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IMMUNOREACTIVITY OF CHILDREN OF DIFFERENT AGES WITH THE CEREBRO-ASTHENIC SYNDROME, WHO WERE PREMATURELY BORN WITH PERINATAL DEFEAT OF CENTRAL NERVOUS SYSTEM

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The immune status and nature of immune frustration of children of different ages with the cerebral asthenic syndrome (CAS), who were prematurely born with perinatal defeat of the central nervous system (CNS) was studied. The obtained data argue that these children have the expressed imbalance among Th-cages (Th1, Th2, Treg) and cytokines that shows the potential risk of inadequate reaction of these children on infectious and noninfectious immunogenes.

KEY WORDS: cerebro-asthenic syndrome, perinatal defeat of central nervous system, prematurely born children, immunoglobulins, cytokines

ИММУНОРЕАКТИВНІСТЬ ДІТЕЙ РІЗНОГО ВІКУ З ЦЕРЕБРАСТЕНІЧНИМ СИНДРОМОМ, ЩО НАРОДИЛИСЯ НЕДОНОШЕНИМИ З ПЕРИНАТАЛЬНИМ УРАЖЕННЯМ ЦЕНТРАЛЬНОЇ НЕРВОВОЇ СИСТЕМИ

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Вивчено імунний статус і характер імунних розладів у дітей різного віку з церебрастенічним синдромом (ЦАС), що народилися недоношеними з перинатальним ураженням центральної нервової системи (ЦНС). Отримані дані свідчать про те, що ці діти мають виражений дисбаланс серед Тh-клітин (Th1, Th2, Treg) і цитокінів, що вказує на потенційний ризик неадекватного реагування цих дітей на інфекційні та неінфекційні імуногени.

КЛЮЧОВІ СЛОВА: церебрастенічний синдром, перинатальне ураження центральної нервової системи, недоношені діти, імуноглобуліни, цитокіни

ИММУНОРЕАКТИВНОСТЬ ДЕТЕЙ РАЗНОГО ВОЗРАСТА С ЦЕРЕБРАСТЕНИЧЕСКИМ СИНДРОМОМ, РОДИВШИХСЯ НЕДОНОШЕННЫМИ С ПЕРИНАТАЛЬНЫМ ПОРАЖЕНИЕМ ЦЕНТРАЛЬНОЙ НЕРВНОЙ СИСТЕМЫ

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Изучен иммунный статус и характер иммунных расстройств у детей разного возраста с церебрастеническим синдромом (ЦАС), родившихся недоношенными с перинатальным поражением центральной нервной системы (ЦНС). Полученные данные свидетельствуют о том, что эти дети имеют выраженный дисбаланс среди Th-клеток (Th1, Th2, Treg) и цитокинов, что указывает на потенциальный риск неадекватного реагирования этих детей на инфекционные и неинфекционные иммуногены.

КЛЮЧЕВЫЕ СЛОВА: церебрастенический синдром, перинатальное поражение центральной нервной системы, недоношенные дети, иммуноглобулины, цитокины

INTRODUCTION

Formation and development of immune system begins at early children's age and resistance of an organism to infectious and noninfectious diseases depends on its nature of functioning. In recent years indicators of survival of the prematurely born children with low body weight has significantly improved. However during postnatal development, prematurely born children have difficulties of medical and social adaptation, a delay of physical and psycho-emotional development could be attended with high incidence.

According to A.A. Baranov, in recent years pathology of nervous system in the perinatal period has increased almost in two times [1]. The injury of a brain, concerned with cerebral hypoxia, occurs to 60-80 % of newborns [2]. Hypoxic-ischemic encephalopathies are the reason of high mortality in the neonatal period and lead to various nervous disorders in children, defining the quality of life in future [3, 4, 5].

OBJECTIVE

According to all mentioned above and also close interrelation of nervous and immune system, the purpose of our work is the study of the immune status and nature of immune frustration in children of different ages with the cerebro-asthenic syndrome (CAS), who were prematurely born with a perinatal lesion of central nervous system.

MATERIALS AND METHODS

We have surveyed 56 children with CAS at the age of 6-7 years and 58 children at the age of 12-14 years, born prematurely with perinatal lesions of CNS (primary group). The group of comparison comprised children with CAS of the same age (44 children at the age of 6-7 years and 48 children at the age of 12-14 years), born in time without perinatal CNS disorders. The control group was made by almost healthy children of the same age (30 children - 6-7 years and 30 children - 12-14 years).

Criteria of an exception were chronic diseases of inner organs: cardiovascular system diseases, chronic infectious diseases, chronic diseases of endocrine system, serious chronic illness of nervous system (epilepsy, infantile cerebral paralysis, hydrocephaly, consequences of heavy craniocerebral traumas).

Examination of children was conducted on the basis of the Regional center of children's immunology Kharkiv Regional children's clinical hospital № 1 (№ 1 RCCH) and the Department of the general and clinical immunology and allergology of V.N. Karazin Kharkiv National University.

The diagnosis of cerebral asthenic syndrome was determined according to ICD-10, verified on the base of pathognomonic clinical manifestations of the disease and data of laboratory and instrumental examinations.

The program of immunological investigations included evaluation of lysozyme, secretory immunoglobulin A (IgA), monomeric IgA, immunoglobulin G (IgG) levels in an oral secret, the main classes of immunoglobulins (IgA, IgG, IgM) in serum of blood, immunoglobulin E (IgE), circulating immune complex (CIC), complement, population and subpopulation structure of lymphocytes in peripheral blood, the content of the main inflammatory and anti-inflammatory cytokines. The content of lysozyme in an oral secret was determined early in the morning fasting by a diffusion method in an agar [6]. The concentration of immunoglobulin of various classes in an oral secret and serum of blood was defined by spectrofluorimetry [7], concentration of immunoglobulin E - enzyme-linked immunosorbent assay (IgE-ELISA) - according to the enclosed instruction. The level of CIC in serum of blood was determined by the method of selective precipitation with polyethylene glycol (PEG) – 6000 [8]. The activity of complement was judged on 50% hemolysis test system [8].

Population structure of lymphocytes in peripheral blood was determined by a method of a flowing laser cytometry with use of monoclonal antibodies of different specificity, on the FACSC Calibur device (USA). The maintenance of Th1 and Th2-cells was evaluated according to the content of IL-4 and IFN γ in cytoplasm of lymphocytes [9, 10].

Proliferative activity of lymphocytes was estimated at reactions of a blast-cell transformation of lymphocytes with phytohemagglutinin [11]. Intensity of reaction estimated morphologically in a percentage of formed blastic variant.

The content of cytokines in serum of blood was determined by the enzyme-linked immunoassay method according to the enclosed instruction. It was used commercial test systems

produced by «Vektor-Best» (village Koltsovo, the Novosibirsk Region, Russia).

The statistical data processing was carried out by Microsoft XL 2007 and the Med Stat program (serial № MS000055) by DIVP TOV «Alpha», Donetsk, according to the recommendations of statistical processing of biomedical data [12, 13]. It was carried out the checking of selections on a normality of distributions (criterion χ^2), calculated average arithmetic (M), and an average error of the average size (m), determined reliability of distinctions by criterion of Student's t distribution. The critical level of significance was equal to 0,05 [14, 15, 16].

RESULTS AND DISCUSSION

The study of local oral immunity showed that in children with CAS who were prematurely born with perinatal lesion of CNS and children with CAS, born in time without perinatal disorders of CNS, the content of lysozyme, s-IgA, m-IgA, IgG, authentically did not differ from the children of control group. In children of 6-7 years and children of 12-14 years all studied indicators were within

physiological age norm. Also reliable distinctions were not revealed in these groups of children in the content of the main classes of immunoglobulin (IgA, IgG, IgM), IgE, CIC and the complement in serum of blood.

Study of population and subpopulation structure of lymphocytes of peripheral blood of children of 6-7 years and 12-14 years with CAS who were prematurely born with perinatal lesion of CNS, revealed a tendency of decrease in the content of T-general lymphocytes (CD3+), CD4+-cells, decrease in proliferative activity of lymphocytes in blast-transformation reaction (BTR) with phytohemagglutinin (PHA), reliable reduction in contents Treg-of cells and relative increase of the contents among subpopulation of Th-of lymphocytes of Th1-of cells (tab. 1). These children have the imbalance in the ratio of Th1\Th2-cells, Th1/Treg-cells, which was connected with increasing of a share of Th1-lymphocytes among subpopulation of Th-of cells, and also an imbalance between Th2 and Treg caused by decrease of share of Treg-cells in population of lymphocytes.

Table 1

Population and subpopulation structure of lymphocytes of peripheral blood of children of 6-7 years and 12-14 years with CAS who were prematurely born with perinatal lesion of CNS and born in time without perinatal lesion of CNS, ($M \pm m$)

Indices	Children with perinatal lesion of CNS		Children without perinatal lesion of CNS		Control group	
	6-7 years	12-14 years	6-7 years	12-14 years	6-7 years	12-14 years
Lymphocytes, %	30,9 ± 1,80	31,2 ± 1,81	31,7 ± 1,80	31,8 ± 1,80	34,1 ± 1,83	3,21 ± 1,72
Absolute number	2,23 ± 0,13	2,11 ± 0,11	2,39 ± 0,13	2,20 ± 0,12	2,48 ± 0,12	2,23 ± 0,11
CD 3 + cl, %	57,23 ± 3,14	58,9 ± 3,41	58,1 ± 3,19	59,6 ± 3,46	62,5 ± 3,31	65,7 ± 3,42
CD4 + cl, %	35,9 ± 2,01	36,0 ± 2,01	36,3 ± 2,01	36,9 ± 2,00	39,7 ± 2,02	39,1 ± 2,01
CD8 + cl, %	21,1 ± 1,21	22,6 ± 1,32	22,0 ± 1,24	22,4 ± 1,24	22,3 ± 1,15	24,9 ± 1,22
CD19 + cl, %	23,0 ± 1,18	21,9 ± 1,07	23,6 ± 1,18	23,1 ± 1,18	23,6 ± 1,17	22,3 ± 1,09
CD16 + cl, %	11,4 ± 1,12	11,9 ± 1,12	11,3 ± 1,12	11,6 ± 1,12	11,3 ± 1,03	12,0 ± 1,19
BTR with PHA, %	42,0 ± 7,32	45,8 ± 7,44	45,1 ± 7,31	46,7 ± 7,45	56,7 ± 7,93	59,7 ± 9,04
BTR, %	9,3 ± 0,87	9,4 ± 0,88	9,0 ± 0,88	9,0 ± 0,89	7,9 ± 0,61	8,1 ± 0,82
Th1- cl, %	6,7 ± 0,69*†	7,3 ± 0,78*†	5,2 ± 0,58*	5,6 ± 0,62*	4,0 ± 0,43	4,3 ± 0,49
Th2- cl, %	4,3 ± 0,57	4,8 ± 0,53	46,0 ± 0,52	4,9 ± 0,54	4,9 ± 0,51	5,2 ± 0,54
Treg, %	7,1 ± 0,73*	7,3 ± 0,73*	8,6 ± 0,89	8,9 ± 0,91	10,2 ± 1,00	10,9 ± 1,04
Th1/Th2	1,55 ± 0,16*†	1,51 ± 0,16*†	1,13 ± 0,12*	1,14 ± 0,12*	0,81 ± 0,08	0,82 ± 0,08
Th1/Treg	0,94 ± 0,98*†	1,00 ± 0,06*†	0,60 ± 0,06*	0,62 ± 0,06*	0,39 ± 0,04	0,39 ± 0,04
Th2//Treg	0,60 ± 0,06*	0,65 ± 0,06*	0,53 ± 0,06	0,55 ± 0,06	0,48 ± 0,05	0,47 ± 0,05

Note: * $p < 0,05$ - between indicators of children with CAS and children of control group,

† $p < 0,05$ - between indicators of children who were been prematurely born with perinatal lesion of CNS and children who were born in time without perinatal lesion of CNS.

Children of 6-7 years and 12-14 years with CAS born in time without perinatal defeat of CNS, similar changes in population and subpopulation structure of lymphocytes of peripheral blood were observed (tab. 1). However, in comparison of children born with perinatal defeat of CNS, with children without perinatal defeat of CNS it was not observed reliable decreasing in quantity Treg-cells in blood and an imbalance in the content of Th2-cells and Treg-cells, in comparison with norm. Increasing of the share of Th1-cells in peripheral blood, and also imbalance of Th1/Th2 and Th1/Treg, children prematurely born with perinatal lesion of CNS was much higher, than in time born children without perinatal defeat of CNS and statistically reliable ($p < 0,05$).

The obtained data testifies that the children with CAS in the main and comparison group have frustrations in T-cellular of immunity which are considerably related to an imbalance among subpopulations of the Th-cells which are possessing regulatory potential. Degree of frustration of children of 6-7 years and 12-14 years who were prematurely born with perinatal lesion of CNS is much higher, than of the children of the same age, who were born in time without perinatal lesion of CNS (tab. 1).

For the assessment of the cytokine status of children with CAS there have been chosen cytokines with regulatory properties: IL-1 β , IL-2, IL-4, IL-6, INF γ , IL-10, TNF α .

IL-1 – plays an important role in development of reactions of adaptive immunity, assists to activation and maturation of B - cells, an expression on T – cells of receptor to IL-2, formation of molecules of the major histocompatibility complex (MHS), pertains to the category of pro-inflammatory cytokines.

IL-2 – plays the central role in development T-cellular immunity, it is a factor of the growth and differentiation of T-lymphocytes, stimulates differentiation of Th1 and cytolytic T lymphocyte (CTL), activates the lytic activity of a NK-cells.

IL-4 and IL-6 take part in development of the antibody response, stimulate proliferation and differentiation of B-lymphocytes into antibody producers. IL-4 activates production of IgE and development of allergic reactions, induces formation of Th2 cells, suppresses development of Th1 cells and Th17 cells.

IL-6 – has the pro-inflammatory properties.

INF γ – stimulates differentiation of Th0 in Th1–cell, suppresses development of Th2 cells, activates macrophages of the NK-cells, stimulates IgG2 development, suppresses production of IgG1, IgG3, IgE.

IL-10 suppresses the function of Th1–cells, production of INF γ , IL-2, synthesis of pro-inflammatory cytokines – IL-1, IL-6, FNO α , it is a co-stimulant of reproduction and developing of B-cells, it belongs to the family of anti-inflammatory cytokines.

TNF α has antineoplastic and antiviral action, stimulates microbicidal activity of neutrophils, macrophages, and has pro-inflammatory properties.

The investigations which were made showed us that the children of 6-7 years and 12-14 years with CAS who were prematurely born with perinatal lesion of CNS, had the increase respectively in 2,4 and in 2,1 times of the contents of IL-1 β , decrease of the content in 1,46 and 1,35 times of IL-2 and in 1,20 and 1,19 times of IL-10 in peripheral blood (tab. 2). The children of this group had the imbalance in the ratio IL-1/IL-10 (children of 6-7 years - $0,64 \pm 0,06$ and children of 12-14 years $0,59 \pm 0,60$, in control group respectively $0,22 \pm 0,02$ and $0,24 \pm 0,02$), IL-2/IL-4 (respectively $0,11 \pm 0,01$ and $0,12 \pm 0,01$, in control $0,18 \pm 0,01$), IL-2/IL-6 ($0,10 \pm 0,01$ and $0,11 \pm 0,01$ in control $0,15 \pm 0,01$ and $0,16 \pm 0,01$) ($p < 0,05$).

In children of 6-7 years and 12-14 years with CAS who were born in time without perinatal lesion of CNS, it was noted only reliable increasing of concentration IL-1 β , in comparison with children of control group. They, as well as the children of a primary group, had the imbalance in the ratio IL-1/IL-10 (children of 6-7 years $0,42 \pm 0,04$, children of 12-14 years $0,39 \pm 0,04$, in control respectively $0,22 \pm 0,02$ and $0,24 \pm 0,02$). The ratio of IL-2/IL-10 and IL-2/IL-6 was normal. It is necessary to notice that content of IL-1 β of peripheral blood of children with CAS, without perinatal lesion of CNS was lower, than in children with CAS, with perinatal lesion of CNS, IL-10-is slightly higher, and the content of IL-2 of children who were born in time without perinatal lesion of CNS, as opposed to children who were been prematurely born with perinatal lesion of CNS, authentically did not differ from values of norm.

Table 2

**Contents of cytokines in serum of blood in children of 6-7 years
and 12-14 years with CAS who were prematurely born with perinatal lesion
of CNS and born in time without perinatal lesion of CNS, (M ± m)**

Indicators	Children with perinatal lesion of CNS		Children without perinatal lesion of CNS		Control group	
	6-7 years	12-14 years	6-7 years	6-7 years	12-14 years	6-7 years
IL-1 β pg/ml	4,3 ± 0,41*†	3,9 ± 0,38*†	3,3 ± 0,35*	2,9 ± 0,30*	1,8 ± 0,20	1,9 ± 0,20
IL-2 pg/ml	1,3 ± 0,11*†	1,4 ± 0,11*†	1,6 ± 0,14	1,7 ± 0,14	1,9 ± 0,20	1,9 ± 0,20
IL-4 pg/ml	11,4 ± 1,10	11,1 ± 1,11	11,0 ± 1,10	10,7 ± 1,10	10,3 ± 1,12	10,1 ± 1,10
IL-6 pg/ml	12,5 ± 1,18	12,0 ± 1,18	12,2 ± 1,15	11,9 ± 1,17	12,1 ± 1,13	11,5 ± 1,14
IL-10 pg/ml	6,7 ± 0,70	6,6 ± 0,70	7,7 ± 0,81	7,4 ± 0,76	8,1 ± 0,80	7,9 ± 0,80
TNF pg/ml	0,7 ± 0,08	0,6 ± 0,07	0,7 ± 0,08	0,6 ± 0,07	0,7 ± 0,07	0,6 ± 0,6
INF γ pg/ml	9,8 ± 0,91	9,7 ± 0,92	9,7 ± 0,92	9,6 ± 0,93	9,9 ± 0,97	9,8 ± 0,96

Note: * p < 0,05 - between indicators of children with CAS and children of control group.

† p < 0,05 - between indicators of children who were been prematurely born with perinatal lesion of CNS and children who were born in time without perinatal lesion of CNS.

The obtained data testify that children with CAS who were prematurely born with perinatal disorders of CNS had the expressed imbalance in the contents of the main regulatory of cytokines. Revealed imbalance among Th-cells (Th1, Th2, Treg) and cytokines, of children with CAS who were prematurely born with perinatal lesion of CNS, which regulate an orientation and full value of development of immune reaction indicates to the potential risk of inadequate reaction of these children on infectious and noninfectious immunogenic that can lead to development of an immunodeficient status and synchronization of an infection or, per contra, to hyper reactions, an autosensibilization and developing of autoimmune processes. The obtained data testify that this category of children needs special attention and dispensary registration.

CONCLUSIONS

Children with CAS who were prematurely born with perinatal lesion of CNS, the

frustrations in immune system concern T-cell part of immunity and are mainly connected with an imbalance of subpopulations in Th-cell (Th1, Th2, Treg) and in the cytokine network, the increased content of IL-1β and the decreased content of IL-10 and IL-2, which are responsible for the development of immune reaction. The obtained data indicates the risk of inadequate reaction on infectious and noninfectious immunogenes in these children that can lead to the development of an immunodeficiency state and chronization of an infection or, on the contrary, to hyper reactions, autosensibilization and development of autoimmune processes.

PROSPECTS FOR FUTURE STUDIES

Further it is obviously very important the selection of the immunocorrecting preparation for studying of efficiency of correction of the revealed violations.

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