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ФАКТОРЫ ВРОЖДЕННОГО И АДАПТИВНОГО ИММУНИТЕТА В ПАТОГЕНЕЗЕ ВНУТРИУТРОБНОЙ ГЕНЕРАЛИЗОВАННОЙ ЦИТОМЕГАЛОВИРУСНОЙ ИНФЕКЦИИ

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Резюме. Цель: роль факторов врожденного и адаптивного иммунитета в развитии внутриутробной генерализованной цитомегаловирусной инфекции.

Под наблюдением находилось 47 новорожденных детей с врожденной генерализованной цитомегаловирусной инфекцией, которые составили I группу. На основании данных клинико-лабораторного обследования все исследуемые новорожденные были разделены на две подгруппы. Подгруппу 1.1 (29 человек) составили новорожденные с тяжелой формой ЦМВИ и подгруппу 1.2 (18 человек) – новорожденные со среднетяжелым течением ЦМВИ. В контрольную группу вошли 26 новорожденных без герпесвирусной инфекции. Определение количества моноцитов, экспрессирующих Toll-рецепторы (TLR), проводили методом лазерной проточной цитометрии (Beckman Coulter) с использованием реагентов Beckman Coulter, HyCultBiotechnology: FITC-CD282$^+$, CD284$^+$, CD286$^+$ и PE-CD14$^+$. Концентрацию IFNγ, IFNα, IL-6, IL-8 в сыворотке крови новорожденных определяли методом ИФА с использованием тест-систем BenderMedsistems.

Внутриутробная генерализованная ЦМВИ с полной клинической симптоматикой у новорожденных характеризовалась снижением количества моноцитов, экспрессирующих TLR-2 и TLR-6, что было сопряжено со снижением уровня IFNα, IFNγ, повышением уровня IL-6, IL-8 и MCP-1. Подгруппа с неполной клинической симптоматикой ЦМВИ характеризовалась снижением уровня IFNα, в сочетании с повышением уровня IL-6. Выявленные иммунные нарушения приводят к редукции противовирусного иммунного ответа, определяют тяжесть заболевания у перинатально инфицированных новорожденных.

Ключевые слова: врожденный иммунитет, TLR-рецепторы, цитокины, внутриутробная инфекция, цитомегаловирус, новорожденные дети

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FACTORS OF CONGENITAL AND ADAPTIVE IMMUNITY IN THE PATHOGENESIS OF INTRAUTERINE GENERATED CYTOMEGALOVIRUS INFECTION

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Abstract. Subject: to assess a role of factors of innate and adaptive immunity in the development of intrauterine generalized cytomegalovirus infection.

The study included 47 newborns with congenital generalized cytomegalovirus infection comprising group I. Based on the data of clinical and laboratory examination, all newborns studied were divided into two subgroups. Subgroup 1.1 (29 subjects) consisted of newborns with severe CMVI and subgroup 1.2 (18 subjects) – newborns with moderate CMVI. The control group included 26 newborns without herpesvirus infection.

Determination of the number of monocytes expressing Toll receptors (TLR) was performed by laser flow cytometry (Beckman Coulter) using Beckman Coulter, HyCultBiotechnology reagents: FITC-CD282$^+$, CD284$^+$, CD286$^+$, and PE-CD14$^+$. The newborn serum concentration of IFNγ, IFNα, IL-6, IL-8 was determined by ELISA using BenderMedsistems test systems.

Intrauterine generalized CMVI with complete clinical symptoms in newborns was characterized by a decrease in the number of monocytes expressing TLR-2 and TLR-6, which was associated with a decrease in the level of IFNα, IFNγ, an increase in the level of IL-6, IL-8 and MCP-1. The subgroup with incomplete clinical symptoms CMVI was characterized by a decrease in the level of IFNα, in combination with an increase in the level of IL-6. The identified immune disorders lead to a reduction in the antiviral immune response and determine the severity of the disease in prenatally infected newborns.

Keywords: innate immunity, TLR receptors, cytokines, intrauterine infection, cytomegalovirus, newborn children

Introduction

Intrauterine infectious pathology in newborns is recorded in 11 to 45% of deaths as well as in 14-18% of stillbirths.

Herpesvirus infections hold a leading place among the causes of premature birth, infant mortality, and morbidity in newborns, being one of the most difficult problems to solve in modern medicine. It was shown that in the structure of infant mortality, 10 to 61% cases result from intrauterine infections, among which cytomegalovirus infection (CMVI) prevails [3]. In newborns, cytomegalovirus infection may be also characterized by generalization of the process and accompanied by the DIC development. Often, newborns recovered after CMVI have persistent neurological symptoms by the end of the first year of life, development of early disability being the most important medical and social problem of modern perinatology.

The development and severity of intrauterine infection in neonates is determined by the type of pathogen, its virulence, duration of infection, tropism of the pathogen to the organs and tissues of the fetus, maternal protective reserves, fetal potential to mount immune response [6] as well as reduced T-cell functional activity underlying the pathogenesis of severe forms of cytomegalovirus infection [2].

Taking into account the activation and regulation of adaptive immune response depends on the functioning of the innate immune system, a large number of studies has been recently carried out to study Toll-like receptors (TLR), which ligation by microbial factors leads to inflammatory response mediated by the production of cytokines, which further determines the nature, modality and intensity of body adaptive immune response [7]. In the resting state, non-activated TLRs are found monomers on the cell membrane. After the TLR interacts with the ligand, certain genes are activated and expressed to ensure destruction of invading pathogens.

As a result of TLR activation, a wide range of biological reactions occurs – from induced production of proinflammatory cytokines and interferons ensuring execution of innate immunity reactions, to the expression of co-stimulating molecules which promote activation of T-lymphocytes and stimulate development of adaptive immune response [4].

In recent years, evidence has been accumulated that defects in the TLR system such as decreased expression and function due to mutations or epigenetic disorders in TLR regulation underliedevelopment of immunodeficiency states, the implementation and generalization of infection.

It is evident that characteristics of immunological disorders in neonatal CMVI characterized by suppressed immune response should be throughly investigated.

Objective: the role of innate and adaptive immunity factors in the development of intrauterine generalized cytomegalovirus infection.

Materials and methods

The study included 47 newborns with congenital generalized cytomegalovirus infection comprising group I. Based on the data of clinical and laboratory examination, all newborns were divided into two subgroups. Subgroup 1.1 (29 subjects) consisted of newborns with a severe CMVI and subgroup 1.2 (18 subjects) – newborns with moderate CMVI. The control group included 26 newborns without herpesvirus infection.

Determination of the number of TLR expressing monocytes was performed by flow cytometry (Beckman Coulter) by using HyCultBiotechnology reagents: FITC-CD282$^+$, CD286$^+$ and PE-CD14$^+$. The level of serum IFNγ, IFNα, IL-6, IL-8 was determined by ELISA (Bender MedSistems).

Quantity of CMV DNA in neonatal blood and urine of newborns was assessed by real-time PCR (Corbet-Researc, Australia). The determination of specific IgG and IgM antibodies against cytomegalovirus (CMV) in the blood serum of newborns was carried out by enzyme immunoassay using reagents from the "Vector-Best" company (Novosibirsk).

Analyzing statistical significance of differences between the study groups, the Mann–Whitney and Wilcoxon test was used. The correlation coefficient was calculated according to Spearman test. The statistical significance level (p) in our study was set at 0.05.

Results and discussion

The analysis of the conducted studies showed that all children in group I were born to women with burdened obstetric and gynecological history, from high-risk pregnancies, so that we analyzed anamnestic data of mothers and paired newborns in the surveyed subgroups.

Comparative analysis of gynecological diseases revealed that the history of endocervicitis in the mothers of paired children from both subgroups of group I was revealed at significantly higher rate than in the control group (by 24.1 and 22.2%, respectively). Similar changes were found while studying the frequency of detected adnexitis (27.6 and 27.7%, respectively).

Mothers of the examined children from both subgroups of group I vs. control group had significantly higher rate of previous of the urinary system diseases (58.6 and 55.6%, respectively), diseases of the gastrointestinal tract (48.3 and 44.4%, respectively). From the anamnesis of mothers of subgroup 1.1 it was found that 17.2% of the examined previous pregnancies were completed with non-developing pregnancies, 34.5% – spontaneous miscarriage at age of 5-11 weeks, which was significantly higher than in the control. A history of premature birth was noted in 31.5% of women.

The leading place in the pathology of pregnancy and childbirth in mothers from subgroups 1.1 and 1.2 was held by threatened termination of pregnancy (48.3% and 44.4%, respectively), which occurred significantly more often than in the control, early gestosis) – 34.5 and 33.3%, respectively). Polyhydramnios was a characteristic clinical symptom in mothers from subgroup 1.1, which was more common than in the control group and subgroup 1.2; similar data were also found for the early maturation of the placenta.

When analyzing the clinical symptoms in the examined newborns with cytomegalovirus infection, it was revealed that in the subgroup with severe CMVI it had significantly higher rate of hepatitis than the subgroup with moderate disease (37.9 and 5.6%, respectively), whereas pneumonia (51.7 and 11.1%, respectively), hemorrhagic syndrome (44.8 and 11.1%, respectively); meningoencephalitis and myocarditis were diagnosed only in newborns with severe generalized CMVI (6.9 and 10.3%, respectively). All newborns with cytomegalovirus infection had perinatal CNS lesion.

Studying the mechanisms of innate immunity found that only newborns with CMVI in subgroup 1.1 had decreased number of monocytes expressing TLR-2 (CD282$^+$), compared with subgroup 1.2 and the control group (45.4±8.9%, 65.1±6.1% and 76.2±5.6%, respectively). Similar changes were shown for TLR-6 (CD14$^+$CD286$^+$) (44.6±5.9%, 57.8±2.2% and 61.1±1.4%, respectively). Signaling from TLR-2 is the main factor in the activation of T- and B-lymphocytes.

Interferon production including interferon alpha and gamma in newborns with cytomegalovirus infection was significantly decreased. Owing to interferons, cells become resistant to the virus. A decrease in the level of interferon inevitably leads to significantly lower response against invading viral agent. Insufficient activation of a specific antiviral response occurs, which further may be one of the factors in developing severe form of the disease. In this regard, the study of the cytokine system as regulators of inflammation processes is of great interest.

During the study we found a direct correlation between the number of TLR-2 expressing monocytes and level of IFNγ ($r = 0.60$, $p < 0.05$).

When conducting a study of the level of interferon alpha, it was found that in both subgroups it was significantly decreased compared to the control (8.3±0.9 pg/ml and 10.7±1.1 pg/ml *versus* 15.9±2, 1 pg/ml, respectively), with the peak decrease in subgroup 1.1 (8.3±0.9 pg/ml), which may be associated with lowered number of monocytes expressing TLR-2 in such newborns.

It should be noted that the level of IFNγ in group 1.1 was significantly decreased (42.1±9.7 pg/ml) both compared with group 1.2 and control group. A decrease in the production of IFNα and IFNγ, which can be both a cause and a consequence of acute and chronic viral diseases, indicates a congenital or acquired deficiency in the interferon system and can be considered a marker of severe generalized cytomegalovirus infection.

Taking into account that interleukin 6 participates as a cofactor in the maturation and differentiation of B-lymphocytes, we studied its level in the newborn cohort. It was found that during severe generalized CMVI, the amount of IL-6 was significantly higher than the value of the control group (25.8±5.2 pg/ml *versus* 9.0±1.6 pg/ml. disease, the concentration of IL-6 was also higher than in the control group (18.3±4.2 pg/ml and 9.0±1.6 pg/ml, respectively).

IL-8 is a major chemokine involved in neutrophil activation, apoptosis, chemotaxis, and migration of immune cells during inflammation and antiviral response. In the peripheral blood serum of healthy newborns, the level of IL-8 averaged 26.3±7.7 pg/ml, whereas in subgroup 1.1 it reached 71.3±5.8 pg/ml, being higher by 2.7-fold than in control group.

MCP-1 is a potent chemoattractant for monocytes/macrophages and activated T-lymphocytes, produced by many cell types. MCP-1 production is initiated by inflammatory mediators: IL-1 , TNFα, IFNγ, and lipopolysaccharides (LPS). After analyzing the data obtained, it was revealed that in subgroup II the content of MCP-1 was significantly higher than the level of the control group (1083.2±258.4 pg/ml *versus* 600.5±53.9 pg/ml) compated to newborns in subgroup I.2, where no change in the level of MCP-1 was observed compared with the control group.

Infectious diseases in newborns are the most important medical and social problem of modern perinatology, which is due to the severity of the course of the disease and high frequency of complications. An increase in the frequency of fetal and neonatal infections is caused by a prominent spread of infection, including cytomegalovirus, in the urogenital tract in pregnant women, which in turn results in complicated adaptation during the neonatal period.

Innate immunity plays an important role in protecting newborns from infection because in the first month of life there is no immunological memory and the ability to develop specific antibodies to pathogens is significantly reduced. Therefore, protection against infection in newborns mainly depends on innate immunity and its humoral component.

Cytomegalovirus is able to evade immune control, including impaired both the quantitative composition of immune cells and their synthetic function. Our analysis of immunological parameters showed that the formation of cytomegalovirus infection was accompanied by impaired immunity in newborns. The subgroup with severe CMVI and full clinical picture was characterized by decreased in the number of monocytes expressing TLR-2 and TLR-6, decreased level of IFNα, IFNγ, increased level of IL-6, IL-8, and MCP-1. In the subgroup with moderate CMVI, there was a decrease in the IFNα content in combination with an increase in the IL-6 level.

The consequence of decreased count of monocytes expressing TLRs may result in no proper response against invading virus, prolonged persistence and chronicity of infection, because our data showed that the number of monocytes expressing TLRs correlates with the cell activity. Such monocytes are incapable of adequate activation – sensing and signal transmission after infection; suppressed production of pro-inflammatory cytokines which affects the timely activation of antigen-specific arm in the immune system. Consequently, the observed decrease in the level of monocytes expressing TLR-2 and TLR-6 can account for the high sensitivity to CMV and is consistent with the data [5] on the participation of TLR in the formation of generalized CMVI.

Cytokine production forms the basis for the immune response and has a direct effect on the mechanisms of immunoresistance in cytomegalovirus infection.

In our study, an increased amount of IL-6 was found in subgroups with varying degrees of CMVI severity. We hypothesized that these changes can lead to a shift in the differentiation of T-helpers towards Th2 subset and lacked effective antiviral response.

An increase in the level of IL-8 and MCP-1 in newborns with CMVI can lead to elevated CMV replication due to the use of chemokine receptors, damage to the vascular endothelium, and a reduction in the antiviral interferon activity, which is consistent with the previous dat a [1].

Among the factors of immune defense, the interferon system, which plays an important role both in mounting antiviral immunity and regulation of the immune response in viral infection, has been currently attracting much attention. The state of the neonatal interferon system determines host immunological maturity. These data on decreased

IFNα production indicate at imbalance in this protective mechanism resulting in the onset and severe course of intrauterine cytomegalovirus infection. A low level of IFNγ demonstrates impairment in the functional activity of $CD4^+$-lymphocytes in newborns, which is of decisive importance in the formation of immune dysfunction.

Thus, the development of intrauterine cytomegalovirus infection in newborns is associated with impaired cytokine balance with a tendency to the Th2-type, impaired processes of intercellular interaction. Deficiency in the IFN-reaction of peripheral blood leukocytes particularly IFNα and IFNγ. Decrease in the number of monocytes, expressing TLR-2 and TLR-6, which indicates suppression of the early stages of the immune response, impairment of the synthesized function of immunocompetent cells. Identified immune disorders leads to uncontrolled viremia, determines the severity of the disease in prenatally infected newborns.

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