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4 RESULTS OF THE STUDY OF THE MICROBIAL SPECTRUM IN CHILDREN WITH AUTISM SPECTRUM DISORDERS ASSOCIATED WITH GENETIC DEFICIENCY OF THE FOLATE CYCLE

INTRODUCTION

Evidence from a number of recent independent meta-analyses and systematic reviews of randomized controlled trials, published in peer-reviewed medical journals indexed in the PubMed (MEDLINE) abstract bibliographic electronic database, indicates that genetic deficiency of the folate cycle (GDFC) is associated with the clinical phenotype of autism spectrum disorders (ASD) in children [1–5]. Results of a meta-analysis of randomized controlled trials by B. Q. Guo et al. in 2020, which included 31 trials involving 3304 children, including 1641 patients with ASD, without genetic clarification of the diagnosis, demonstrated that hyperhomocysteinemia, a phenomenon specific to GDFC, is associated with ASD and is a class feature of such children (Hedges's $g = 0.56$; 95 % CI = 0.36–0.76, $p < 0.001$) [6]. N. S. Mohammad et al., using the ANN (artificial neural network) model in a controlled clinical study involving 138 children with ASD and 138 healthy individuals, showed that the determination of pathogenic polymorphic variants of the genes GCPII C1561T, SHMT1 C1420T, MTHFR C677T, MTR A2756G, and MTRR A66G for diagnostic purposes allows determining the risk of developing ASD in a carrier with an accuracy of 63.8 % [2]. These data allow us to consider GDFC as the main factor in the genetic predisposition to the development of ASD in children.

Currently, the pathways of damage to the nervous system in children with GDFC have been discovered, which are implemented in the pathogenesis of encephalopathy in ASD. It has been shown that biochemical disorders caused by GDFC lead to damage to the immune system with the induction of immunodeficiency and associated immune dysregulation [7]. H. K. Hughes et al. in a systematic review of immune system dysfunction in patients with ASD demonstrated a number of characteristic pathological changes in immune status that may have pathogenetic significance and be targets of therapeutic interventions, including an abnormal cytokine profile with increased concentrations of pro- and decreased levels of anti-inflammatory cytokines, various changes in the absolute and relative number of lymphocytes and their subpopulations, laboratory signs of systemic and intracerebral inflammation, defects in the functioning of the adaptive and innate immune systems, deviations in serum concentrations of immunoglobulins of different classes, and serological signs of autoimmunity to both connective tissue autoantigens and neurons and glia [8].

The presence of immune dysfunction predicts a decrease in the body's resistance to microbial factors. Indeed, many reports have accumulated to date about the abnormal development of opportunistic and conditionally pathogenic infections in children with ASD, which can be explained by the damage to the immune system induced by GDFC. Binstock T. first pointed out the selectively reduced immunoresistance in children with ASD, identifying a subgroup of patients with the so-called intramonocytic pathogens – measles virus, cytomegalovirus, herpes virus type 6 and Yersinia enterocolitica [9]. Such children were characterized by suppression of hematopoiesis, impaired peripheral immunity, increased permeability of the blood-brain barrier and manifestations of demyelination in the white matter of the cerebral hemispheres – signs, as it is now known, typical of GDFC [10]. G. L. Nicolson et al. in a controlled clinical study using blood PCR showed

abnormally frequent detection of Mycoplasma pneumoniae, Chlamydia pneumoniae and herpes virus type 6 in children with ASD compared with healthy people [11]. A. Sakamoto et al. in a specially designed study found that congenital CMV infection with CNS involvement in children with ASD occurs significantly more often (7.4 %) than in the general population (0.31 % of cases) ($p = 0.004$). CMV was identified by real-time PCR of dried neonatal blood samples and cord blood samples obtained immediately after delivery [12]. S. Valayi et al. in a controlled clinical study demonstrated that specific IgM to EBV in the serum of children with ASD were significantly more common than in healthy individuals ($p < 0.05$) [13]. H. Jyonouchi et al. in a specially designed study showed an association of ASD with a primary deficiency of specific antipolysaccharide antibodies, which may explain the known predisposition to the development of chronic streptococcal infection in such children [14]. H. K. Hughe and P. Ashwood in a controlled clinical study found that seropositivity to Candida albicans in children with ASD occurs in 36.5 % of cases, while in healthy children it occurs in only 14.3 % of cases (OR = 3.45; 95 % CI = 1.0409–11.4650; $p = 0.041$). An association of seropositivity to Candida with manifestations of gastrointestinal dysfunction was shown [15]. T. Nayeri et al. conducted a meta-analysis of randomized controlled clinical trials, which demonstrated the association of ASD with toxoplasmosis, and that the presence of toxoplasmosis infection increases the risk of developing ASD in a child by 1.93 times (95 % CI = 1.01–3.66) [16]. M. Kuhn et al. reported a series of clinical cases of the combination of borreliosis and ASD in children and a significant reduction in the manifestations of ASD as a result of long-term therapy with ampicillin and azithromycin for borreliosis [17].

As can be seen from the above data, the spectrum of microorganisms that are atypically common in children with ASD compared to healthy people has expanded significantly since the publication of T. Binstock, however, the principle highlighted by the author still remains unchanged, since intracellular/intramonocytic pathogens predominate. X. Kong et al. justify the identification of subtypes of children with ASD depending on lesions of the oral and intestinal microbiota, given the important role of opportunistic and conditionally pathogenic microorganisms in the pathogenesis of mental illness [18].

It has now been established that infectious agents are active components of the pathogenesis of encephalopathy in children with ASD associated with GDFC. Microorganisms are capable of exerting both direct damaging effects on the brain parenchyma and being involved in indirect immune-mediated mechanisms of cerebral damage by inducing systemic inflammation and anti-brain autoimmunity. The direct damaging effect of infectious agents can consist in the induction of encephalitis and neurodegenerative processes. Thus, a number of cases of the development of autism symptoms after temporal partial necrotic-hemorrhagic encephalitis of HSV-1 etiology have been described [19–21]. This is one of the examples of the direct damaging effect of infectious agents on the CNS tissue in patients with ASD. In parallel, it has been established that HHV-6 carries out transolfactory migration to the brain [22] and thereby affects the structures of the mesolimbic system of the temporal lobes, inducing a neurodegenerative process called temporal mesial sclerosis [23], the clinical and radiological features of which are noted in many children with ASD [24]. The association of HHV-6 and temporal mesial sclerosis in humans is confirmed by the results of the latest meta-analysis of randomized controlled clinical trials performed on brain biopsies obtained from areas of neurodegenerative damage [25]. This is a second, neurodegenerative, form of direct damage to the CNS by microorganisms, which may be an important component of the pathogenesis of encephalopathy in children with ASD associated with GDFC.

If we talk about the indirect effects of infectious agents on the CNS tissue in children with ASD, then at least two ways of such damage should be distinguished. First, microorganisms can be triggers of anti-brain autoimmunity to myelin [26] and neurons [27] of the brain. Thus, cases of the development of autism symptoms in children with autoimmune limbic encephalitis [28, 29] with a positive response to immunomodulatory therapy [30] have been described, and viruses of the herpes family [31], as well as Toxoplasma [32] and Mycoplasma [31], can provoke a breakdown of immune tolerance to autoantigens of hippocampal neurons in such cases. The role of group A beta-hemolytic streptococcus, which is frequently found in children with ASD, in inducing autoimmune subcortical encephalitis, some of the symptoms of which may resemble ASD, is now well known and characterized [33, 34]. On the other hand, infectious agents in the context of immune dysregulation caused by GDFC can induce a state of systemic inflammation with the induction of hypercytokinemia with neurotoxic effects. The phenomenon of systemic hypercytokinemia with a pro-inflammatory profile in children with ASD is supported by the results of two recent meta-analyses of randomized controlled clinical trials [35, 36]. It has been shown that herpes virus type 6, which is often reactivated in autistic disorders, can induce a state of hyperactivation of macrophages with the development of hypercytokinemia, similar in nature to that observed in children with ASD [37]. In addition, when discussing the inflammatory mechanism of microbe-induced cerebral damage, it is worth mentioning the possibility of involvement of the functional microbiota-gut-brain axis [33, 38]. By enhancing inflammation in the intestine, infectious agents can induce further intracerebral inflammation in children with ASD by abnormal spread of the inflammatory process from the intestinal compartment through the blood and pathologically permeable blood-brain barrier to the brain parenchyma [39]. The role of the functional microbiota-gut-brain axis in the pathogenesis of encephalopathy in children with ASD is currently being discussed in a number of systematic reviews [33, 38] and the results of clinical trials [39].

Currently, there is a lack of systematization of knowledge on the microbial spectrum in patients with ASD, which appears to be quite specific and sharply different from that in healthy children. The relationship between microbial load and the state of the immune system requires significant clarification, and the role of microorganisms in the induction of cerebral damage and other complications in children with ASD remains poorly understood. Therefore, conducting specially planned studies in the outlined direction is an urgent task of modern neuroimmunology and immunopsychiatry.

The aim of the research: to study the structure of the microbial spectrum in children with ASD associated with GDFC, according to the evidence base accumulated to date and to study the association of the identified microorganisms with indicators of immune status to improve understanding of the pathogenesis of encephalopathy and improve diagnostic, monitoring and treatment algorithms.

MATERIALS AND METHODS OF THE RESEARCH

Data on the selection of patients for the study and control groups, the principles of making a clinical diagnosis of ASD, ethical and organizational aspects, the diagnosis of pathogenic polymorphic nucleotide substitutions in the genes of folate cycle enzymes, and the laboratory methods used to study associated biochemical disorders are given in the **Section Materials and methods in Chapter 2**. Data on the principles

and approaches to laboratory assessment of the immune status of patients in the observation groups are contained in the **Section Materials and methods in Chapter 3**.

Pathogenic polymorphic variants of folate cycle genes were determined by restriction polymerase chain reaction (PCR) based on the detection of the MTHFR C677T nucleotide substitution in monoform (68 patients), as well as – in combination with other nucleotide substitutions – MTHFR A1298C, MTRR A66G and/or MTR A2756G (157 individuals). These individuals constituted the study group (SG).

The control group (CG) included 51 clinically healthy children (37 boys and 14 girls) of similar age distribution who did not suffer from GDFC.

Special laboratory paraclinical examination of children in the observation groups was performed taking into account modern ideas about the microbial spectrum in patients with ASD. Thus, the diagnosis of reactivated herpesvirus infections and TTV infection was performed by PCR of blood leukocytes (Department of Neurobiochemistry of the Institute of Neurosurgery of the National Academy of Medical Sciences of Ukraine) according to the data of the study by G. L. Nicolson et al. [11]. Detection of beta-hemolytic streptococcus group A was performed by bacteriological culture from the oropharyngeal mucosa on a selective nutrient medium or by specific antitoxic immunity in blood serum (antistreptolysin-O, antistreptodornase, antihyaluronidase) (ELISA; MDI Limbach Berlin GmbH, the Federal Republic of Germany), as stated in the systematic review by D. Dop et al. [34]. Infection caused by Candida albicans was diagnosed based on specific IgM in serum (ELISA; MDI Limbach Berlin GmbH, the Federal Republic of Germany) according to the results of the study by H. K. Hughes and P. Ashwood [15]. Infections caused by Mycoplasma and Chlamydia pneumoniae were detected based on specific IgM in serum (ELISA, Sinevo, Ukraine) [47]. Borreliosis and yersiniosis were identified based on the Versten blot analysis with simultaneous detection of IgM and IgG to a number of surface and deep antigens of the indicated pathogens (Sinevo, Ukraine) according to the data of M. Kuhn et al. [17] and T. Binstock [9]. Toxoplasmosis was diagnosed based on specific IgA in serum (ELISA, Sinevo, Ukraine), as shown by T. Nayeri et al. in the corresponding meta-analysis of randomized controlled clinical trials [16]. Transferred congenital CMV neuroinfection was identified based on the data of anamnestic studies of the newborn's serum (PCR, Department of Neurobiochemistry of the Institute of Neurosurgery of the National Academy of Medical Sciences of Ukraine and other laboratory centers) according to the data of A. Sakamoto et al. [12] and a specific neuroimaging pattern, which was interpreted according to the results of an 18-year longitudinal clinical study by R. Pinillos-Pisón et al. [40].

Thus, SG patients underwent a comprehensive assessment of the microbial spectrum in accordance with the evidence accumulated to date, however, the specificity of the approach consisted precisely in the simultaneous search for all microbial agents, which could provide a holistic picture of the child's current infection, since the scientific articles published so far usually dealt with the diagnosis of only some pathogens from a known list, which does not allow for a comprehensive analysis of the microbial load and an adequate assessment of the immune–microbiological connections and their place in the pathogenesis of the disease.

Thus, the analysis of the microbial spectrum was carried out by taking into account possible connections with the immune status of the child, which is a specific feature of the approach to data analysis, which was not previously used in children with ASD according to the data of available publications in PubMed and Embase. Such an approach can allow not only to study the microbial load in SG children, but also to explain the

reasons for the formation of a specific pattern of microbial load and to investigate the clinical significance of infectious agents with the identification of potential mechanisms of involvement of microorganisms in the pathogenesis of encephalopathy in children with ASD associated with GDFC.

Statistical processing of the material was carried out by comparative and structural analyses. To determine the probability of differences between the indicators in the observation groups, the parametric student's T-test with a confidence interval of *p* and the nonparametric criterion – the number of signs *Z* according to Yu. Urbach. Differences were considered probable at $p < 0.05$ and $Z < Z_{0.05}$. To study the associations between the studied indicators, the odds ratio (OR) and 95 % confidence interval (95 % CI) were used.

Microsoft Excel was used for statistical calculations.

The study was carried out as a fragment of research work commissioned by the Ministry of Health of Ukraine (state registration number 0121U107940).

RESULTS AND DISCUSSION OF THE RESEARCH

The results of the applied laboratory tests for the comprehensive identification of opportunistic and conditionally pathogenic microorganisms in SG children are generally consistent with the data of previous separate clinical studies in this area. Positive test results were obtained for all potential microbes that are believed to be involved in the pathogenesis of encephalopathy in ASD. The results of studying the microbial spectrum in SG children significantly differ from the data in CG for all studied pathogens. Therefore, the obtained data are consistent with the ideas formed in recent years about the specific microbial spectrum in children with ASD, which does not correspond to that in healthy individuals. Although all infectious agents currently characterized as associated pathogens in autistic disorders are detected in children with ASD associated with GDFC, the proportion of positive cases differs sharply among different microorganisms, which suggests a heterogeneity of their distribution in the studied cohort of patients and, as a result, a different significance in the development of encephalopathy in the entire group. These differences should be taken into account both when planning laboratory tests to study the microbial load in patients with ASD associated with GDFC, and when conducting clinical and laboratory monitoring and determining the prerogative of certain therapeutic interventions.

Data on the structure of SG compared to CG by the detected opportunistic and conditionally pathogenic microorganisms are shown in **Fig. 4.1**.

The data in **Fig. 4.1** demonstrate the unequal prevalence of various studied microorganisms in SG patients and a significant difference in SG and CG in the specific gravity of detection of all studied infectious agents ($p < 0.05$; $Z < Z_{0.05}$). The results obtained indicate the predominance of viral agents over bacterial, fungal and protozoan. Therefore, opportunistic viral infections in a state of reactivation are the most frequent finding when assessing the microbial load in children with ASD associated with GDFC. Viral agents in such cases are detected at least three times more often than all studied bacterial, fungal and protozoan agents taken together. Therefore, the study of viral opportunistic agents should receive maximum attention when planning diagnostic algorithms for assessing the microbial spectrum in children with ASD associated with GDFC, and the pathogenetic pathways of virus-induced CNS damage should be a potential primary object of clinical research in this area.

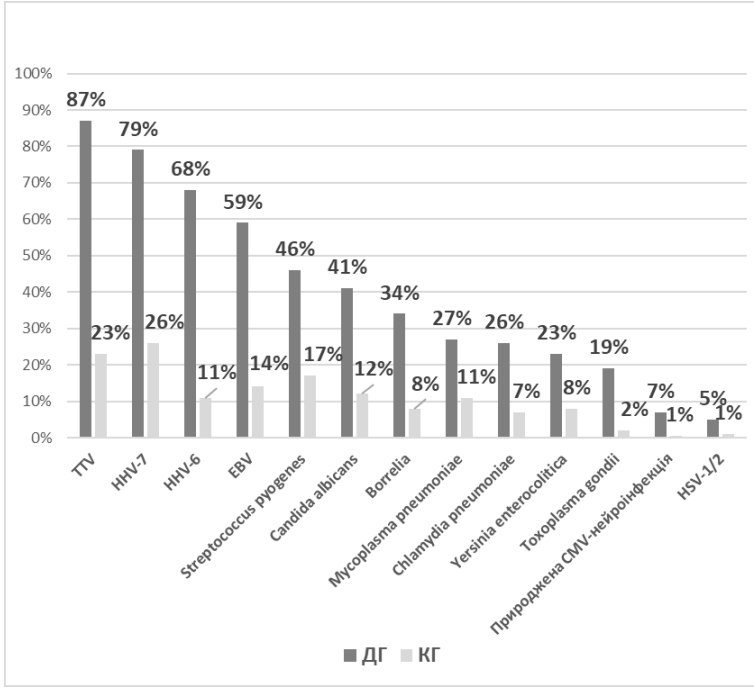

Fig. 4.1 Structure of SG (n = 225) and CG (n = 51) according to the detected microorganisms of the studied spectrum

Among the viral agents, TTV is most often detected in children with ASD associated with GDFC – in almost 9 out of 10 examined children, which is at least four times more than in healthy children of the CG ($p < 0.05$; $Z < Z_{0.05}$). HHV-7 and HHV-6 are found in 7 and 6 out of 10 examined SG patients, respectively, which is 3 and 6 times less common than in the CG ($p < 0.05$; $Z < Z_{0.05}$). As the results of this study show, TTV, HHV-7 and HHV-6 are the most frequent pathogens in children with ASD associated with GDFC. While the reactivation of herpesviruses in such cases has been reported previously [11], we did not find any relevant reports regarding TTV in the available scientific literature, therefore we believe that we have discovered the indicated association for the first time in the world.

EBV, Streptococcus pyogenes, Candida albicans, and Borrelia were found in children with ASD associated with GDFC at a moderate frequency. The proportion of cases of identification of these pathogens in an active state in SG ranged from 59 % to 34 %, while in CG – from 17 % to 8 % ($p < 0.05$; $Z < Z_{0.05}$).

Mycoplasma pneumoniae, Chlamydia pneumoniae, Yersinia enterocolitica were detected in 27–23 % of SG children and in 11–8 % of CG patients ($p < 0.05$; $Z < Z_{0.05}$). We propose to designate these microorganisms as pathogens with a low frequency of distribution in children with ASD associated with GDFC.

Congenital CMV infection and reactivated HSV-1/2 infection were pathogens with an extremely low proportion in SG (7–5 % vs. 1 % in CG; ($p < 0.05$; $Z < Z_{0.05}$).

The division of the identified microorganisms into four groups according to their specific gravity of distribution can be useful for rational planning and organization of diagnostic studies and therapeutic interventions, taking into account the economic and technical aspects of the problem. We propose a stepwise approach to assessing the microbial spectrum and a stepwise approach to prescribing antimicrobial drugs, taking into account the identified four groups of microorganisms with different specific gravity of distribution. Initially, it seems appropriate to identify microbes with a high specific gravity, and in subsequent stages – with a consistently lower specific gravity of distribution. Accordingly, as we believe, antimicrobial therapy should be carried out.

Of particular note is the identification of the association of EBV, Streptococcus pyogenes, and Candida albicans, which were simultaneously detected in the same patient in at least 80 % of cases of identification of such microorganisms. The relationship between EBV and Streptococcus pyogenes is now well known. Infectious mononucleosis, the primary form of EBV infection, often begins with streptococcal angina. The association of EBV and rheumatoid streptococcus has several aspects. The relationship is known at the level of intermicrobial interaction and at the level of the characteristics of human immune status disorders. If we talk about intermicrobial interaction, it has been established that streptococcus peptidoglycans, through their effect on TLR-2, activate EBV from a latent state in infected cells of the lymphoblastoid lineage of the tonsils, and EBV, by disrupting antibody genesis and inducing neutropenia, promotes the growth of streptococcus [41]. The immunological aspect of the interaction between these microbes lies in the typical associations of genetic disorders of antibody formation in humans. It was found that primary total deficiency of IgG3 (G3(g) allotype), which contributes to EBV reactivation, is associated with primary selective deficiency of specific IgG2 to streptococcal polysaccharides with preservation of the general IgG2 pool (G2(n) negative allotype) [42]. Due to its synergistic effect, coinfection of EBV and Streptococcus pyogenes promotes the accelerated development of associated autoimmune complications, as shown by T. Watanabe et al. on the example of acute glomerulonephritis [43]. The combination with candidiasis, in our opinion, can be explained not only by the peculiarities of the immune status, but also by the use of antibiotics for streptococcal infection, which, as is known, promotes the growth of candida.

It is important to establish why a specific microbial spectrum is formed in children with ASD associated with GDFC, which involves abnormally high susceptibility to some microorganisms and normal resistance to others. One of the explanations may relate to the features of the immune status in these patients, since the structure of the specified microbial spectrum is dominated by opportunistic and conditionally pathogenic microorganisms with low virulence, the reactivation of which usually occurs under conditions of immunosuppression. As noted above, various laboratory manifestations of immunodeficiency and related immune dysregulation have been described in patients with ASD [7, 8], therefore it seems appropriate to study the associations of certain disorders of the immune status and certain microorganisms that have undergone activation in the patient's body. The results of the study of such immuno-microbiological associations in SG children are given in **Table 4.1**.

Table 4.1 Results of the association study (OR; 95 % CI) of immune disorders and identified microorganisms in children with ASD associated with GDFC (n = 225)

Indicator	NK	NKT	CD8+ T lympho-cytes	CD4+ T lympho-cytes	ДІГ	ГІГ	МПО	Combined violations
TTV	2.3767; 1.1772–4.7983*	2.2713; 1.1259–4.5818*	1.8452; 0.9231–3.6885	1.7642; 0.8831–3.5246	0.3950; 0.1865–0.8366	0.4493; 0.2147–0.9403	0.3152; 0.1485–0.6690	0.6483; 0.3201–1.3131
HHV-6	3.7316; 1.8118–7.6856*	2.1711; 1.077–4.3767*	3.9184; 1.8989–8.0857*	0.7756; 0.3835–1.5684	0.6957; 0.3465–1.3967	0.4487; 0.2197–0.9165	0.5269; 0.2605–1.0659	0.3374; 0.1601–0.7111
HHV-7	3.8864; 1.8758–8.052*	2.8095; 1.3893–5.6814*	3.5561; 1.7296–7.3116*	0.6625; 0.3293–1.3327	0.5546; 0.2749–1.1190	0.7018; 0.3455–1.4256	0.6754; 0.3375–1.3514	0.8546; 0.4242–1.7218
EBV	3.7059; 1.7914–7.6663*	2.4879; 1.2311–5.0276*	2.8607; 1.4103–5.8026*	0.8144; 0.4035–1.6437	0.9214; 0.4627–1.8348	0.8526; 0.4288–1.6951	0.7381; 0.3642–1.4958	0.7300; 0.3643–1.4630
Str.	0.5068; 0.2445–1.0504	0.8922; 0.4491–1.7723	0.5833; 0.2898–1.1741	0.6130; 0.3052–1.2312	6.6667; 3.0563–14.5419*	4.5588; 2.1939–9.4728*	5.0679; 2.4247–10.5927*	0.7018; 0.3455–1.4256
Candida	0.8024; 0.4016–1.6032	0.5997; 0.2927–1.2288	0.2936; 0.1373–0.6280	0.6483; 0.3201–1.3131	0.5404; 0.2659–1.0981	0.5844; 0.2869–1.1902	3.9184; 1.8989–8.0857*	0.9263; 0.4580–1.8732
Toxoplas-ma	0.5678; 0.2761–1.1675	0.8802; 0.4416–1.7545	0.7083; 0.3546–1.4150	9.4286; 4.2272–21.0303*	0.4242; 0.2069–0.8698	0.3837; 0.1843–0.7990	0.6327; 0.3098–1.2924	7.4582; 3.4436–16.153*
CMV-infec-tion	0.7655; 0.3826–1.5317	0.9333; 0.4702–1.8527	0.4242; 0.2069–0.8690	0.5678; 0.2761–1.1670	0.3122; 0.1484–0.6570	0.3775; 0.1825–0.7809	0.1970; 0.0884–0.4392	9.7101; 4.3616–21.6175*

*Note: *α = 0.05*

It should be noted that Mycoplasma pneumoniae, Chlamydia pneumoniae, Borrelia, Yersinia enterocolitica were not associated with any of the identified disorders in the immune status of SG patients, therefore these data were not included in **Table 4.1**. The lack of associations in these cases can be explained by the properties of these microorganisms, which have a higher virulence than other studied microbes, and can usually affect the body of an immunocompetent person. In contrast, other microbes that have demonstrated certain associations with immune disorders have pronounced opportunistic or conditionally pathogenic properties, are usually characterized by low virulence and undergo reactivation from a latent or persistent state mostly in an immunocompromised organism. Analysis of associations for HSV-1/2 was not performed due to the small number of observations.

As shown in **Table 4.1**, viral infections were associated with disorders in the effector chain of adaptive and innate cellular immunity, which corresponds to the classical postulates of clinical immunology about the prerogative of cellular mechanisms of immune surveillance during the reactivation of viral agents in the human body [44]. HHV-6, HHV-7 and EBV were associated with deficiencies of NK, NKT and CD8+ cytotoxic T lymphocytes, which, according to the evidence accumulated so far, play an important role in the implementation of the immune response to viral pathogens [44, 45]. In particular, NK and NKT lymphocytes, using different mechanisms of recognition of virus-infected cells, participate in spontaneous and antibody-dependent cell-mediated cytotoxicity reactions and exert a number of regulatory effects aimed mainly at potentiation of effector mechanisms of cellular immunity [45, 46]. Instead, CD8+ cytotoxic T lymphocytes eliminate the virus in situ by implementing a specific immune cytotoxicity response [44]. It should be noted that TTV has also been associated with NK and NKT cell deficiencies, but not with CD8+ cytotoxic T cell deficiencies. The data obtained are fully consistent with the results of a systematic review by J. S. Orange on the clinical manifestations of primary NK cell deficiency in humans [46].

Unlike viral agents, streptococcal infection developed mainly in cases of disorders of the humoral component of adaptive immunity – hypo- and dysimmunoglobulinemia, which corresponds to the classical ideas about the predominant role of immunoglobulins in protection against streptococci and the well-known clinical picture of humoral immunodeficiencies, for example, Bruton's disease or common variable immunodeficiency, in which lesions caused by pyogenic opportunistic cocci predominate [47, 48]. Antibodies exert both direct and indirect antimicrobial effects in streptococcal infection in humans. The direct effects include the effects of agglutination, precipitation and neutralization [33]. Indirectly, antibodies help destroy streptococcal cells by opsonization and induction of immune phagocytosis, activation of the complement system by the classical pathway, and by promoting antibody-dependent cell-mediated cytotoxicity reactions, in which macrophages play the role of effector cells [34]. Previously, the association of streptococcal infection with humoral immunodeficiency in children with ASD was reported by H. Jyonouchi et al. [14]. Streptococcus was also associated with a deficiency of phagocyte myeloperoxidase. The role of phagocytosis in the neutralization of bacterial agents in the human body is now well known [49]. Selective abnormal susceptibility to streptococcal infection in primary neutrophil myeloperoxidase deficiency was first reported by P. Cocchi et al. back in 1973 [50].

In contrast, Candida albicans was associated only with neutrophil myeloperoxidase deficiency, which is consistent with current understanding of the key role of the myeloperoxidase-mediated microbicidal system of phagocytes in controlling candidal infection in humans [49] and with the results of a systematic review by W. M. Nausee on the problem of primary phagocyte myeloperoxidase deficiency in humans, which noted candidiasis as the leading clinical manifestation of this immunodeficiency [51].

Toxoplasmosis was associated with CD4+ T lymphocyte deficiency and combined immune disorders, in which both cellular and humoral immunity were involved simultaneously. The crucial role of CD4+ T lymphocytes in the control of Toxoplasma infection is well known due to numerous observations of severe Toxoplasma reactivation in AIDS of HIV etiology and idiopathic CD4+ T-cell lymphopenia [52].

Congenital CMV neuroinfection was associated only with combined immune disorders, which is consistent with the classical notion of the need for deep immunosuppression to reactivate this opportunistic agent in the human body [44].

Thus, it was established that different microorganisms are associated with different disorders in the immune status of patients with ASD associated with GDFC. We can talk about specific immuno-microbiological relationships, which are consistent with the classical notion of differences in the mechanisms of immune surveillance of opportunistic and conditionally pathogenic microbial agents of different nature in the human body. These data allow us to assume that it is the immunodeficiency caused by GDFC that is the cause of the formation of a specific microbial spectrum in children with ASD associated with GDFC. The identified connections allow, by assessing the immune status, to predict which microbes are most likely to multiply in the child's body, properly adjusting the direction of the diagnostic microbiological search and the program of further antimicrobial treatment. Conversely, the prevalence of certain microorganisms may indicate specific disorders in the immune system, which should be taken into account when planning the patient's immunological examination and selecting immunotherapy to correct immune status disorders. It is possible to distinguish subgroups of children with ASD associated with GDFC according to the prevailing microorganisms – viral, bacterial, fungal or protozoan – given the obvious differences in rational algorithms for diagnostic search and programs of antimicrobial and immunotropic treatment of such patients.

CONCLUSIONS TO THE SECTION 4

Children with ASD associated with GDFC are characterized by a specific microbial spectrum with a predominance of intracellular opportunistic and conditionally pathogenic microorganisms, which is determined by the features of immune status disorders provoked by GDFC, which should be taken into account when implementing the algorithm of rational microbiological search, assessment of immune status, and antimicrobial and immunotropic treatment in children with ASD.

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