DOI: 10.15587/978-617-8360-21-4.CH10

10 EFFICACY OF COMBINED USE OF TRANSFER FACTORS BASED ON COLOSTRUM IMMUNE EXTRACT IN NK AND NKT CELL DEFICIENCY IN CHILDREN WITH AUTISM SPECTRUM DISORDERS ASSOCIATED WITH GENETIC DEFICIENCY OF THE FOLATE CYCLE

JUSTIFICATION

Recent decades of research have shown that immunodeficiency and related immune dysregulation are important components in the pathogenesis of multisystemic damage in children with autism spectrum disorders (ASD). Data from 5 meta-analyses and systematic reviews of randomized controlled trials supporting the association of ASD and genetic deficiency of the folate cycle (GDFC) shed light on key factors in the genetic predisposition to the development of severe neuropsychiatric syndromes in children [1, 2, 3, 4, 5]. The results of the last 3 systematic reviews of controlled clinical trials have characterized the spectrum of immunological disorders characteristic of children with ASD, one of the main components of which is the deficiency of natural killer (NK) cells, natural killer T lymphocytes (NKT) and, conversely, an abnormally increased number of CD3 + T lymphocytes in the peripheral blood [6, 7, 8]

Immune system disorders shed light on the origin of a number of immune-dependent clinical manifestations (allergic, autoimmune, immunoinflammatory, etc.) characteristic of children with ASD, which cannot be explained solely by the existing mental dysfunction [9, 10, 11].

Correction of immune disorders in children with ASD appears to be a promising tool not only for restoring immune resistance to microorganisms, but also for preventing the development of numerous immune-dependent complications that affect both the CNS and other organs and systems of the child's body.

Currently, there is a lack of clinical studies on the testing of immunocorrective interventions in children with ASD with signs of immune dysfunction. A recent report on the effectiveness of combined immunotherapy with Propes and Inflamaferin in eliminating key disorders of innate immunity in children with ASD associated with GDFC, namely, NK and NKT cell deficiency, indicates the potential curability of this immune dysfunction and may serve as an incentive for initiating further research in this direction [12]. It is necessary to find alternative, cheaper and more convenient immunocorrective agents, but at least equivalently effective to the approved peptide immunotherapy. Such immunocorrective agents may be transfer factors based on the immune extract of bovine colostrum, which in placebo-controlled clinical studies have so far demonstrated the ability to eliminate or at least attenuate many immune-dependent disorders characteristic of children with ASD, namely, to improve immune status, increase resistance to respiratory and intestinal infections, reduce manifestations of intestinal dysbiosis, normalize intestinal wall permeability, have antiallergic effects and attenuate manifestations of autoimmunity, as well as normalize some neurological and psychiatric disorders [13, 14, 15]. There is a justified need to test transfer factors to correct key cellular immune disorders in children with ASD associated with GDFC, which would provide practical medicine with an effective means of preventing immune-dependent complications that affect the severity of the condition, quality of life, and endurance of patients with neuropsychiatric disorders.

The aim of the research: to study the efficacy and safety of the combined use of classical transfer factor and trifactor transfer factor in NK and NKT cell deficiency in children with ASD associated with GDFC, taking into account the effect on the number of CD3 + T lymphocytes in the blood.

Research objectives:

1. To assess the dynamics of the number of NK cells in peripheral blood using classical transfer factor and trifactor transfer factor in children with ASD associated with GDFC.

2. To investigate the dynamics of the number of NKT cells in the blood using classical transfer factor and trifactor transfer factor in children with ASD associated with GDFC.

3. To establish the dynamics of the number of CD3 + T lymphocytes in the blood using classical transfer factor and trifactor transfer factor in children with ASD associated with GDFC.

4. To assess the effectiveness and safety of the use of classical transfer factor and trifactor transfer factor for the correction of key signs of immune dysregulation in children with ASD associated with GDFC.

Materials and methods. To achieve the goal and fulfill the objectives, the medical records of 225 children aged 2 to 9 years with GDFC and ASD (study group, SG) were studied. The SG included 183 boys and 42 girls. These children were patients of the Institute of Immunology and Allergology of the Bogomolets National Medical University (NMU) of the Ministry of Health of Ukraine (from 2012 to 2018) and the Vivere clinic, specializing in neuroimmunology, for the period from 2019 to 2022. Registration dossier of the Vivere clinic No. 10/2212–M dated 12/22/2018. Further processing of clinical material after receiving medical data in the clinic was carried out at the Institute of Experimental and Clinical Medicine of the Bogomolets National Medical University (NMU) in accordance with the cooperation agreement No. 150221 dated 02/15/2021 and based on the relevant conclusion of the NMU bioethical expertise commission according to the data of protocol No. 140 dated 12/21/2020. The clinical diagnosis of ASD for patients in the observation groups was made by experienced child psychiatrists specializing in the problem of psychospeech disorders in children, according to the validated diagnostic criteria of DSM–IV–TR.

Pathogenic polymorphic variants of nucleotide substitutions in the genes of folate cycle enzymes for diagnosing GDFC in patients of the observation groups were identified using the polymerase chain reaction (PCR) method with restriction in the Department of Neurobiochemistry of the Romodanov Institute of Neurosurgery of the National Academy of Medical Sciences of Ukraine (from 2012 to 2018) (**Figs. 10.1 and 10.2**) and the Sinevo laboratory, Ukraine (from 2019 to 2022).

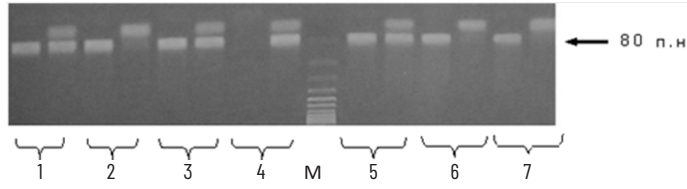

Fig. 10.1. Electrophoregram of allele-specific PCR products of the A1298C polymorphism of the MTHFR gene. Pair of lanes 2,6,7 – genotype AA; pair of lanes 1,3,5 – genotype AC; pair of lanes 4 – genotype CC, M – marker 100 bp

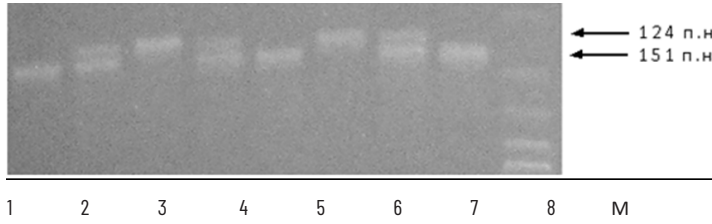

Fig. 10.2. Electrophoregram of restriction analysis products of the A66G polymorphism of the MTRR gene. Lanes 3,6 – genotype AA; lanes 2,4,7 – genotype AG; lanes 1,5,8 – genotype GG, M – marker 100 bp

At the same time, MTHFR C677T nucleotide substitutions were detected both in mono-form (68 SG patients; 30 % of cases) and in combination with other pathogenic nucleotide substitutions, in particular with MTHFR A1298C, MTR A2756G and/or MTRR A66G (157 SG patients; 70 % of cases). The genome, which included double pathological nucleotide substitutions MTHFR C677T + MTHFR A1298C, was observed in 26 (12.5 %), MTHFR C677T + MTRR A66G – in 19 (8.5 %), and MTHFR C677T + MTR A2756G – in 25 (11 % of cases) of SG children. The genome containing the triple pathological nucleotide substitutions MTHFR C677T + MTRR A66G + MTR A2756G occurred in 23 (10.5 %), MTHFR C677T + MTHFR A1298C + MTR A2756G – in 22 (9.5 %), and MTHFR C677T + MTHFR A1298C + MTRR A66G – in 21 (9 % of cases) of SG children. Finally, the genome containing all four studied pathogenic nucleotide substitutions, MTHFR C677T + MTHFR A1298C + MTR A2756G + MTRR A66G was identified in 21 (9 % of cases) of SG children (**Fig. 10.3**).

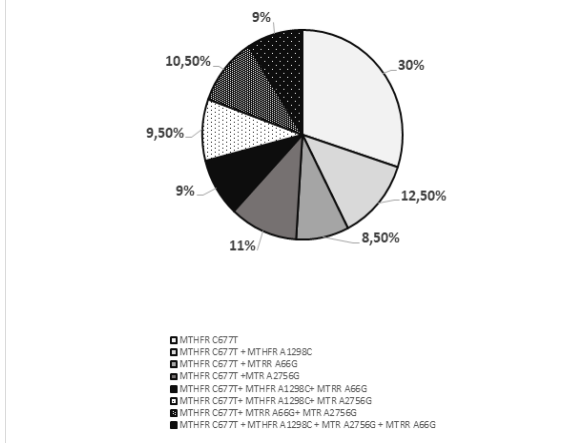

Fig. 10.3. SG structure (n = 225) based on genetic testing for GDFC

The number of CD3 + T-lymphocytes, NK- and NKT-cells in the peripheral blood of patients in the observation groups was measured using laser flow cytometry (Epics XI cytometer, USA) using the indirect immunofluorescence method using monoclonal antibodies to CD-markers of lymphocytes (single and triple label; Beckman Coulter reagents, USA). NK-cells were understood as a subpopulation of lymphocytes with the CD3-CD16 + CD56 + phenotype, and NKT-cells were understood as a subpopulation of lymphocytes with the CD3 + CD16 + CD56 + phenotype (**Fig. 10.4**).

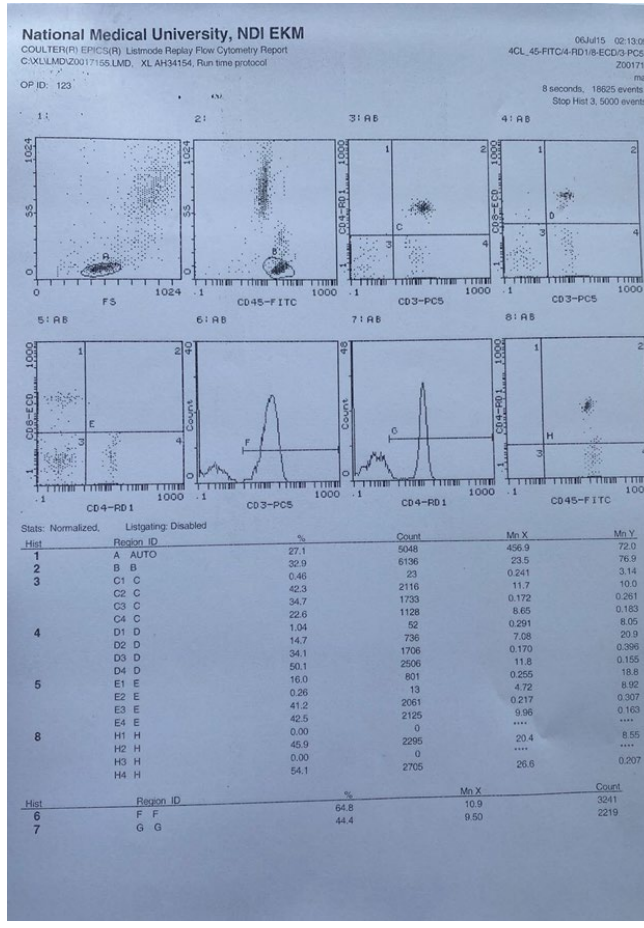

Fig. 10.4. Example of a flow laser cytofluorimetry protocol for studying the subpopulation composition of blood lymphocytes by CD markers on the Epics XI device at the Institute of Experimental and Clinical Medicine (NDI ECM) of the National Medical University

The mean number of CD3 + T lymphocytes in SG patients' peripheral blood at the study's beginning was 4.24±0.15x10^9/l. The number of CD3 + T lymphocytes in the peripheral blood of SG children exceeded the upper limit of normal in 72 % of cases, and in the remaining 28 % of cases it was normal. The mean number of NK cells in SG patients' peripheral blood at the study's beginning was 0.08±0.004x10^9/l, and the mean number of NKT lymphocytes was 0.03±0.009x10^9/l. At the beginning of the study, combined NK and NKT cell deficiency occurred in 82 % of SG patients, while NK cell deficiency in general occurred in 65 %, and in isolated form in only 9 % of cases. In contrast, NKT cell deficiency in general occurred in SG in 73 %, and in isolated form in 17 % of cases (**Fig. 10.5**).

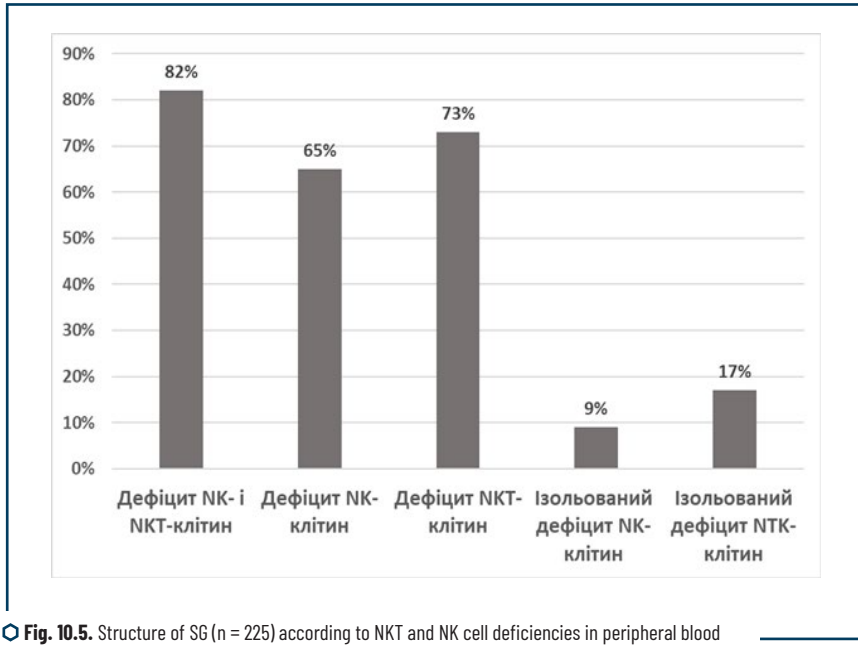

Fig. 10.5. Structure of SG (n = 225) according to NKT and NK cell deficiencies in peripheral blood

For the purpose of immunocorrection, SG patients used the original standardized immune extracts of bovine colostrum Transfer Factor Classic 4Life (UltraFactor XF® – 600 mg) 2 capsules 3 times a day during meals and Transfer Factor Tri–Factor 4Life (UltraFactor XF® 600 mg + OvoFactor® + NanoFactor®) 1 capsule 3 times a day during meals for 3 consecutive months. UltraFactor XF® is a specific concentrate of ultrafiltrate of immune proteins and other peptides of bovine colostrum, OvoFactor® is a specific concentrate of immune proteins and other peptides from chicken egg yolks, and NanoFactor® is a proprietary concentrate of nanofiltrate of bovine colostrum. Measurement of the number of CD3 + T–lymphocytes, NK– and NKT–cells in the peripheral blood of SG patients during the observation period was carried out 4 times a month – before the start of the study, after the first, second and third months of transfer factor application.

The same SG was used as in the case of the testing of Propes and Inflamafertin, but in a different time period, after the restoration of the deficiency of NK– and NKT–cells after a certain time (on average – from 6 to 12 months) after the complete cessation of the therapeutic effect of the tested peptide drugs.

The control group (CG) included medical records of 52 children with GDFC and ASD of similar age (2 to 8 years) and gender distribution (37 boys and 15 girls) who similarly had NK– and NKT–cell deficiencies. CG patients did not receive transfer factors during the observation period. CG children also underwent four-time monthly monitoring of the numbers of CD3 + T–lymphocytes, NK– and NKT–cells for 3 consecutive months to assess immune status during the natural course of the disease.

Statistical processing of the obtained material was carried out using comparative and structural analyses. To study the distribution of variants in the variation series, the Shapiro–Wilk test was used. To establish the probability of the obtained differences between the values of the studied laboratory indicators in the observation groups, the parametric Student's T-test with an additional measurement of the confidence probability indicator p and the nonparametric Z-test according to Urbach V.Yu. [16] were used. Differences were considered probable in the case of obtaining $p < 0,05$ and $Z < Z_{0,05}$.

To study the relationship between the appointment of transfer factors and the dynamics of the studied indicators of immune status, the calculation of the odds ratio (OR) and 95 % confidence interval (95 % CI) was used. The information was processed using the Microsoft Excel computer program (Redmond, WA).

Sources of funding. This clinical study was implemented as a fragment of scientific research work with funds from the State Budget of Ukraine under No. 0121U107940.

Research results and their discussion. The results of the structural analysis in the observation groups indicate that the number of NK cells reached the lower limit of normal in 109 of 146 patients (75 % of cases) with a baseline deficiency of these lymphocytes, and the average number of NK cells in the blood in the SG increased almost threefold during the 3-month course of transfer factors. In contrast, the number of NKT cells normalized in 127 of 164 patients (77 % of cases) with a baseline deficiency of these cells, and the average number of NKT cells in the blood in the SG increased almost 4-fold during the course of immunotherapy compared to the baseline level. In contrast, the number of NK cells increased to the lower limit of normal in only 7 of 36 patients (15 %), and the number of NKT cells in only 9 of 42 people (21 %) of the CG with a baseline low number of these lymphocytes in the peripheral blood at the end of the observation period, which was a significant difference from the data in the SG ($p<0,05$; $Z<Z_{0,05}$). The results of the structural analysis in the observation groups indicate that NK and NKT cell deficiencies are not only common, but also quite persistent laboratory phenomena in children with ASD associated with GDFC, which usually do not undergo spontaneous resolution during the natural course of the disease. The combined use of transfer factors contributes to the restoration of previously reduced numbers of NK and NKT lymphocytes in the peripheral blood in the majority of children with ASD associated with GDFC, compensating for key disorders of cellular immunity and thereby modifying the immune status of patients.

Comparative analysis data indicate no differences in the achieved proportion of complete responders to the tested combination of transfer factors for correction of NK and NKT cell deficiency among SG patients at the end of the observation period ($p > 0,05$; $Z > Z_{0,05}$). This means that the combined use of transfer factors is equally effective in NK and NKT lymphocyte deficiencies in children with ASD associated with GDFC, with initial low numbers of these cells in the peripheral blood.

At the same time, the comparative analysis data did not reveal a significant difference in the proportion of responders to immunocorrective interventions using transfer factors in isolated NK and NKT cell deficiencies and combined disorders, when a single patient has a simultaneous decrease in the number of both NK and NKT lymphocytes ($p > 0,05$; $Z > Z_{0,05}$). This may mean that NK and NKT cells respond to transfer factors separately, in an independent manner, and patients with isolated and combined disorders are equally sensitive to the immunocorrective interventions used.

Data from dynamic observation of the studied laboratory indicators of immune status in SG and CG indicate a gradual increase in the number of NK and NKT cells in the peripheral blood of SG patients at each control point with the achievement of the maximum level at the end of the course of immunocorrection and the absence of significant changes in the average numbers of NK and NKT lymphocytes in the peripheral blood of CG individuals during the observation period. The average number of NK cells in the peripheral blood during the observation period in SG increased 3 times, and NKT cells – more than 4 times (**Figs. 10.6** and **10.7**). There was a significant difference between the initial mean numbers of NK and NKT cells in SG at the beginning of the study and at the end of the 3rd month of immunocorrection ($p > 0,05$; $Z > Z_{0,05}$). A similar significant difference in the mean numbers of NK and NKT lymphocytes was found among SG and CG patients at the 3rd month of observation ($p > 0,05$; $Z > Z_{0,05}$). However, no significant difference was found in the mean numbers of NK and NKT cells in CG at all control points during the entire observation period ($p > 0,05$; $Z > Z_{0,05}$). This may indicate that the use of transfer factors not only normalizes the previously reduced number of NK and NKT lymphocytes in the peripheral blood of children with ASD associated with GDFC, but also provides a progressive increase in the number of these cells over time throughout the course of immunocorrection. We can speak of a time-dependent effect of the use of transfer factors in NK and NKT cell deficiencies in children with ASD associated with GDFC. This progressive manner of response to transfer factors by NK and NKT lymphocytes in SG allows us to consider the hopes of achieving the success of immunocorrection interventions by extending the duration of their use without increasing the dosages of immunobiological agents as justified, when the lower limit of the norm of the studied laboratory indicator after the previous course of immunocorrection was not reached.

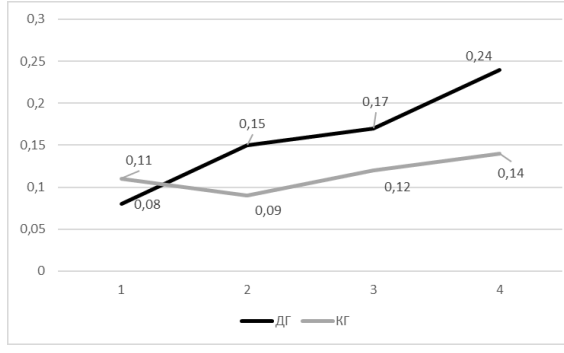

Fig. 10.6. Dynamics of the average number of NK cells in the peripheral blood of patients SG (n = 225) and CG (n = 52) during the observation period

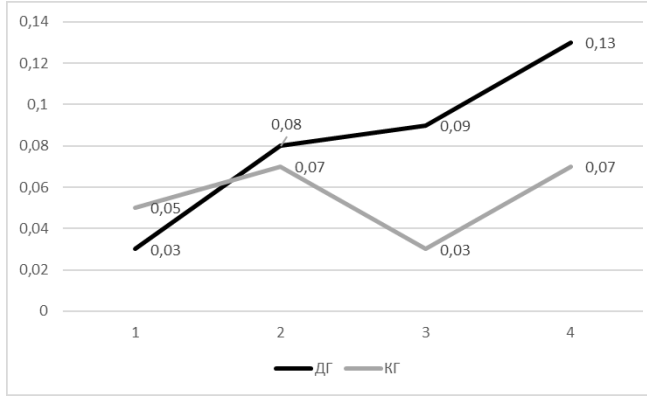

Fig. 10.7. Dynamics of the average number of NKT cells in the peripheral blood of patients SG (n = 225) and CG (n = 52) during the observation period

If we talk about the assessment of the dynamics of the number of CD3 + T lymphocytes in patients of the observation groups, then there was no significant difference in the average numbers of these cells in the peripheral blood of patients in both the SG and the CG at all control points during the study period ($p > 0,05$; $Z > Z_{0,05}$). There was also no significant difference in the average numbers of CD3 + T-lymphocytes in the peripheral blood between the SG and the CG at all control points during the observation period ($p > 0,05$; $Z > Z_{0,05}$) (**Fig. 10.8**). This indicates that the use of transfer factors does not affect the number of CD3 + T-cells in the peripheral blood in children with ASD associated with GDFC. Such patients are characterized by a state of immune dysregulation, which is associated with a number of immune-dependent complications, and an abnormally increased number of CD3 + T-lymphocytes in the peripheral blood is an important component of the specified immune dysregulation. When testing transfer factors, it was important to check not only the effectiveness of correcting NK and NKT cell deficiencies, but also their safety in terms of their impact on immunoregulatory disorders. Transfer factors do not increase the number of CD3 + T-cells in the blood, which may indicate the absence of a negative effect on the existing immune dysregulation in children with ASD, one of the manifestations of which is a large number of cells of the specified lymphocyte population in the blood.

To verify the available data on the association of the used immunocorrective interventions and the normalization of the number of NK and NKT cells in the peripheral blood of SG patients at the end of the observation period, the odds ratio (OR), standard error of the odds ratio (S) and 95 % confidence interval (95 % CI) were calculated. The "outcome" was understood as the normalization of the number of the studied lymphocyte subpopulations, and the "risk factor" was the use of transfer factors. This would avoid errors in assessing the conjugation between the studied processes at the previous stages of statistical analysis. The results obtained are presented in **Table 10.1**.

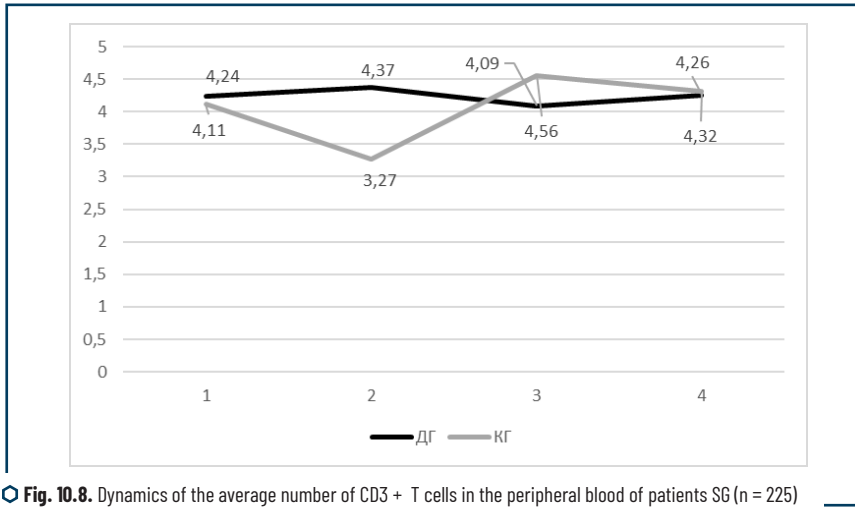

Fig. 10.8. Dynamics of the average number of CD3 + T cells in the peripheral blood of patients SG (n = 225) and CG (n = 52) during the observation period

Table 10.1. Results of OR, S and 95 % CI measurements in assessing the association of immunocorrective interventions and normalization of immune status indicators in SG patients (n = 225)

Indicator	NK cells	NKT cells
Odds ratio (OR)	12.205	12,586
Standard error of odds ratio (S)	0,462	0.420
95 % confidence interval (95 % CI)	4,934–30,191	5,526–28,661

As can be seen from the data in **Table 10.1**, the calculation of OR and 95 % CI confirms the previously obtained results on the close relationship between the use of transfer factors and the normalization of the studied indicators of immune status in patients with SG. The fact, identified at the previous stage of statistical analysis of the data, of almost equally high sensitivity of NKT and NK lymphocytes to combined immunocorrective interventions using transfer factors, was demonstrated again. Thus, the use of transfer factors in SG was associated with an almost 12-fold increase in the chances of normalization of previously reduced numbers of NKT– and NK–cells in peripheral blood.

Transfer factors were well tolerated. No adverse reactions were noted during their use during the observation period.

Research results and their discussion. A wide range of immune system dysfunctions in children with ASD have been described and characterized. The results of this scientific search are summarized in the data of three recent systematic reviews of clinical studies.

Thus, the data of the systematic review by Mead J. et al. demonstrated signs of immune dysregulation in ASD: neuroinflammation, autoantibodies, abnormally enhanced T-cell response, impaired activity of natural killer cells and monocytes. These immune aberrations were associated with the severity of mental disorders in ASD, in particular, impaired social interaction, stereotyped behavior and reduced communication skills. This review notes that experimental animal models have made it possible to achieve the elimination of clinical symptoms of ASD after removing from the body those immune factors that were involved in aberrant immune reactions [6].

Accordingly, Noriega D.B., Savelkoul H.F. in a systematic review of ASD in children described signs of immune dysregulation, including hyperproduction of pro- and suppression of synthesis of anti-inflammatory cytokines, as well as increased permeability of the blood-brain barrier, abnormal production of anti-brain autoantibodies, and modification of NK functional activity [7]. According to the data of a systematic review by Hughes H.K. et al. on the state of the immune system in children with ASD, there is a disturbed cytokine balance, quantitative disorders of immunocompetent cells, signs of persistent neuroglial inflammation in the CNS, defects in the functioning of the adaptive and innate immune systems, including NK dysfunction, as well as pathological deviations in serum concentrations of immunoglobulins of different classes and subclasses, signs of autoimmune reactions to neurons, myelin and extracerebral autoantigens [8].

Since children with ASD are a rather heterogeneous group, the features of immunological disorders may differ in certain subgroups of patients. Accordingly, a specific form of immunodeficiency has been described in children with ASD associated with GDFC, the key disorders in which there is a deficiency of NK and NKT lymphocytes [17]. The results of a number of studies demonstrate various immunological disorders, including NK deficiency, in GDFC and folic acid deficiency in humans [18]. The mechanism of immunosuppression and immune dysregulation in such cases is seen in gene regulatory and epigenetic disorders associated with impaired DNA, protein, lipid and nucleoprotein methylation processes [11], as well as with a state of persistent oxidative stress and critical weakening of the antioxidant system [19], deficiency of vitamins, trace elements and essential nutrients due to immunoinflammatory damage to the intestinal wall [20].

The mechanism of immune transfer from mother's breast milk to the intestine of infants is one of the main components of the unprecedented advantage of mammals in the struggle for existence in the biological world. The use of transfer factors based on immune extracts of colostrum in the clinic artificially recreates the physiological mechanism of immune transfer as an inherent property of mammals, which was supported by natural selection in the process of evolution of biological organisms, convincingly demonstrating its effectiveness in the long struggle for the existence of mammals with representatives of other classes of biological beings [21].

The data obtained in this clinical study indicate the appropriate immunomodulatory effect of the tested immunocorrective strategy using transfer factors in a specific form of immunodeficiency observed in children with ASD associated with GDFC. Immunodeficiency and the associated immune dysregulation in children with ASD are most likely responsible for the development of a number of immune-dependent complications that affect both the severity of mental disorders and the level of health of the child in general.

As is known, in ASD there is an abnormally high microbial load with a predominance of opportunistic pathogens [22], persistent immunoinflammatory enterocolitis [23], various allergic manifestations [24], a state of systemic inflammation with hypercytokinemia [25] and signs of autoimmunity against neurons, myelin and some extracerebral autoantigens [26]. Normalization of the impaired immune status may be the key to preventing the development of a number of immune-dependent complications in ASD children associated with GDFC, in whom there is a deficiency of NK and/or NKT cells. The development and implementation of effective immunomodulatory strategies with a targeted effect on the affected immune components is believed to contribute to the improvement of the clinical condition of children with ASD and may significantly improve the consequences of the disease. Therefore, the success of transfer factors demonstrated in the results of this clinical study allows us to hope for the earliest possible finding of an answer regarding effective, safe and convenient immunocorrective interventions for children with ASD, who show signs of immunodeficiency and immune dysregulation.

The composition of transfer factors based on a standardized immune extract of bovine colostrum was studied in detail by Sacerdote P. et al. in 2013 [27], and a comprehensive characterization of the proteome of bovine milk in the context of immune transfer was provided by Zhang L. et al. in 2015 [28]. It has been shown that transfer factors contain more than 100 proteins and peptides, including lysozyme, lactoferrin, alpha/beta defensins, cytokines, chemokines, immunoglobulins, soluble lymphocyte receptors, etc., which pass from blood serum to breast milk for the purpose of subsequent immune transfer to the infant's intestine during breastfeeding. Currently, immunomodulatory and anti-inflammatory effects of transfer factors based on an immune extract of bovine colostrum have been demonstrated in humans. In particular, the results of double-blind placebo-controlled randomized clinical trials indicate the potential effectiveness of transfer factors in frequent respiratory and intestinal infections in children, for the prevention of sepsis episodes in premature infants and in HIV-infected children with insufficient response to antiretroviral therapy [29, 30, 31, 32]. In accordance with this, Huppertz H.I. et al. in a double-blind placebo-controlled randomized clinical trial showed that immune bovine colostrum extract leads to a decrease in the symptoms of diarrhea that has already begun, caused by enterotoxigenic strains of E. coli, prevents the development of exicosis and reduces the need for infusion therapy [29]. Patıroğlu T., Kondolot M. in a double-blind placebo-controlled randomized clinical trial involving 31 patients found that oral immunotherapy with immune bovine colostrum extract leads to a sharp decrease in the frequency and severity of upper respiratory tract infections in patients with primary selective IgA deficiency without changing the content of secretory IgA in saliva [30]. On the other hand, Lee J. et al. in a double-blind placebo-controlled randomized clinical trial demonstrated that immune extract of bovine colostrum reduces the number of episodes of sepsis in premature infants with low body weight and signs of immune system immaturity. In parallel, they achieved an increase in the concentration of secretory immunoglobulin class A and lactoferrin in biological media, which indicated an increase in immunoresistance due to optimization of the work of local mucosal immunity. There was also a decrease in the content of transforming growth factor beta, interleukin-1beta and interleukin-6 in urine and saliva, which indicated the implementation of an anti-inflammatory effect [31]. Byakwaga H. et al. in a placebo-controlled randomized clinical trial involving 75 patients, it was shown that an immune extract of bovine colostrum leads to an increase in the

number of CD4 + T-helper cells in the blood of patients with HIV-induced AIDS who poorly respond to highly active antiretroviral therapy administered according to an international protocol [32].

The results of this clinical trial expand the list of potential indications for the use of transfer factors based on immune extract of bovine colostrum for the correction of immune disorders in children with ASD associated with GDFC.

Data from previous controlled clinical trials dedicated to finding ways to compensate for the deficiency of NK and NKT cells indicate the potential benefit of combined immunotherapy with Propes and Inflamafertin in both children with ASD associated with GDFC [12] and adults with chronic fatigue syndrome/myalgic encephalomyelitis in GDFC [33], but these encouraging data need to be verified in larger controlled clinical trials with greater validity of the results obtained. Propes is a biological agent containing alpha- and beta-defensins, which has pronounced immunoactivating and lymphoproliferative effects. At the same time, Inflamafertin, which includes alarmins and adrenomedullin, on the contrary, has an anti-inflammatory effect mediated by interleukin 10, which is important in preventing autoimmune complications during drug-induced immune activation. As the accumulated experience of using another highly active immunomodulatory agent – recombinant interleukin 2 – indicates, therapeutic immune activation can cause an undesirable increase in the risk of developing autoimmune complications [34], therefore, the combination of the immunoactivating drug Propes with an anti-inflammatory tolerogenic immunotropic agent seems to be the key to achieving a safe immunomodulatory therapeutic effect. As the results of this clinical study show, the combined use of transfer factors is associated with results in terms of correction of NK- and NKT-cell deficiencies and tolerability of corrective interventions similar to those obtained with the use of Propes and Inflamafertin, however, transfer factors turned out to be significantly cheaper, and therefore more accessible to patients, and technically more convenient to use, since they do not require long-term intramuscular injections and special storage conditions.

Conclusions. The results obtained in this single-center retrospective controlled non-randomized clinical study indicate that the combined use of transfer factors based on standardized immune extracts of bovine colostrum is an effective strategy for the correction of NK- and NKT-cell deficiencies in children with ASD associated with GDFC, with an appropriate safety profile. These biological immunotropic agents are able to normalize the previously reduced number of NK and NKT cells in the peripheral blood in the specified category of patients already during a 3-month immunocorrection course with the same effect on NKT and NK lymphocytes in a time-dependent manner without increasing the number of CD3 + T cells in the peripheral blood of patients. It seems promising to continue clinical studies in the field of the use of transfer factors in children with ASD, who show signs of immunodeficiency and immune dysregulation.

REFERENCES

1. Pu, D., Shen, Y., Wu, J. (2013). Association between MTHFR Gene Polymorphisms and the Risk of Autism Spectrum Disorders: A Meta-Analysis. Autism Research, 6 (5), 384–392. https://doi.org/10.1002/aur.1300

2. Shaik Mohammad, N., Sai Shruti, P., Bharathi, V., Krishna Prasad, C., Hussain, T., Alrokayan, S. A. et al. (2016). Clinical utility of folate pathway genetic polymorphisms in the diagnosis of autism spectrum disorders. Psychiatric Genetics, 26 (6), 281–286. https://doi.org/10.1097/ypg.0000000000000152
3. Rai, V. (2016). Association of methylenetetrahydrofolate reductase (MTHFR) gene C677T polymorphism with autism: evidence of genetic susceptibility. Metabolic Brain Disease, 31 (4), 727–735. https://doi.org/10.1007/s11011-016-9815-0
4. Sadeghiyeh, T., Dastgheib, S. A., Mirzaee-Khoramabadi, K., Morovati-Sharifabad, M., Akbarian-Bafghi, M. J., Poursharif, Z. et al. (2019). Association of MTHFR 677C > T and 1298A > C polymorphisms with susceptibility to autism: A systematic review and meta-analysis. Asian Journal of Psychiatry, 46, 54–61. https://doi.org/10.1016/j.ajp.2019.09.016
5. Li, Y., Qiu, S., Shi, J., Guo, Y., Li, Z., Cheng, Y., Liu, Y. (2020). Association between MTHFR C677T/A1298C and susceptibility to autism spectrum disorders: a meta-analysis. BMC Pediatrics, 20 (1). https://doi.org/10.1186/s12887-020-02330-3
6. Mead, J., Ashwood, P. (2015). Evidence supporting an altered immune response in ASD. Immunology Letters, 163 (1), 49–55. https://doi.org/10.1016/j.imlet.2014.11.006
7. Noriega, D. B., Savelkoul, H. F. J. (2013). Immune dysregulation in autism spectrum disorder. European Journal of Pediatrics, 173 (1), 33–43. https://doi.org/10.1007/s00431-013-2183-4
8. Hughes, H. K., Mills Ko, E., Rose, D., Ashwood, P. (2018). Immune Dysfunction and Autoimmunity as Pathological Mechanisms in Autism Spectrum Disorders. Frontiers in Cellular Neuroscience, 12. https://doi.org/10.3389/fncel.2018.00405
9. Alhuzimi, T. E., Alharbi, M. H. (2022). Perceptions of Parents on Management of Food Allergy in Children with Autism Spectrum Disorder (ASD) in Saudi Arabia. Children, 10 (1), 48. https://doi.org/10.3390/children10010048
10. Tordjman, S., Charrier, A., Kazatchkine, M., Roubertoux, P., Botbol, M., Bronsard, G., Avrameas, S. (2023). Natural IgG Anti-F (ab')2 Autoantibody Activity in Children with Autism. Biomedicines, 11 (3), 715. https://doi.org/10.3390/biomedicines11030715
11. Alshamrani, A. A., Alshehri, S., Alqarni, S. S., Ahmad, S. F., Alghibiwi, H., Al-Harbi, N. O. et al. (2023). DNA Hypomethylation Is Associated with Increased Inflammation in Peripheral Blood Neutrophils of Children with Autism Spectrum Disorder: Understanding the Role of Ubiquitous Pollutant Di(2-ethylhexyl) Phthalate. Metabolites, 13 (3), 458. https://doi.org/10.3390/metabo13030458
12. Maltsev, D., Stefanyshyn, V. (2021). Efficacy of combined immunotherapy with propes and inflamafertin in selective deficiency of nk and nkt cells in children with autism spectrum disorders associated with genetic deficiency of the folate cycle. Current Pediatric Research, 25 (4), 536–540.
13. Artym, J., Zimecki, M. (2023). Colostrum and Lactoferrin Protect against Side Effects of Therapy with Antibiotics, Anti-inflammatory Drugs and Steroids, and Psychophysical Stress: A Comprehensive Review. Biomedicines, 11 (4), 1015. https://doi.org/10.3390/biomedicines11041015
14. Artym, J., Zimecki, M. (2023). Colostrum Proteins in Protection against Therapy-Induced Injuries in Cancer Chemo- and Radiotherapy: A Comprehensive Review. Biomedicines, 11 (1), 114. https://doi.org/10.3390/biomedicines11010114

15. Bagwe-Parab, S., Yadav, P., Kaur, G., Tuli, H. S., Buttar, H. S. (2020). Therapeutic Applications of Human and Bovine Colostrum in the Treatment of Gastrointestinal Diseases and Distinctive Cancer Types: The Current Evidence. Frontiers in Pharmacology, 11. https://doi.org/10.3389/fphar.2020.01100
16. Urbakh, V. Iu. (1975). Statisticheskii analiz v biologicheskikh i meditcinskikh issledovaniiakh. Moscow: Meditcina, 295.
17. Maltsev, D. (2020). Features of folate cycle disorders in children with ASD. Bangladesh Journal of Medical Science, 19 (4), 737–742. https://doi.org/10.3329/bjms.v19i4.46634
18. Wu, J., You, Q., Lyu, R., Qian, Y., Tao, H., Zhang, F. et al. (2023). Folate metabolism negatively regulates OAS-mediated antiviral innate immunity via ADAR3/endogenous dsRNA pathway. Metabolism, 143, 155526. https://doi.org/10.1016/j.metabol.2023.155526
19. Liu, X., Lin, J., Zhang, H., Khan, N. U., Zhang, J., Tang, X. et al. (2022). Oxidative Stress in Autism Spectrum Disorder – Current Progress of Mechanisms and Biomarkers. Frontiers in Psychiatry, 13. https://doi.org/10.3389/fpsyt.2022.813304
20. Indika, N.-L. R., Frye, R. E., Rossignol, D. A., Owens, S. C., Senarathne, U. D., Grabrucker, A. M. et al. (2023). The Rationale for Vitamin, Mineral, and Cofactor Treatment in the Precision Medical Care of Autism Spectrum Disorder. Journal of Personalized Medicine, 13 (2), 252. https://doi.org/10.3390/jpm13020252
21. Lyons, K. E., Ryan, C. A., Dempsey, E. M., Ross, R. P., Stanton, C. (2020). Breast Milk, a Source of Beneficial Microbes and Associated Benefits for Infant Health. Nutrients, 12 (4), 1039. https://doi.org/10.3390/nu12041039
22. Nicolson, G. L., Gan, R., Nicolson, N. L., Haier, J. (2007). Evidence for Mycoplasma ssp., Chlamydia pneunomiae, and human herpes virus-6 coinfections in the blood of patients with autistic spectrum disorders. Journal of Neuroscience Research, 85 (5), 1143–1148. https://doi.org/10.1002/jnr.21203
23. Torrente, F., Ashwood, P., Day, R., Machado, N., Furlano, R. I., Anthony, A. et al. (2002). Small intestinal enteropathy with epithelial IgG and complement deposition in children with regressive autism. Molecular Psychiatry, 7 (4), 375–382. https://doi.org/10.1038/sj.mp.4001077
24. Li, H., Liu, H., Chen, X., Zhang, J., Tong, G., Sun, Y. (2020). Association of food hypersensitivity in children with the risk of autism spectrum disorder: a meta-analysis. European Journal of Pediatrics, 180 (4), 999–1008. https://doi.org/10.1007/s00431-020-03826-x
25. Saghazadeh, A., Ataeinia, B., Keynejad, K., Abdolalizadeh, A., Hirbod-Mobarakeh, A., Rezaei, N. (2019). A meta-analysis of pro-inflammatory cytokines in autism spectrum disorders: Effects of age, gender, and latitude. Journal of Psychiatric Research, 115, 90–102. https://doi.org/10.1016/j.jpsychires.2019.05.019
26. Edmiston, E., Ashwood, P., Van de Water, J. (2017). Autoimmunity, Autoantibodies, and Autism Spectrum Disorder. Biological Psychiatry, 81 (5), 383–390. https://doi.org/10.1016/j.biopsych.2016.08.031
27. Sacerdote, P., Mussano, F., Franchi, S., Panerai, A. E., Bussolati, G., Carossa, S. et al. (2013). Biological components in a standardized derivative of bovine colostrum. Journal of Dairy Science, 96 (3), 1745–1754. https://doi.org/10.3168/jds.2012-5928

28. Zhang, L., Boeren, S., Hageman, J. A., van Hooijdonk, T., Vervoort, J., Hettinga, K. (2015). Bovine Milk Proteome in the First 9 Days: Protein Interactions in Maturation of the Immune and Digestive System of the Newborn. PLOS ONE, 10 (2), e0116710. https://doi.org/10.1371/journal.pone.0116710
29. Huppertz, H., Rutkowski, S., Busch, D. H., Eisebit, R., Lissner, R., Karch, H. (1999). Bovine Colostrum Ameliorates Diarrhea in Infection with Diarrheagenic Escherichia coli, Shiga Toxin-Producing E. coli, and E. coli Expressing Intimin and Hemolysin. Journal of Pediatric Gastroenterology and Nutrition, 29 (4), 452–456. Portico. https://doi.org/10.1002/j.1536-4801.1999.tb02450.x
30. Patıroğlu, T., Kondolot, M. (2011). The effect of bovine colostrum on viral upper respiratory tract infections in children with immunoglobulin A deficiency. The Clinical Respiratory Journal, 7 (1), 21–26. https://doi.org/10.1111/j.1752-699x.2011.00268.x
31. Lee, J., Kim, H.-S., Jung, Y. H., Choi, K. Y., Shin, S. H., Kim, E.-K. et al. (2015). Oropharyngeal Colostrum Administration in Extremely Premature Infants: An RCT. Pediatrics, 135 (2), e357–e366. https://doi.org/10.1542/peds.2014-2004
32. Byakwaga, H., Kelly, M., Purcell, D. F. J., French, M. A., Amin, J., Lewin, S. R. et al. (2011). Intensification of Antiretroviral Therapy With Raltegravir or Addition of Hyperimmune Bovine Colostrum in HIV-Infected Patients With Suboptimal CD4 + T-Cell Response: A Randomized Controlled Trial. The Journal of Infectious Diseases, 204 (10), 1532–1540. https://doi.org/10.1093/infdis/jir559
33. Maltsev, D., Stefanyshyn, V. (2022). The efficacy of combined immunotherapy with Propes and Inflamafertin in adult patients with genetic deficiency of the folate cycle and selective deficiency of NK and NKT cells. Immunology, 167 (3), 443–450. https://doi.org/10.1111/imm.13548
34. Ko, B., Takebe, N., Andrews, O., Makena, M. R., Chen, A. P. (2023). Rethinking Oncologic Treatment Strategies with Interleukin-2. Cells, 12 (9), 1316. https://doi.org/10.3390/cells12091316