-1β	15.03 ± 1.28	< 0.05	8.9 ± 0.94	> 0.05	5.32 ± 1.73	< 0.05
IL-4	4.60 ± 0.85	> 0.05	6.30 ± 0.62	< 0.05	2.06 ± 0.94	> 0.05
TNF-α	7.9 ± 0.42	< 0.05	5.6 ± 0.49	> 0.05	2.69 ± 1.67	< 0.05

*Notes: P1 – the first group relative to the; P2 – reliability of indicators of children of the second and control groups; P3 – reliability of indicators of children of the first and second groups.

interleukins is more significant, with a less significant reaction of anti-inflammatory interleukin-4. This can be explained by keeping the inflammatory process at the minimal, subclinical level of the local nasopharynx focus due to streptococcal invasion, as a result of which the level of pro-inflammatory interleukins starts from their increased content, and the anti-inflammatory ones are inhibited due to inhibition of cellular factors of immunity.

The clinical picture of the acute period in children with IM with present Streptococcus pyogenes at concentrations of 10^{-5} and higher on the nasal and oropharyngeal mucosa was characterized by a higher body temperature response, more pronounced intoxication and morphological changes in the tonsils, a much more pronounced increase in regional (submandibular, cervical) lymph nodes, liver and spleen. The blood of such patients included significantly lower content of lymphocytes and higher content of neutrophils. However, what we have suggested requires further study.

In the children of the studied groups, a decrease in the levels of IL-1β, TNF-α was found, which was more significant in the children of the second group (P3 < 0.05), where no mathematical difference of interleukins in the blood in comparison with healthy children was established (P2 > 0.05).

At the same time, in the children of the first group, the level of pro-inflammatory interleukins remained at high numbers for the reconvalisation period (P1 < 0.05).

The content of anti-inflammatory interleukin-4 in the blood of children to the recovery period was higher than in the acute period. However, in the recovery period only in the children of the second group a significant difference in the levels of IL-4 compared with health indicators was found (P2 < 0.05).

In the children of the first group, the course of the disease was longer (17.56 ± 1.56 and 9.12 ± 1.26, P < 0.05).

In our opinion, this is due to the additional antigenic effects of streptococcus even at the local level in the children of the first group, as we discussed above.

Our findings suggest that in the acute period of infectious mononucleosis in the observation groups, an increase in the level of pro-inflammatory IL-1β, TNF-α in the serum (P1 < 0.05, P2 < 0.05) is found. In the patients of the first group, this increase was more significant and significantly different in comparison with the patients of the second group (P3 < 0.05). In the dynamics of infectious mononucleosis in children without background infection, a rapid and statistically significant decrease in the levels of proinflammatory cytokines was observed, whereas in the children who had isolated streptococcus in concentrations 10^{-5} and above from the naso- and oropharyngeal mucosa, a continued maintenance of elevated levels of proinflammatory cytokines continued (P1 < 0.05, P3 < 0.05).

In the period of early convalescence in children of the second group, higher levels of serum IL-4 were detected compared with patients with a background infection. The established absence of significant changes in the content of IL-4 in children of the first group at different periods of the disease may indicate a disbalance in the cytokine-mediated mechanisms of inflammation regulation. The detected disbalance of cytokine response in patients, in whom

a nasal and oropharyngeal streptococcus was isolated from the mucosa at concentrations of 10^{-5} and higher, causes a deficiency in the compensation of cytokine homeostasis, and therefore it may be one of the factors that maintains inflammation and supports the pathological state.

Thus, the identified disbalance of the body's immune system of a child with infectious mononucleosis, depending on the presence and absence of streptococcus on the mucous membrane of the nasopharynx, leads to changes in the clinical picture of IM and affects the duration of the disease.

Conclusions

1. Analysis of the results of examination of children of the compared groups found that the presence in patients of streptococcus on the nasal and oropharyngeal mucosa in the acute period of infectious mononucleosis leads to a more pronounced reaction of proinflammatory interleukins in their blood and inhibition of the response of anti-inflammatory interleukin-4 ($P < 0.05$).

2. Later, there is a slow decrease in the levels of interleukin - 1, TNF-α and inhibition of the increase in the content of IL-4.

3. Streptococcus pyogenes in concentrations of 10^{-5} and higher on the mucous membrane of the nose and oropharynx in a child with mononucleosis can contribute to the occurrence of adverse outcomes of the disease.

Prospects for the future: the results of the study revealed the importance of the microbial flora of the nasal and oropharyngeal mucosa in the formation of the cytokine response in children with mononucleosis, which allows us to predict the severity and course of the disease in the early stages of the disease. This may be a promising direction for improving the treatment of patients and reducing adverse outcomes.

The study was conducted strictly in accordance with the Helsinki Declaration after the approval of the Regional Ethics Committee of the Kharkiv National Medical University.

Conflict of Interest: The authors declare no conflict of interest.

References

1. Abbott RJ, Quinn LL, Leese AM et al. CD8+ T cell responses to lytic EBV infection: late antigen specificities as subdominant components of the total response. J Immunol. 2013;191:5398–5409.

2. Adeishvili PS, Polesko IV, Osipov GA,et al.. Issledovanie mikrobiotsenoza rotoglotki metodom mass-spektrometrii mikrobnyih markerov u detey s infektsionnyim mononukleozom. Detskie infektsii. 2012; 11(1):12-16.

3. Antonova MV. The dynamics of the immune response in Epstein-Barr viral infectious mononucleosis in children. Med Sci Educat Urals, 2018, 19(1) (93), 21-28.

4. Albegova, BZ, Revazova AB, Tadeeva SH. Infektsionnyiy mononukleoz assotsiirovannyiy s virusom Epshteyna-Barr u detey. Vladikavkazskiy mediko-biologicheskiy vestnik. 2014, 20(30): 64-67.

5. Ali AS, Al-Shraim M, Al-Hakami AM, Jones IM. Epstein-Barr Virus: Clinical and Epidemiological Revisits and Genetic Basis of Oncogenesis. Open Virol J. 2015 Nov 3. 9:7-28.

6. Azzi T, Lunemann A, Murer A et al. Role for early-differentiated natural killer cells in infectious mononucleosis. Blood, 2014;124:2533-2543.

7. Balfour HH, Jr, Odumade OA, Schmeling DO et al. Behavioral, virologic, and immunologic factors associated with acquisition and severity of primary Epstein-Barr virus infection in university students. J Infect Dis, 2013;207:80-88.

8. Bobruk S. V. The degree of indicators level violation of local immunity in children with infectious mononucleosis. J Educat, Health and Sport, 2017;7(3):576-585.

9. Chijioke O, Muller A, Feederle R et al. Human natural killer cells prevent infectious mononucleosis features by targeting lytic Epstein-Barr virus infection. Cell Rep, 2013;5:1489-1498.

10. Cunha BA, Petelin A, George S. Fever of unknown origin (FUO) in an elderly adult due to Epstein-Barr virus (EBV) presenting as "typhoidal mononucleosis," mimicking a lymphoma.Heart Lung, 2013 Jan-Feb. 42(1):79-81.

11. Dunmire SK, Hogquist KA, Balfour HH. Infectious Mononucleosis. Curr Top Microbiol Immunol, 2015, 390 (Pt 1):211-40.

12. Engelmann I, Nasser H, Belmiloudi S et al. Clinically severe Epstein-Barr virus encephalitis with mild cerebrospinal fluid abnormalities in an immunocompetent adolescent: a case report. Diagn Microbiol Infect Dis, 2013 Jun. 76(2):232-4.

13. Kramarev SO, Vygovskaya OV. Infectious Mononucleosis in Children: Features of Modern Clinic, Immunogenesis, Treatment // Health of Ukraine, 2016; 36 (1):17-25.

14. Krasnov MV, Stekolschikova IA , Borovkova MG, Andreeva LV. Infektsionnyiy mononukleoz u detey Sovremennyie problemyi nauki i obrazovaniya, 2015, 2(Part 2). https://science-education.ru/ru/article/view?id=18371.

15. Langer-Gould A, Wu J, Lucas R et al. Epstein-Barr virus, cytomegalovirus, and multiple sclerosis susceptibility: A multiethnic study. Neurology, 2017 Sep 26. 89 (13):1330-1337.

16. Lyadova TI, Volobueva OV, Gololobova OV et al. Types of the immune response in various forms of Epstein-Barr virus infection. Int Med J, 2017, (1):70-76.

17. Rickinson AB, Fox CP. Epstein-barr virus and infectious mononucleosis: what students can teach us. J Infect Dis, 2013 Jan. 207(1):6-8.

18. Rostgaard K, Wohlfahrt J, Hjalgrim H. A genetic basis for infectious mononucleosis: evidence from a family study of hospitalized cases in Denmark. Clin Infect Dis, 2014 Jun. 58(12):1684-9.

19. Tso KK, Yip KY, Mak CK et al. Complete genomic sequence of Epstein-Barr virus in nasopharyngeal carcinoma cell line C666-1. Infect Agent Cancer, 2013;8(1):29.

20. Filatova EN, Anisenkova EV, Presnyakova NB et al. Antiapoptoticheskoe deystvie retseptora cd95 v naivnyih cd8 t-limfotsitah u detey s ostryim infektsionnyim mononukleozom. Infektsiya i Immunitet. 2016; 6(3):207-218.

✉ *Address for correspondence:*
Guz Olena, Phd student
Department of Pediatric Infectious Diseases
Kharkiv National Medical University
Avenue Heroes of Stalingrad № 160
Kharkiv, Ukraine
tel. + 380-950028637
e-mail: guz.elena.doctor@gmail.com