CLINICAL

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CHARACTERISTICS OF THE ANTIENDOTOXIC ANTIBODIES (AEAs) SYSTEM IN CHILDREN WITH ASTHMA.

– THE INFLUENCE OF COLOSTRO NONI

SUMMARY

The research objective is to study the influence of Colostro Noni on the Immune System and on the levels of humoral AEAs in patients with bronchial asthma during the remission period.

85 ill children with asthma were under our care; the group included 46 boys (54.1%) and 39 girls (45.9%). Patients aged from 7 to 15 years (11±1.4); 34 (40%) patients had the disease for 2-5 years, 40 patients (47.1%) had the disease for 6-9 years, and 11 patients (12.9%) had been ill for 10 years and more.
The remission period was about 6-24 months (average 8.2 ±2.3 months).

The control group (CG) was made up of 16 intact peers who belonged to the second health group and had not had any respiratory and contagious diseases for the last three months.
The number of boys and girls among these children was equal. All the examined children had history taking, general physical, and dynamic examination (ECG, spirography).

Immunomodulatory effect of Colostro Noni on cellular immune link consisted in accurate (p<0.05-0.001) increase of average number of CD3+, CD4+ cells, and significant rise (by 2 %) of lymphocytes carrying on their surface CD8+ receptors.
– These changes took place against the decline of CD20+ number; this fact bore a record to normalization of immune response of the child with asthma.
The changes in humoral immune link with application of Colostro Noni consisted in an accurate (p<0.001) decline of IgE and serum IgA as well as slight increase of IgM.
This fact bears the record to the decrease of allergization of the ill children.

Colostro Noni was well tolerated by all the patients with asthma and did not show any negative side effect.

KEY WORDS **ANTIENDOTOXIC ANTIBODIES (AEAs), ASTHMA, COLOSTRO NONI**

INTRODUCTION

The treatment and prevention of **asthma in children** remain nowadays one of the most urgent problems.
Despite huge amount of remedies and drug-free modalities **it is not always possible to control the disease**, while the mechanisms of persistence of the respiratory tracts inflammation have not been studied enough.
– The pathogenesis of asthma is inseparably associated with production of Th2 (type 2 T *helper* cells) by T cell-mediated immunity at early postnatal period of life. In this respect, a role of endotoxin (lipopolysaccharide) of Gram-negative bacteria stimulating immune response through Th1 (type 1 T *helper* cells) contributing to the reduction of asthma risk and its clinical aspects, is very important. At the same time, the treatment with endotoxin could intensify the allergy and asthma. However, these questions are not covered enough in literature.
– Disorders of the mucous membrane of a respiratory tract and digestive tube in children provide conditions for pathological effects of endotoxin coming by exogenic and endogenic means, specifically when there is a disorder of the scavenger receptors of the immune-competent cells.
– An important part in the clearance of the endotoxins is taken by natural AEAs, which are considered to be a general integrated marker simultaneously reflecting body reaction to endotoxins and characterizing the inborn immunity to Gram-negative bacteria.
In our opinion, the study of the endotoxins role by the example of *E. coli* and the system of endotoxic antibodies including antibodies of A, M, G class in children with asthma allows to specify and complete the mechanisms of persistence of respiratory tracts inflammation and develop treatment and rehabilitation facilities aimed to decrease the pathologic influence of the endotoxins taking into account a condition of humoral antiendotoxic immunity within the general strategy of asthma treatment.

The research objective is to study the influence of **Colostro Noni** on the Immune System and on the levels of humoral AEAs in patients with bronchial asthma during the remission period.

MATERIALS AND METHODS

85 ill children with asthma were under our care; the group included 46 boys (54.1%) and 39 girls (45.9%).

Patients aged from 7 to 15 years (11±1.4); 34 (40%) patients had the disease for 2-5 years, 40 patients (47.1%) had the disease for 6-9 years, and 11 patients (12.9%) had been ill for 10 years and more.

The remission period was about 6-24 months (average 8.2 ±2.3 months).

Diagnosis of bronchial asthma was conformed in compliance with the order of the Ministry of Healthcare of Ukraine No 767 December 27, 2005.

Intermitting course of asthma was detected in 28 children (32.9%), mild long-lasting course in 29 children (34.2%), and moderately severe persistent course in 28 children (32.9%).

– The control group (CG) was made up of 16 intact peers who belonged to the second health group and had not had any respiratory and contagious diseases for the last three months. The number of boys and girls among these children was equal.

All the examined children had history taking, general physical and dynamic examination (ECG, spirography).

The study of the immune *status* was carried out with the help of a set of standard unified tests of the first-second level including evaluation of the total number of leucocytes and lymphocytes, indexes of phagocytosis, level of T- and B-lymphocytes, IgA, IgM, IgG and IgE representing the functioning of the main links of the immune system (phagocytic, cellular and humoral) and the level of circulating immune complexes.

Cellular link of the Immune System was estimated with indirect immunofluorescence. Panels of monoclonal antibodies were used (R.E. Kravetsky Cancer Institute, Kiev) and the results were analyzed by immunology analyzer. CD3+, CD4+, CD8+ were determined and immunoregulatory index (CD4+/CD8+) was estimated.

The amount of B-lymphocytes (CD20+) and the concentration of IgA, IgM, and IgG was determined for specification of humoral component of Immune System with the help of microturbidity method. Monotypic serum obtained at SRI of epidemiology and microbiology named after H.F. Gamaley (Moscow, Russia), test serum of human blood made with the help of a set for immunoglobulin assessment according to microturbidity method (SRI of epidemiology and microbiology, Gorky-City, Russia) (with the concentration of IgG-8.47 mg/ml; IgM-1.04 mg/ml; IgA-1.53 mg/ml) were applied for IgA, IgM, and IgG testing.

Immunoenzyme analyzer was used for the definition of optical medium opacities resulting from antigen-antibody reaction. All measurements were carried out by the wavelength of 492 nm.

Concentration of antibodies were calculated according to the existing calibration curve formulas; the results received were transformed into mg/ml, multiplying by rarefaction of serum.

Level of IgE was determined by immunofluorescent assay (IFA).

Levels of antibodies of A, M, G class specific to lipopolisaccharides (LPSs) of *E. coli* (*Escherichia coli* K30), anti-LPS-IgA, anti-LPS-IgM, anti-LPS-IgG respectively, were determined by enzyme-linked immunosorbent assay according to protocols developed at the Laboratory of Clinical Immunology of the Central Research Laboratory of S.I. Georgievsky Crimea State Medical University.

LPS was obtained from biomass of Gram-negative bacteria *Escherichia coli* K30 by phenol-water extraction and further cleared from RNA by cetavlon (Serva) (Vestfal, Yann. 1967).

100 mkl of solution of LPS *Escherichia coli* K30 (10 mkl/ml) was added to wells of polystyrene plates (All-Russian SRI Medpolimer, Russia) and incubated for 12-18 hours at the temperature of 37° for AEAs determination. Wells were cleaned (5 times in a minute) with a phosphate buffer (0.033 M), pH 7.4, containing a 1% NaCl solution and 0.05% Tween-20 (PBS-T) for disposal of unused LPS and blocking of free binding sites. Then 100 mcl of rare serum of patients (anti-LPS-IgA and anti-LPS-IgM were 50 times diluted by PBS-T; during the evaluation of anti-LPS, IgG level the serum was diluted 1:100, it was further diluted till the proportion became 1:400) and conjugate of goat's affinity purified antibodies against human immunoglobulins with horseradish peroxidase (Sigma, A8786) (the proportion of anti-LPS-IgG of a human is 1:4000; the proportion of anti-LPS-IgA and anti-LPS-IgM is 1:5000) were added to each well. Each reagent was incubated for 60 minutes at 37°.

Non-specifically bounded components were removed with the help of PBS-T (5 times in a minute) at each stage.

For the recording of the peroxidase reaction we added 100 mcl of substrate buffer (30 MW phosphate citrate buffer pH 5.0 containing 0.33 mg/ml of o-phenylenediamine and 0.02% H_2O_2) to each well and incubated for 1 hour at 37°. The reaction was stopped by adding 25 mcl 3M of sulphuric acid.

Optical density of the final product of enzyme reaction was specified by enzyme-linked immunosorbent assay with 492 nm wavelength.

The study of the phagocytic component of the Immune System was carried out by methods based on ability of neutrophilic leukocytes to absorb and digest microbes.

Phagocytic index of neutrophilic leukocytes means a number of phagocytes out of 100 neutrophilic leucocytes; 32-85% is a normal rate of phagocytic index of neutrophilic leukocytes.

Phagocytic index (PI) is an average number of bacteria ingested by one neutrophilic leukocyte; index of 4-6 n.u. is normal.

The index of phagocytosis completeness was detected by the relation between the phagocytic index obtained after incubation (30 minutes) with a microbe test.

The phagocytic index received after 120 minutes of incubation.

Index of phagocytosis completeness of healthy children is >20%.

A level of circulating immune complex was defined in compliance with the Digeon method (1987).

The level of average weight molecules (AWMs) was determined with the help of the absorption spectrum of the protein-free fractions of blood serum in ultraviolet light by the wavelength of 250-300 nm.

Statistical data processing was carried out by methods of parametric and nonparametric statistics using Statistica 6.0 statis-

tical program (Statsoft, USA).

The main statistical characteristics were determined, namely, average deviation, error of average and standard deviation. Hypothesis testing of equality of the two means was fulfilled using Wilcoxon signed rank test and Mann-Whitney test.

During careful history taking we found out that 61 patients (71.8%) with asthma had hereditary load. The parents and/or brothers/sisters of 51 patients (60%) had allergic diseases. The relatives suffered from asthma (21 patients/24.7%), food allergy (18 patients/21.1%), drug allergy (8 patients/9.4%), atopic dermatitis (9 patients/ 10.6%), allergic rhinitis (17 patients/ 20.0%), pollinosis (13 patients/15.3%) and other allergic diseases (5 patients/ 5.9%). Asthma was recorded among relatives in the second degree of 12 patients (14.1%).

Half of the mothers (43/50.6%) of ill children had burdened obstetric history.

The following factors were widespread: obstructed labor (poor uterine contraction, accelerated labor, caesarian section) (23/27.1%), threatened miscarriage (52/61.2%), preeclampsia during the first or second half of pregnancy (19 /22.4%), premature labor (17/20.0%).

15 children (17.6%) were born with asphyxia, 11 patients (12.9%) had fetal hypotrophy. There are records about former perinatal Central Nervous System damages in the history of 43 patients (50.6%). Early bottle-feeding (during first three months of life) got 30 ill children (35.3%). 16 patients (18.8%) had unfavorable living conditions (old buildings with high humidity level, animals living in the house).

– The evaluation of allergological examination showed that **84** out of **85 children** (98.8%) had **multivalent sensitization**. The most frequent sensitizations as a part of multiple allergy were sensitizations to dust (68/80.0%) and book dust (33/38.8%). Epidermal (42/49.4%), fungal (43/50.6%), ingestant (38/44.7%) and pollen allergens (38/44.7%) took the second place for their frequency of occurrence. Food allergy (29/34.1%) and drug allergy (16/18.8%) were on the third place.

Asthma recurrence definitely arose after contact with cause-relevant allergen. Among other triggers contagious diseases (ARVI, recurrence of chronic pathology of upper respiratory tract) were the most frequent (56 persons/65.9%). Such deterioration in condition was caused by emotional stress (56/65.9%) and physical activity (48/56.5%).

Bronchoconstriction was promoted by changes of atmospheric conditions

Comorbidity	Frequency, n	
	Absolute number	%
Upper respiratory Tract pathology		
Allergic rhinitis	66	77.6
Chronic vasomotor rhinitis	14	16.5
Deflection of nasal septum	16	18.8
Chronic tonsillitis	28	32.9
Hypertrophy of palatine tonsils of I-II grade	26	30.6
Skin and mucous membrane lesions		
Atopic dermatitis	31	36.5
Recurrent urticaria, Quincke's edema	19	22.4
Syndrome of connective tissue displasia		
Postural disorder	39	45.9
Varus, valgus joints, supermotility of joints	17	20.0
Chest distortion	9	10.6
Dysplastic shape of the skull, facial asymmetry, teeth deformity	25	29.4
Changes of Heart-Vascular System		
Heart connective tissue displasia	23	27.1
Mitral valve prolapse	21	24.7
Rhythm disturbance	3	3.5
Comorbidity of Digestive Tract		
Chronic gastritis	11	12.9
Chronic gastroduodenitis	7	8.2
Duodenal ulcer	2	2.4
Chronic cholecystitis	3	3.5
Gallbladder dyskinesia	60	70.6
Abnormal development of gallbladder	13	15.3
Intestinal dyskinesia	9	10.6
Chronic colitis	5	5.9
Functional dyspepsia	22	25.9
Intestinal dysbacteriosis	21	24.7
Other diseases and conditions		
Vegetative-vascular dystonia	71	83.5
Contamination by *M. tubercolosis*	12	14.1
Hyperplasia of thyroid gland	45	52.9

TAB. 1
Comorbidity in the children with asthma.

(36/42.4%), strong odour (21/24.7%) and tobacco smoke (34/40.0%).
We recorded many comorbidities in the enrolled children (TABLE 1).
Disorders of the Upper Respiratory Tract and the Digestive Tract were the most frequent.
It should be mentioned that 45 children (52.9%) had disorders of GIT.
We also detected allergic dermatitis and symptoms of the syndrome of connective tissue displasia.

Disease-free survival patients with asthma complained of: intermittent cough (42/49.4%), single asthma attacks (51/60.0%), exertional dyspnea (36/42.4%), respiratory difficulty (34/40.0%), asthenia (27/31.8%) and performance decrement (18/21.2%).
The main part consisted of gastroenterological complaints recorded in 45 (52.9%) patients. These patients had: intermittent sicchasia (20/23.5%), loss of appetite (33/38.8%), stomachache unrelated to meal (20/23.5%), erucration (15/17.6%), tendency to constipation (12/14.1%), flatulence (11/12.9%), and periodic vomiting (4/4.7%).
While the examined patients with asthma were at disease-free survival of the main illness and this period lasted at least three months, we did not find out any significant deviations of their condition during the physical examination.
80 patients (94.1%) used salbutamol and its derivates for jugulation of asphyxia, and only 5 patients (5.9%) eased their condition by using Berodual fixed combination which were taken through pressurized metered dose inhaler or nebulizer. At the stage of sanatorium and resort therapy only 18 out of 85 ill children (21.2%) received backbone therapy of the main disease for more than 2-3 months.
Among them, 2 patients (2.4%) with moderate severe disease took average dose of inhaled corticosteroids. Eight persons (9.4%) with asthma of 3rd type and 2 patients (2.4%) with asthma of 2nd type took anti-inflammatory therapy as well as small doses of inhaled corticosteroids. Antileukotriene drugs were taken by 5 patients (5.9%) for the same reason. Combined backbone therapy was recorded in 3 ill children (3.5%).
In compliance with the set objectives all children with asthma were divided into two Groups.
– **The first Group** had standard sanatorium and resort therapy including **hypoallergic balanced diet, active climatotherapy, seabathing** (promenades by the sea, rubdown with seawater, remedial swimming in a pool with seawater), **heliotherapy** (ultraviolet irradiation according to time-lapse plan), **therapeutic exercise**, **electrosleep**, **inhalation** with refined seawater and mineral water, **hydrotherapy** and **balneotherapy**, **massotherapy**, **mud applications** using electolysis of interscapular region.
– **The second Group** was treated with **Colostro Noni** (Guna Laboratories, Milan - Italy) for recovering intestinal microflora, for preserving histological integration of the mucous membrane of the digestive tract, for the regulation of enzymatic functions improving cellular metabolism and increasing protein synthesis, for the rebalancing of the system (water-electrolyte balance).
Ill children were treated with 1 sachet every day during 14 consecutive days.
Groups for research were formed by random sampling technique.

RESULTS AND DISCUSSION

During the undertaken research significant changes of Immune System among patients with asthma were recorded in comparison with healthy peers (TABLE 2).

The decrease of levels of CD3+ ($p<0.05$), CD4+ ($p<0.05$), and CD8+ cells in the blood serum of the patients with asthma was detected; the level of CD20+ lymphocytes increased ($p<0.05$). Immunoregalatory balance had only reducing trend. Besides we recorded an increase of IgA levels ($p<0.05$) and decrease of IgM, while

Indexes	Control group n=16	Patients with asthma, n=85
T-lymphocytes (CD3+), %	61.200±0.572	54.000±3.258 p<0.05
T-*helpers*/inductor (CD4+), %	39.233±0.481	31.857±1.421 p<0.05
T-*killers*/suppressor cells (CD8+), %	26.433±0.771	22.714±2.008
B-lymphocytes, (CD20+), %	16.667±0.384	19.000±1.000 p<0.05
Immunoregulatory balance (CD4+/CD8+), n.u.	1.515±0.048	1.401±0.096
IgA, mg/ml	1.316±0.626	1.760±0.552
IgM, mg/ml	1.633±0.883	1.390±0.759
IgG, mg/ml	9.107±0.347	9.633±0.890
IgE, IU/ml	25.067±1.725	210.050±26.040 p<0.001
Phagocytic number, n.u.	4.600±0.176	2.600±0.400 p<0.05
Phagocytic index, %	65.500±1.255	62.200±3.104
Phagocytosis completion index, %	24.300±0.442	15.400±1.116 p<0.001
Circulating immune complexes, c.u.	0.041±0.002	0.076±0.003 p<0.001

TAB. 2
Changes of the indexes of the Immune System in patients with asthma (M±m).

the number of IgE was rather high (p<0.001), and IgG was normal. Phagocytic link of Immune System was also affected. We noticed a decrease of phagocytic index (1.8-time), phagocytic index of neutrophilic leucocytes (by 3.300%) and index of phagocytosis completion (by 8.9%) (p<0.001) in children with asthma even during a remission period.
The level of circulating immune complexes was almost 2 times higher than in the control group (p<0.001).
From the very beginning the patients with asthma had abnormal AWM (TABLE 3).
If a patient had the disease this index was 28.9% higher (p<0.05).

In patients with asthma a disorder of the system of AEAs specific to LPSs (anti-LPS-IgA, anti-LPS-IgM, anti-LPS-IgG) of *E. coli* was identified. At individual evaluation of the tests we determined their various trends: 15 patients (17.7%) had the same indexes as healthy children, 21 patients (24.7%) had much higher results, and the indexes of 49 children (57.6%) were much lower.
As in the TABLE 4, in children with asthma average levels of anti-LPS-IgM and anti-LPS-IgG decreased greatly (p<0.05 and p<0.001) and the level of anti-LPS-IgA did not differ from that one of the control group.

The received decrease of two types of AEAs as for anti-endotoxin of *E. coli* is an unfavorable factor promoting ill effect of endotoxins which is a part of general immune disorders in children with asthma.
Analyzing the data in TABLE 5, we can see that at disease-free survival levels of anti-LPS-IgM and anti-LPS-IgG are lower than in the control group of children (reliability from p<0.05 to p<0.001).
The decrease of anti-LPS-IgM and anti-LPS-IgG levels could have taken place as a result of the use of given genuine AEAs for neutralization of LPSs which got into systemic circulation in amounts exceeding physiological limit.
With the increase of disease-free survivals (TABLE 5) the imperfect indexes did not improve (except anti-LPS-IgA); in other words the duration of the remission did not influence greatly the levels of anti-LPS-IgM and anti-LPS-IgG. Their number remained accurately lower (p<0.05-0.001) than in the control group.
However, it should be pointed out that the average anti-LPS-IgG level at a remission period of more than 6 months was 0.022 nominal units of optic density (p<0.001) higher than the average anti-LPS-IgG level at a remission period of 3-6 months, anti-LPS-IgM differed only by 0.009 nominal units of optic density.
The average level of anti-LPS-IgA grew together with the increase of the period of remission. It should be mentioned that at a disease-free survival of more than 6 months, the anti-LPS-IgA level was even higher than in the control group.
In this paper we also studied the dependence of AEAs to endotoxins of *E. coli* in relation to asthma severity (TABLE 6).
As we can see the anti-LPS-IgA level

Index	Control group n=16	Patients with asthma, n=85
AWM, n.u.	96.590±6.600	124.496±3.392 p<0.05

TAB. 3
Number of molecules of average weight in healthy children and in patients with asthma (M±m).

Indexes	n	Anti-LPS-IgA, nominal units of optic density	Anti-LPS- IgM, nominal units of optic density	Anti-LPS-IgG, nominal units of optic density
Children with asthma	85	0.138±0.005	0.274±0.015	0.092±0.008
Control group	16	0.135±0.009	0.329±0.029	0.123±0.007
Reliability (p)		-	<0.05	<0.001

TAB. 4
Levels of specific antibodies for LPSs of *E. coli* in children with asthma (M±m).

N	Indexes	n	Anti-LPS-IgA, nominal units of optic density	Anti-LPS- IgM, nominal units of optic density	Anti-LPS-IgG, nominal units of optic density
1	Remission of 3-6 month	25	0.121±0.007	0.227±0.009	0.079±0.003
	p 1-3		-	<0.05	<0.001
2	Remission in more than 6 months	60	0.151±0.008	0.218±0.011	0.101±0.005
	p 1-2		-	-	<0.001
	p 2-3		-	<0.05	<0.005
3	Control group	16	0.135±0.009	0.329±0.029	0.123±0.007

TAB. 5
Levels of specific antibodies to LPSs of *E. coli* in children with asthma at disease-free survival (M±m).

among patients with intermittent and slightly obstinate asthma were even higher (25.2% and 19.3% respectively) than the control index. But with moderately severe obstinate asthma this level was 4.4% lower than in the control group. The reasons of such differences were not defined, but the tendencies of increase and decrease of anti-LPS-IgA were accurate.

Anti-LPS-IgA level among patients with asthma was lower irrespective of the severity of the disease.

However, we recorded a decrease of 32% ($p<0.05$) among patients with disease of the 1st and the 2nd type, and of 37.4% ($p<0.001$) in children with the 3rd type.

We also mentioned a decrease of average levels of anti-LPS-IgG. Its amount was 15.4% lower than in the control group at intermittent period, 11.4% lower at slightly obstinate period and 26% lower at moderately severe obstinate asthma ($p<0.001$).

– The decrease of anti-LPS-IgM and anti-LPS-IgG levels and therefore the decrease of possibilities for binding of endotoxins of *E. coli* is nevertheless connected with a severity type of the disease.

The reduced level of anti-LPS-IgM and anti-LPS-IgG can indicate an asthma severity type and volume of disorder of immune response to the endotoxins.

We have determined a relation between disorders of anti-endotoxic link of Immune System and asthma duration in children (TABLE 7).

The analysis of anti-LPS-IgA levels showed that its amount did not depend upon the asthma duration.

It should be emphasized that if the disease duration was 6-9 years the levels were the lowest, but if the duration was 10 years and more the level of anti-LPS-IgA looked up.

The level of anti-LPS-IgM was beyond all doubts lower in children with asthma ($p<0.05$) regardless of its duration.

Its level of reduction was only 26.1% when the disease duration was less than 5 years, 34.0% with disease duration of 6-9 years, and 37.1% when the disease lasted more than 10 years.

The average level of anti-LPS-IgG differed greatly from the level of healthy children. Thus, its level decreased by 33.3% from norm when the disease lasted for 2-5 years, 35.0% with disease duration of 6-9 years, and 19.5% with duration of more than 10 years.

The above-mentioned data allow to conclude that the larger asthma duration a child has the less remarkable changes of AEAs.

The most serious changes were traced at disease duration of 6-9 years. It is the most critical period for anti-endotoxic immunity formation of a child with asthma.

In our research we discovered some gender differences at AEAs levels of patients with asthma (TABLE 8).

The boys from the control group had much lower levels of anti-LPS-IgA and anti-LPS-IgM than the control girls, whereas the average level of anti-LPS-IgG was 1.2-times higher among the examined males.

The analysis of considered indexes among patients with asthma showed that the average level of anti-LPS-IgA was lower by 6% among boys with the disease than among healthy peers, while the same level among girls was 10.5% higher than in the control group. Time-course of anti-LPS-IgM did not depend upon patients' gender and was lower by 34.5% in both groups. We mentioned a more distinct reduction of anti-LPS-IgG among boys with asthma (by 31.8%) than among girls with

N	Asthma severity	n	Anti-LPS-IgA, nominal units of optic density	Anti-LPS- IgM, nominal units of optic density	Anti-LPS-IgG, nominal units of optic density
1	1st type	28	0.169±0.015	0.226±0.020	0.104±0.009
	p1-4		-	p<0.05	-
2	2nd type	29	0.161±0.010	0.225±0.022	0.109±0.008
	p2-4		-	p<0.05	-
3	3rd type	28	0.129±0.009	0.206±0.016	0.091±0.005
	p3-4		-	p<0.001	p<0.001
4	Control group	16	0.135±0.009	0.329±0.029	0.123±0.007

TAB. 6

Levels of specific antibodies to LPSs of *E. coli* in children with asthma at disease-free survival depending upon severity (M±m).

N	Duration of the disease	n	Anti-LPS-IgA, nominal units of optic density	Anti-LPS- IgM, nominal units of optic density	Anti-LPS-IgG, nominal units of optic density
1	2-5 years	34	0.131±0.011	0.243±0.018	0.082±0.005
	p 1-4		-	p<0.05	p<0.001
2	6-9 years	40	0.127±0.008	0.217±0.011	0.080±0.003
	p 2-4		-	p<0.05	p<0.001
3	10 years and more	11	0.138±0.005	0.207±0.007	0.099±0.004
	p 3-4		-	p<0.001	p<0.05
4	Control group	16	0.135±0.009	0.329±0.029	0.123±0.007

TAB. 7

Levels of specific antibodies to LPSs of *E. coli* in children with asthma at disease-free survival depending upon duration of the disease (M±m).

N	Indexes	n	Anti-LPS-IgA, nominal units of optic density	Anti-LPS- IgM, nominal units of optic density	Anti-LPS-IgG, nominal units of optic density
1	Boys (asthma)	46	0.124±0.004	0.203±0.007	0.090±0.003
	p1-3		-	<0.001	<0.001
2	Girls (asthma)	39	0.142±0.008	0.237±0.010	0.092±0.004
	p2-4		-	<0.001	<0.05
	p1-2		<0.05	<0.05	-
3	Boys (control group)	8	0.132±0.012	0.310±0.034	0.132±0.009
4	Girls (control group)	8	0.138±0.015	0.362±0.053	0.107±0.008

TAB. 8

Level of specific antibodies to LPSs of *E. coli* among girls and boys with asthma at disease-free survival (M±m).

N	Groups of children	n	Anti-LPS-IgA, nominal units of optic density	Anti-LPS- IgM, nominal units of optic density	Anti-LPS-IgG, nominal units of optic density
1	asthma and GIT diseases	45	0.136±0.005	0.252±0.012	0.091±0.003
	p 1-3		-	<0.05	<0.001
2	asthma without GIT diseases	40	0.138±0.012	0.366±0.043	0.094±0.010
	p 2-3		-	-	<0.001
	p 1-2		-	<0.05	-
3	Control group	16	0.135±0.009	0.329±0.029	0.123±0.007

TAB. 9

Level of specific antibodies to LPSs of *E. coli* among children with asthma depending upon comorbidity of GIT (M±m).

N	Groups	n	Anti-LPS-IgA, nominal units of optic density	Anti-LPS- IgM, nominal units of optic density	Anti-LPS-IgG, nominal units of optic density
1	asthma and GIT disorder	27	0.135±0.005	0.251±0.013	0.093±0.003
	p 1-4		-	<0.05	<0.001
2	asthma and chronic GIT diseases	18	0.139±0.012	0.253±0.024	0.086±0.003
	p 2-4		-	<0.05	<0.001
	p 1-2		-	-	-
3	asthma and GIT diseases	45	0.136±0.005	0.252±0.012	0.091±0.003
4	Control group	16	0.135±0.009	0.329±0.029	0.123±0.007

TAB. 10

Level of specific antibodies to LPSs of *E. coli* among children with asthma and comorbidity of GIT (M±m).

the same disease (14.0%).

There are also some differences between groups of boys and girls. The anti-LPS-IgA and anti-LPS-IgM levels among boys were much lower than among girls ($p<0.05$). Thus, characteristics of antibodies specific to LPS of *E. coli* among children with BA depend upon such genotopic marker as gender. An obvious decrease of AEAs levels (anti-LPS-IgM and anti-LPS-IgG) protecting from endotoxins among boys can be caused by one of the reasons why BA is more widespread among male patients.

The analysis of anti-endotoxic immunity of patients with GIT disorders revealed that this category of patients has more distinct AEAs disorders (TABLE 9).

In TABLE 9 we can see that the level of anti-LPS-IgA among children with GIT disorders did not obviously differ from the control index, but it tended to reduce if compared with patients without GIT disorders.

The level of anti-LPS-IgM reduced sharply by 23% among patients with both asthma and GIT disorders, while the same level among patients without GIT disorders exceeded the control index by 11.2%. Consequently, existence or lack of GIT comorbidity caused multidirectional changes of anti-LPS-IgM level ($p<0.05$). This confirms a persistent supply of endotoxin into systemic circulation, a leading role of anti-LPS-IgM in LPSs clearance and a need to find ways for correction of LPSs related to nosotropic effect and abnormal protection of AEAs.

Approximately the same reduction of average levels of anti-LPS-IgG was recorded in both groups under discussion.

However, the number changed by 26% if patients had GIT disorders and by 26% if they did not have such diseases.

Hence, a comorbid GIT disease contributes to more serious disorder in humoral component of the Immune System leading to decrease of anti-LPS-IgM and anti-LPS-IgG protection from LPSs.

As shown in TABLE 10, there were no distinct differences among subgroups of children with comorbid functional and chronic GIT diseases.

However, it is worth mentioning that we recorded higher average levels of anti-LPS-IgA and anti-LPS- IgM in patients with chronic GIT diseases.

The level of anti-LPS-IgG among this patients group was lower than among patients with functional disorders of digestive Tract.

– Consequently, children with asthma and comorbid GIT diseases had more distinct disorders in the system of antibodies specific to LPSs of *E. coli*. Thus, such patients need appropriate follow-up care of a gastroenterologist and improved treatment focused on reduction of endotoxin influence on a body of a child.

– Treatment of the detected disorders and results

We detected disorders of cellular, humoral and anti-endotoxic immune system in patients with asthma at disease-free survival and should find the way to treat revealed changes effectively.

– 40 patients with asthma out of 80 formed an experimental group and received standard sanatorium-resort treatment during 21-24 days. The basic group of 45 patients took 1 sachet a day of Colostro Noni (Guna Laboratories, Milan - Italy) during 14 days. Both groups include asthma patients with and without other GIT disorders (TABLE 11).

	Types of therapy			
	Standard sanatorium-resort treatment n=40		Colostro Noni n=45	
	Absolute number	%	Absolute number	%
Asthma without GIT diseases	20	50	20	44.4
Asthma with GIT diseases	20	50	25	55.6

TAB. 11

Division of patients with asthma according to comorbidity and diversional therapy.

During the research we discovered the positive influence of standard sanatorium-resort treatment on phagocytic, cellular and humoral links of the Immune System (TABLE 12).

The positive effect of standard sanatorium-resort treatment on immunological indexes included an increase of average level of CD3+ lymphocytes by 5.43% ($p<0.05$), CD4+ lymphocytes by 5.71% ($p<0.05$), CD8+ lymphocytes only by 0.57% and decrease of CD20+ lymphocytes by 1.0%. However, neither of indexes under consideration reached the levels of healthy peers.

Advanced reduction of **indices** also happened with immunoglobulin rates, except IgG, the level of which did not change during the whole course of the treatment. Thus, the level of IgA decreased by 24.4% ($p<0.05$), and IgE level declined by 44.2% ($p<0.05$). Further reduction of general IgM by 7.9% was a negative symptom.

Phagocytic link of immune system had a positive trend. The average level of phagocytic index of neutrophilic leukocytes increased by 4.2%, phagocytic number improved by 61.5% ($p<0.05$), phagocytosis completion index grew by 50.6% ($p<0.05$). Therefore, influenced by standard sanatorium-resort treatment, the levels of phagocytic index and the index of phagocytosis completeness was 1.5 times higher than the initial levels.

However, indices of phagocytosis were still lower than in the control group.

The level of circulating immune complex in blood serum of the children with asthma decreased by 10.5% ($p<0.05$) in response to therapy.

As we can see from the above, besides changes of IgM levels standard sanatorium-resort treatment improved the Immune System in children with asthma in

Indexes	Control group, n=16	Patients with asthma, n=40	
		Before treatment	After treatment
	1	2	3
CD3+, %	61.200±0.572	54.000±3.258	59.428±3.611 p2-3<0.05
CD4+, %	39.233±0.481	31.857±1.421	37.571±1.586 p2-3<0.05
CD8+, %	26.433±0.771	22.714±2.008	23.285±2.189
CD20+, %	16.667±0.384	19.000±1.000	18.000±1.340
Immunoregulatory balance, n.u.	1.515±0.048	1.401±0.096	1.610±1.081
IgA, mg/ml	1.316±0.626	1.760±0.552	1.330±0.121 p2-3<0.05
IgM, mg/ml	1.633±0.883	1.390±0.759	1.280±0.094 p1-3<0.05
IgG, mg/ml	9.107±0.347	9.633±0.890	9.600±0.730
IgE, IU/ml	25.067±11.725	210.050±26.040	117.170±20.76 p1-3<0.05 p2-3<0.05
Phagocytic index of neutrophilic leucocytes, %	65.500±1.255	62.200±3.104	64.800±1.240
Phagocytic number, n.u.	4.600±0.176	2.600±0.400	4.200±0.400 p2-3<0.05
Phagocytosis completion index, %	24.300±0.442	15.400±1.166	23.200±0.969 p2-3<0.05
Circulating immune complexes, c.u.	0.041±0.002	0.076±0.003	0.068±0.005 p1-3<0.05 p2-3<0.05

TAB. 12

Dynamics of phagocytic, cellular and humoral links of Immune System in patients with asthma in response to standard sanatorium-resort treatment (M±m).

Index	Control group, n=16	Patients with asthma, n=40	
		Before treatment	After treatment
	1	2	3
AWMs, n.u.	96.590±6.600	124.496±3.392 p1-2<0.05	117.920±3.192 p1-3<0.05 p2-3<0.05

TAB. 13

Dynamics of average weight molecules of patients with asthma in response to sanatorium-resort treatment (M±m).

Index	Control group, n=16	Patients with asthma, n=40		Significance of differences	
		Before treatment	After treatment	1-3	2- 3
	1	2	3		
Anti-LPS-IgA, nominal units of optic density	0.135±0.009	0.138±0.005	0.129±0.018	-	-
Anti-LPS-IgM, nominal units of optic density	0.329±0.029	0.219±0.015	0.223±0.037	<0.05	-
Anti-LPS-IgG, nominal units of optic density	0.123±0.007	0.092±0.008	0.084±0.006	<0.001	-

TAB. 14
Dynamics of anti-endotoxic immunity of patients with asthma in response to sanatorium-resort treatment (M±m).

general, but it did not lead to immunorestoration.

Sanatorium-resort treatment contributed to the decrease of the amount of average weight molecules by 5.3% (p<0.05), which confirmed a reduction of anti-endotoxic disorder among these children (TABLE 13).

We registered a trend of anti-endotoxic immunity (TABLE 14).

Average levels of all AEAs altered in response to standard sanatorium-resort treatment. The reasons of such differences were not determined. Despite the fact that anti-LPS-IgA level reduced by 6.5% and anti-LPS-IgG reduced by 8.7% in compliance with the initial one, the average anti-LPS- IgM level of an adult increased by 1.8%.

We analyzed the dynamics of anti-endotoxic immunity in response to standard sanatorium-resort treatment depending upon severity (TABLE 15). It was determined that the level of anti-LPS-IgA decreased irrespective of asthma severity.

Its level decreased by 20.7% (p<0.05) from initial level at intermittent asthma, by 21.7% (p<0.05) at slightly obstinate asthma and by 7.8% at moderately severe obstinate asthma.

The dynamics of anti-LPS-IgM was opposite. Its level improved by 6.2% among patients with the 1st type of asthma, by 4.9% among children with the 2nd type, and by 2.9% among patients with the 3rd type.

During the therapy course the level of anti-LPS-IgG also altered. Almost the same reduction was recorded among patients with intermittent (by 12.5%) and moderately severe obstinate asthma (13.1%). While among children with slightly obstinate asthma we noticed a supreme reduction of the index by 20.2% (p<0.05). Thus, we can make, the conclusion that the more severe the disease is, the more distinct the trend of indexes is in response to standard sanatorium-resort treatment.

Duration of asthma also affected indexes (TABLE 16). Standard sanatorium-resort treatment led to decrease of AEAs regardless of duration of the disease. All indices under review decreased of 3.7-3.8% from the initial level if asthma duration was 2-5 years. When duration of the disorder was 6-9 years the reduction of anti-LPS-IgA by 3.9%, anti-LPS-IgM by 2.8%, and anti-LPS-IgG by 5.5% was recorded.

The maximum levels were detected among patients with asthma duration of more than 10 years. They had a trend of decrease of anti-LPS-IgA by 7.2%, anti-LPS-IgM by 2.9% and anti-LPS-IgG by 11.1% from initial level. Consequently standard sanatorium-resort treatment at most influenced levels antibodies of A and G class to LPSs of *E. coli* if the disease du-

N	Severity of asthma	n	Anti-LPS-IgA, nominal units of optic density		Anti-LPS- IgM, nominal units of optic density		Anti-LPS-IgG, nominal units of optic density	
			Before	*After*	*Before*	*After*	*Before*	*After*
			1	2	3	4	5	6
1	**1st type**	12	0.169 ±0.015	0.134 ±0.010	0.226 ±0.020	0.240 ±0.012	0.104 ±0.009	0.091 ±0.006
	p before/after			<0.05		-		-
2	**2nd type**	14	0.161 ±0.010	0.126 ±0.015	0.225 ±0.022	0.236 ±0.015	0.109 ±0.008	0.087 ±0.008
	p before/after			<0.05		-		<0.05
3	**3rd type**	14	0.129 ±0.009	0.119 ±0.011	0.206 ±0.016	0.212 ±0.011	0.091 ±0.005	0.079 ±0.006
	p before/after			-		-		-
4	**Control group**	16	0.135 ±0.009		0.329 ±0.029		0.123 ±0.007	

TAB. 15
Dynamics of specific antibodies to LPSs of *E. coli* in children with asthma in response to sanatorium-resort treatment depending upon severity (M±m).

N	Duration of the disease	n	Anti-LPS-IgA, nominal units of optic density		Anti-LPS- IgM, nominal units of optic density		Anti-LPS-IgG, nominal units of optic density	
			Before	*After*	*Before*	*After*	*Before*	*After*
			1	2	3	4	5	6
1	**2-5 years**	15	0.131 ±0.011	0.126 ±0.010	0.243 ±0.018	0.234 ±0.008	0.082 ±0.005	0.079 ±0.004
	p before/after			-		-		-
2	**6-9 years**	20	0.127 ±0.008	0.122 ±0.006	0.217 ±0.011	0.211 ±0.01	0.080 ±0.003	0.076 ±0.003
	p before/after			-		-		-
3	**10 years and more**	5	0.138 ±0.005	0.128 ±0.005	0.207 ±0.007	0.201 ±0.005	0.099 ±0.004	0.088 ±0.005
	p before/after			-		-		-
4	**Control group**	16	0.135 ±0.009		0.329 ±0.029		0.123 ±0.007	

TAB. 16
Dynamics of specific antibodies to LPSs of *E. coli* in children with asthma in response to standard sanatorium-resort treatment depending upon duration of the disease (M±m).

ration was more than 9 years.
In our clinical study we discovered some gender differences in AEAs trend in response to standard sanatorium-resort treatment (TABLE 17). The anti-LPS-IgA levels decreased among patients of both genders. But the trend of boys was 4.8%, and the trend of girls was 7.0%. The average level of the index under review was obviously lower ($p<0.001$) among male patients than among healthy peers, while the level of anti-LPS-IgA among female patients with asthma comes close to the level of the control group. The anti-LPS-IgM trend was differently directed among patients of different gender. Thus the average level of anti-LPS-IgM increased by 8.9% among boys, and decreased by 3.8% among girls. However, the received data in both subgroups were obviously ($p<0.05$) lower than in the control group. Standard sanatorium-resort treatment caused reduction of average levels of anti-LPS-IgG. This index decreased by 4.4% among boys with asthma and by 10.9% among girls, namely, 2.5 times more actively.

The analysis of the data show that standard complex of sanatorium-resort treatment lowered the levels of AEAs regardless of co-morbidity of GIT (TABLE 18). With normal activity of digestive system average amount of anti-LPS-IgA decreased by 4.3% compared to initial level, but with disorders of GIT - by 7.4%.
One should note that the same dynamics of anti-LPS-IgA was registered in both groups. Its amount decreased by 21%.
With disorders of GIT the amount of anti-LPS-IgA was declining 1.7 times faster, than with normal activity of Digestive System. With the combination of asthma and pathology of GIT that index decreased by 14.3%, but without it - by 8.5%.

Therefore, our data bore the record to the decrease of AEAs in response to the standard complex of sanatorium-resort treatment that depended on various factors.

The basic group (45 patients) with asthma took Colostro Noni. Some patients who took Colostro Noni stopped making gastroenterological complaints. Half of the children stopped suffering periodic sickness (6 of 12; 50%) and vomit (2 of 4; 50%). 7 of 11 (63.6%) patients noted that they stopped suffering abdominal pain, 4 of 8 (50%) belch, 5 of 8 (62.5%) gaseous distention, 4 of 7 (57.1%) predisposition to oppilations. All children mentioned the improvement of appetite.
– The Immune System of the patients who took Colostro Noni underwent more significant changes than those who didn't take this medication; these changes are set forth in TABLE 19.

N	Indices	n	Anti-LPS-IgA, nominal units of optic density		Anti-LPS- IgM, nominal units of optic density		Anti-LPS-IgG, nominal units of optic density	
			Before	*After*	*Before*	*After*	*Before*	*After*
1	**Boys (asthma)**	22	0.124 ±0.004	0.118 ±0.005	0.203 ±0.007	0.221 ±0.006	0.090 ±0.003	0.086 ±0.003
	p before/after			-		<0.05		-
	p (control)			<0.001		-		<0.001
2	**Girls (asthma)**	18	0.142 ±0.008	0.132 ±0.006	0.237 ±0.010	0.228 ±0.007	0.092 ±0.004	0.082 ±0.005
	p before/after			-		-		-
	p (control)			-		<0.05		<0.01
3	**Boys (control group)**	8	0.132 ±0.012		0.310 ±0.034		0.132 ±0.009	
4	**Girls (control group)**	8	0.138 ±0.015		0.362 ±0.053		0.107 ±0.008	

TAB. 17
Dynamics of specific antibodies to LPSs of *E. coli* in girls and boys with asthma in response to standard sanatorium-resort treatment (M±m).

N	Groups	n	Anti-LPS-IgA, nominal units of optic density		Anti-LPS- IgM, nominal units of optic density		Anti-LPS-IgG, nominal units of optic density	
			Before	*After*	*Before*	*After*	*Before*	*After*
1	**Asthma and diseases of GIT**	20	0.136 ±0.005	0.126 ±0.004	0.252 ±0.012	0.200 ±0.009	0.091 ±0.003	0.078 ±0.003
	p before/after			-		<0.001		-
	p with contr.			<0.001		<0.001		<0.01
2	**Asthma without diseases of GIT**	20	0.138 ±0.012	0.132 ±0.010	0.366 ±0.043	0.289 ±0.015	0.094 ±0.010	0.086 ±0.008
	p before/after			-		-		-
	p with contr.			-		-		<0.001
3	**Control group**	16	0.135 ±0.009		0.329 ±0.029		0.123 ±0.007	

TAB. 18
Dynamics of specific antibodies to LPSs of *E. coli* in children with asthma in response to sanatorium-resort treatment depending on diseases of GIT (M±m).

In response to the medication under investigation the level of CD3+ lymphocytes increased by 8.14% (0.02), CD4+ - by 6.68% (0.001), CD8+ - by 1.89%, but the number of CD20+ cells declined by 1.7%. Therefore, we observed **significant changes of the Immune System**.
However, the indices that we have examined do not correspond to those of healthy counterparts. Moreover, the average number of CD3+ cells after therapy even surpassed the control amount.
Colostro Noni provoked even more significant immune changes than standard complex of sanatorium-resort treatment, as in DIAGRAM 1.

Treatment with Colostro Noni leads to more evident increase of CD3+, CD4+ lymphocytes and decrease of CD20+ cells than standard sanatorium-resort treatment complex. Attention should be paid to the fact that Colostro Noni provoked the **3.3**

time increase of CD8+ lymphocytes compared to the experimental group. This fact is important for the patients with asthma, because they suffer most of all from chronic inflammatory processes in the Respiratory System.

The analysis of humoral immune link showed that Colostro Noni had provoked false decrease of serumal IgA by 16.1% compared to the initial level, the IgE - by 59.3% (p<0,001).

The number of IgM tends to increase.

Its average number inclined by 15.8%, whereas the level of IgG in response to the therapy remained unchanged.

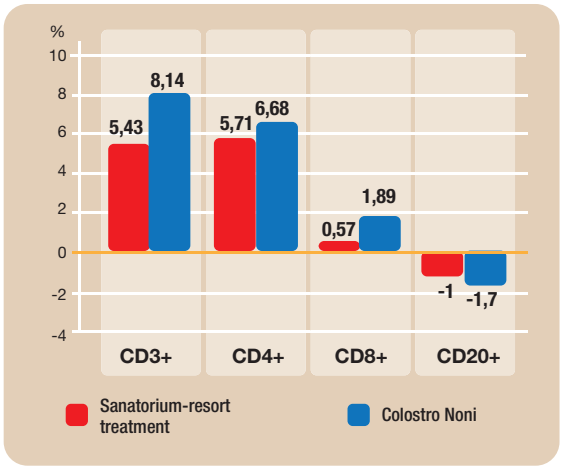
Comparing the dynamics of immunoglobulins in the basic and experimental groups one should note that Colostro Noni provoked not only less significant decrease of serumal IgA and far more substantial drop of IgE level, but **also raised IgM level**. While sanatorium-resort treatment led conversely to decline of average amount of IgM (DIAGRAM 2).

In the basic group of patients there were positive changes in phagocytic immune link. Average phagocytic index increased in response to the therapy by means of the medication under investigation by 6.16%, phagocytic number inclined by 89.1%, phagocytosis completion index - by 54.32%. These changes are more significant than those in the experimental group.

As shown on DIAGRAM 3, the application of Colostro Noni led to **1.5 times faster rise** of phagocytic index and 1.3 time **faster increase** of phagocytic number. Phagocytosis completion index in the basic group compared to the experimental one increased by 3.72%.

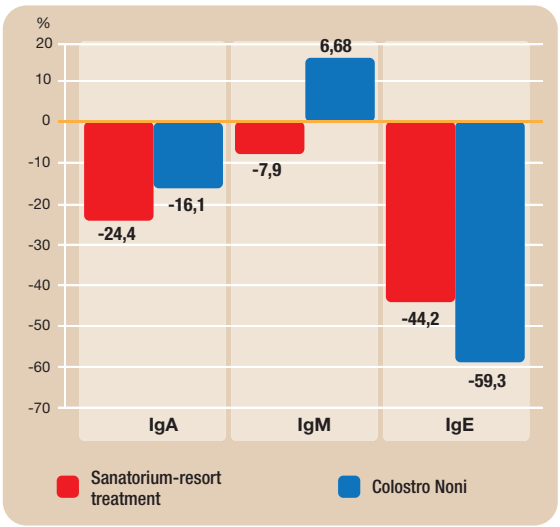
It is important to note that Colostro Noni has led to accurate decrease (p<0.01) of the level of circulating immune complexes in blood serum. This index declined by 1.6 times (35.53%) compared to the initial level. Additionally, the average number of circulating immune complexes reached the control level and it was 3.4 times lower (p<0.05) than in the experimental group.

The number of AWMs (average weight molecules) in blood serum of the patients with asthma of the basic group was declined by 20.4% compared to the initial level (TABLE 20). However this index didn't reach the control level. An additional point is that the medication under investigation led to 3.8 time faster drop of AWMs number in blood serum of the pa-



DIAG. 1

Influence of sanatorium-resort treatment and combined therapy on immune factors.



DIAG. 2

Influence of sanatorium-resort treatment and Colostro Noni on humoral immune link factors.

Indexes	Control group, n=16	Patients with asthma		
		Before treatment n=85	Sanatorium-resort treatment n=40	Colostro Noni n=45
	1	2	3	4
CD3+, %	61.200±0.572	54.000±3.258	59.428±3.611	62.140±0.812 p2-4<0.02
CD4+, %	39.233±0.481	31.857±1.421	37.571±1.586	38.533±0.313 p2-4<0.001
CD8+, %	26.433±0.771	22.714±2.008	23.285±2.189	24.600±0.626
CD20+, %	16.667±0.384	19.000±1.000	18.000±1.340	17.300±0.534
Immune regulatory index, nominal units	1.515±0.048	1.401±0.096	1.610±1.081	1.603±0.034 p2-4<0.05
IgA, mg/ml	1.316±0.626	1.760±0.552	1.330±0.121	1.476±0.077
IgM, mg/ml	1.633±0.883	1.390±0.759	1.280±0,094	1.610±0.418
IgG, mg/ml	9.107±0.347	9.633±0.890	9.600±0.730	9.536±0.256
IgE, IU/ml	25.067±11.725	210.050±26.040	117.170±20.76	85.460±4.950 p1-4<0.001 p2-4<0.001
Phagocytic index	65.500±1.255	62.200±3.104	64.800±1.240	63.033±0.951
Phagocytes number	4.600±0.176	2.600±0.400	4.200±0.400	4.700±0.229 p2-4<0.001
Phagocytosis completion index, %	24.300±0.442	15.400±1.166	23.200±0.969	23.766±0.736 p2-4<0.001
Circulating immune complexes, nominal units	0.041±0.002	0.076±0.003	0.068±0.005	0.049±0.002 p2-4<0.01 p3-4<0.05

TAB. 19

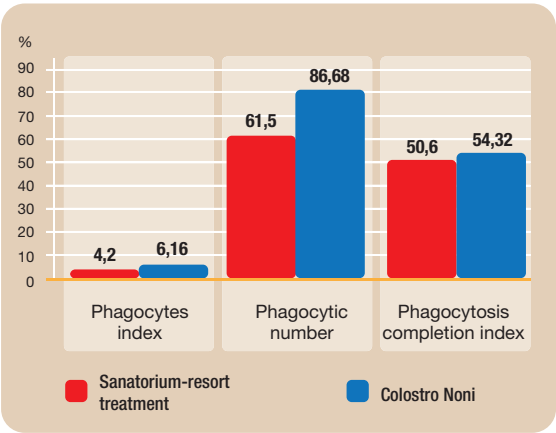
Indexes of phagocytic, cellular and humoral immunity links of the patients with asthma in response to Colostro Noni in the basic group (M±m).

tients with asthma than standard sanatorium-resort treatment complex (p<0,001). Application of Colostro Noni provoked positive changes of humoral antiendotoxic immunity, namely the number of anti-LPS-IgM increased by 35.6%, anti-LPS-IgG - by 29.65%, protective effect of LPSs was also intensified (TABLE 21).

The medication under investigation led to significant decline of anti-LPS-IgA (by 2.2%) that was 3 times less than in response to standard sanatorium-resort treatment complex (DIAGRAM 4). One also should note that in the basic group rise of anti-LPS-IgM was (p<0.05) 20 times larger and the increase of anti-LPS-IgG - 2.4times higher (p<0.05) than in the experimental one.

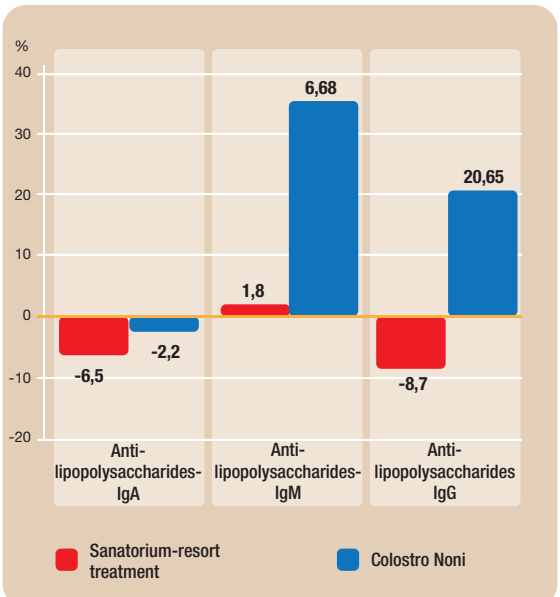
TAB. 20
Dynamics of AWMs of the patients with asthma in response to Colostro Noni (M±m).

Index	Experimental group, n=16	Patients with asthma		
		Before treatment n=85	Sanatorium-resort treatment n=40	Colostro Noni n=45
	1	2	3	4
AWM, nominal units	95.590±6.600	124.496±3.392	117.920±3.192	99.091±1.636 p2-4<0.05 p3-4<0.001



DIAG. 3

Influence of sanatorium-resort treatment and Colostro Noni on humoral immune link factors.



DIAG. 4

Influence of sanatorium-resort treatment and Colostro Noni on humoral link of antiendotoxic immunity.

The analysis of the dynamics of AEAs depending on the disease severity in the basic group showed that with intermittent disease state the number of Anti-LPS-IgA had declined by 8,6%, with mild persistent one - by 1.4%, with persistent one of medium severity level - by 6.2%.

The number of anti-LPS-IgM in response to the combined therapy increased gradually by 21.68% at I stage of asthma (p<0.05). At II and III stage of the disease in children there was the same increase of the indexes by 17.4% and 12.02%, respectively (TABLE 22).

The level of anti-LPS-IgG also changed during the therapy.

Its average value inclined in all involved groups. However, the maximum increase of this index was registered for persistent asthma of intermediate severity. At III stage of the disease its amount increased 19.78%. With intermittent disease state the increase of the index amounted to 11.54%, with mild persistent 8.26%.

Comparative analysis of AEAs of the patients in the basic group showed that at I stage of the disease the average level of anti-LPS-IgA decreased 2.4 times (p<0.05) slower than in the experimental group (DIAGRAM 5). At II stage of asthma the difference between indices amounted to 15.5 times (p<0.001).

The dynamics of anti-LPS-IgA levels at III stage of the disease were almost the same. As it's shown on the DIAGRAM 6 the anti-LPS-IgM level changes with intermittent and mild persistent disease state accounted for 3.5 times (p<0.01), with persistent one of medium severity - 4.1 times (p<0.001).

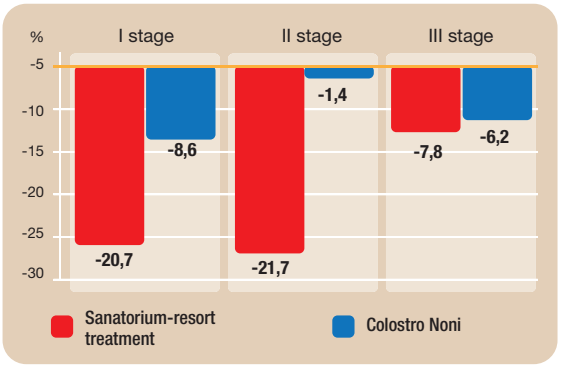
Therefore, the therapy performed by means of the medication under investigation asserted significant influence on anti-LPS-IgM.

The dynamics of anti-LPS-IgG was multidirectional. The standard sanatorium-resort treatment complex led to decrease of this index, but Colostro Noni provoked conversely the rise of the index regardless of asthma severity (DIAGRAM 7).

In our work we defined that the average amounts of AEAs depended on asthma chronicity. The treatment with Colostro Noni led to multidirectional dynamics of anti-LPS-IgA. Its amount increased by 3.05% in cases when duration of the disease was 5 years, from 6 to 9 years - by 1.6%, but when duration amounted for more than 10 years this amount decreased by 3.6% (TABLE 23).

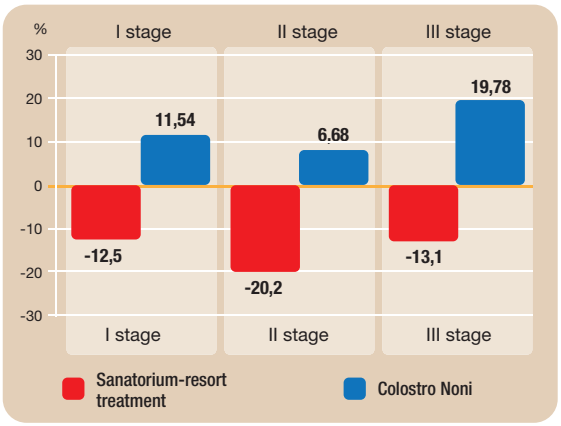
Unidirectional, almost identical dynamics (within 15%) was registered for anti-LPS-IgA regardless of disease duration. The increase (p<0.05-0.02) of the index was registered in all groups.

Positive dynamics of the average amount of anti-LPS-IgG depended on disease duration. When the asthma duration amounted to 2-5 years the number of anti-LPS-IgG increased by 43.9%, 6-9 years - 25.0%, more than 10 years - 13.13%. Therefore, the longer was the duration of



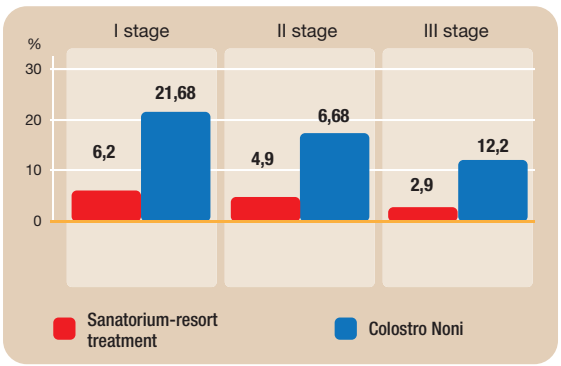
DIAG. 5

Influence of sanatorium-resort treatment and Colostro Noni on anti-LPS-IgA depending on asthma severity.



DIAG. 7

Influence of sanatorium-resort treatment and Colostro Noni on anti-LPS-IgG depending on asthma severity.



DIAG. 6

Influence of sanatorium-resort treatment and Colostro Noni on anti-LPS-IgM depending on asthma severity.

Indexes	Experimental group, n=16	Patients with asthma		
		Before treatment n=85	Sanatorium-resort treatment n=40	Colostro Noni n=45
	1	2	3	4
Anti-LPS-IgA nominal units of optical density	0.135±0.009	0.138±0.005	0,129±0.018	0,135±0.003 p3-4<0.001
Anti-LPS-IgM nominal units of optical density	0.329±0.029	0.219±0.015	0.223±0.037	0.297±0.010 p2-4<0.05 p3-4<0.05
Anti-LPS-IgG nominal units of optical density	0,123±0.007	0.092±0.008	0.084±0.006	0.111±0.003 p2-4<0.01 p3-4<0.02

TAB. 21

Dynamics of antiendotoxic immune indices of the patients with asthma in response to Colostro Noni (M±m).

the child's asthma, the less were the significant dynamics of anti-LPS-IgG. The disease duration amounted to 6-9 years contributed to low increase of anti-LPS-IgA. The increase of anti-LPS-IgM in blood serum in response to Colostro Noni didn't depend on asthma duration.

Comparing the dynamics of the antiendotoxic immunity indexes in the basic and experimental groups depending on the disease duration it was registered that both types of suggested therapy led to multidirectional dynamics of AEAs of patients with asthma.

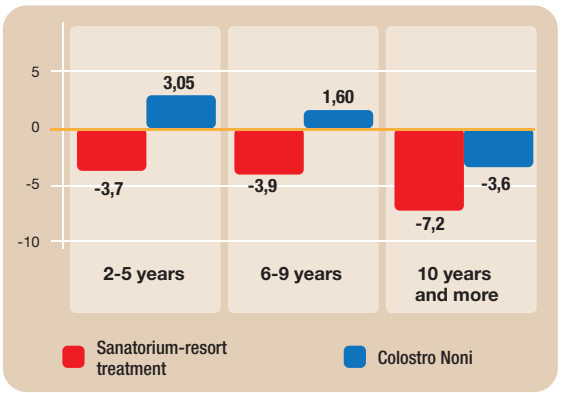
Sanatorium-resort treatment led to decrease of anti-LPS-IgA levels with maximum changes when asthma duration amounted to more than 10 years (DIAGRAM 8). Colostro Noni raised this indexes in cases when disease duration was less than 9 years, but when disease duration accounted for more than 10 years Colostro Noni contributed to the decrease of it. However the decline of this index passed 2 times slower than in the experimental group.

Additionally, one should note that standard sanatorium-resort treatment complex provoked steady decrease of anti-LPS-IgM regardless of disease duration (DIAGRAM 9). Colostro Noni contributed to the stable increase of the index under review regardless of disease duration.

Multidirectional changes in the basic and experimental group were registered for anti-LPS-IgG. Sanatorium-resort treatment lowered progressively anti-LPS-IgG level depending on asthma duration (DIAGRAM 10).

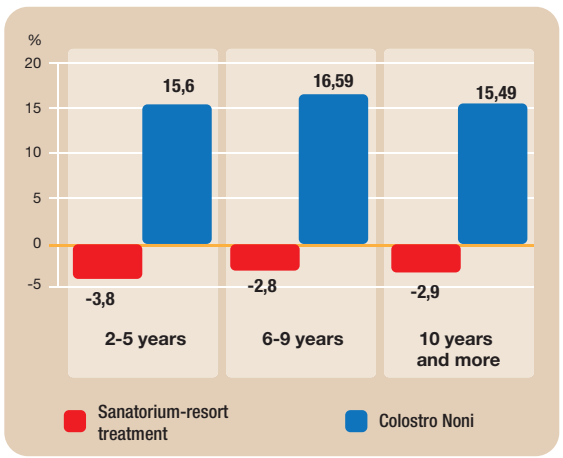
Colostro Noni led conversely to the increase of this index as the disease duration reduced.

We examined the dynamics of AEAs depending on patient's gender (TABLE 24). As for the boys, their anti-LPS-IgA level remained unchanged. As for the girls, there was a positive dynamics with increase by 7.04%. Acquired average number of anti-LPS-IgA of the female patients surpassed



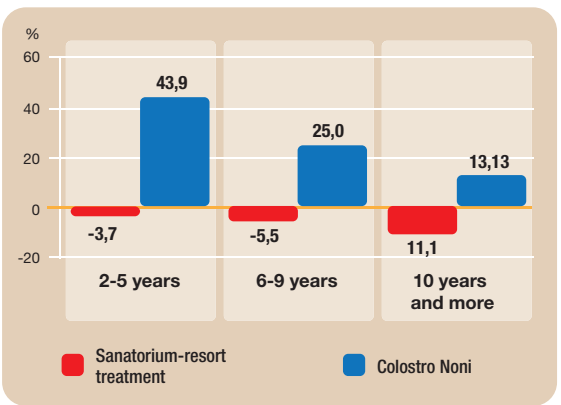
DIAG. 8

Influence of sanatorium-resort treatment and Colostro Noni on anti-LPS-IgA depending on asthma duration.



DIAG. 9

Influence of sanatorium-resort treatment and Colostro Noni on anti-LPS-IgM depending on asthma duration.



DIAG. 10

Influence of sanatorium-resort treatment and Colostro Noni on anti-LPS-IgG depending on asthma duration.

N	Asthma severity level	n	Anti-LPS-IgA, nominal units of optic density		Anti-LPS- IgM, nominal units of optic density		Anti-LPS-IgG, nominal units of optic density	
			Before 1	*After* 2	*Before* 3	*After* 4	*Before* 5	*After* 6
1	**Stage I**	16	0.152 ±0.015	0.139 ±0.010	0.226 ±0.020	0.275 ±0.015	0.104 ±0.009	0.116 ±0.009
	p before/after			-		<0.05		-
2	**Stage II**	15	0.141 ±0.010	0.139 ±0.006	0.224 ±0.022	0.263 ±0.020	0.109 ±0.008	0.118 ±0.006
	p before/after			-		-		-
3	**Stage III**	14	0.129 ±0.009	0.121 ±0.006	0.208 ±0.016	0.233 ±0.015	0.091 ±0.005	0.109 ±0.006
	p before/after			-		-		<0.05
4	**Examination group**	16	0.135 ±0.009		0.329 ±0.029		0.123 ±0.007	

TAB. 22

Dynamics of specific antibodies to LPSs of *E. coli* in children with asthma in response to sanatorium-resort treatment depending on GIT diseases (M±m).

N	Disease duration	n	Anti-LPS-IgA, nominal units of optic density		Anti-LPS- IgM, nominal units of optic density		Anti-LPS-IgG, nominal units of optic density	
			Before	After	Before	After	Before	After
1	**2-5 years**	19	0.131 ±0.011	0.135 ±0.010	0.243 ±0.018	0.281 ±0.010	0.082 ±0.005	0.118 ±0.003
	p before/after			-		<0.05		<0.001
2	**6-9 years**	20	0.127 ±0.008	0.129 ±0.006	0.217 ±0.011	0.253 ±0.010	0.080 ±0.003	0.100 ±0.003
	p before/after			-		<0.02		-
3	**10 years and more**	6	0.138 ±0.005	0.133 ±0.005	0.207 ±0.007	0.239 ±0.006	0.099 ±0.004	0.112 ±0.003
	p before/after			-		<0.02		-
4	**Control group**	16	0.135 ±0.009		0.329 ±0.029		0.123 ±0.007	

TAB. 23

Dynamics of antibodies specific to LPSs of *E. coli* in children with asthma in response to Colostro Noni depending on diseases duration (M±m).

that of their healthy counterparts by 10.14%.

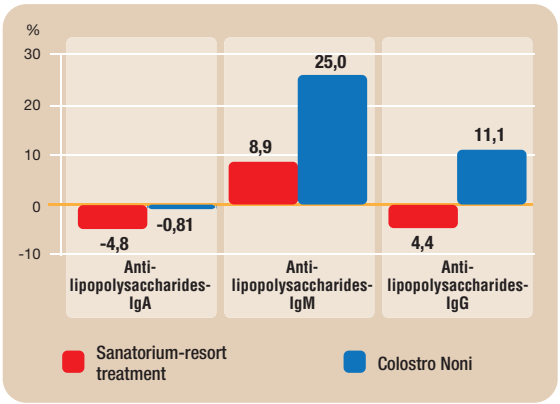
Unidirectional dynamics of anti-LPS-IgM was registered for both boys and girls with asthma. Its amount went up in the male patients by 26.11% - in the female - by 31.22%.

However, neither in boys, nor in girls acquired indices reached the control level.

The therapy with Colostro Noni raised the average amount of anti-LPS-IgG in the patients of both gender. However, the increase of this index in boys amounted to 11.1%, in girls - 20.65% (2 times higher). That's why the average number of anti-LPS-IgG surpassed that of their healthy counterparts by 3.74%.

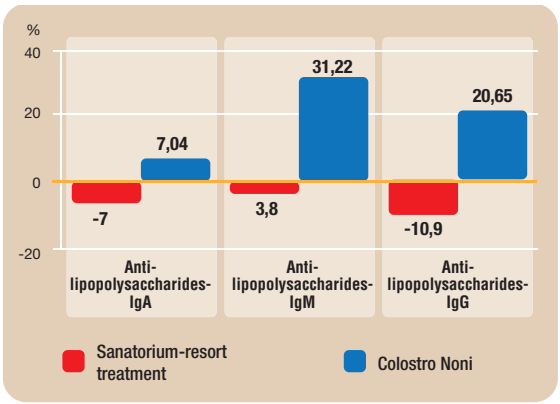
As it's shown on the DIAGRAM 11, sanatorium-resort treatment provoked the decline of the amount of anti-LPS-IgA and anti-LPS-IgG in the male patients and raised the average number of anti-LPS-IgM. Whereas the therapy by means of Colostro Noni didn't influence on anti-LPS-IgA level and raised the other amounts of AEAs.

In the female patients with asthma stan-



DIAG. 11

Influence of sanatorium-resort treatment and Colostro Noni on AEAs level in boys with asthma.



DIAG. 12

Influence of sanatorium-resort treatment and Colostro Noni on AEAs level in girls with asthma.

dard sanatorium-resort treatment complex contributed to the decline of humoral link factors of the antiendotoxic immunity with maximum changes of anti-LPS-IgG and anti-LPS-IgA. The therapy with Colostro Noni led to the increase of all indexes under review. The maximum increase of indexes was registered for anti-LPS-IgM and anti-LPS-IgG (DIAGRAM 12).

The indexes of antiendotoxic immunity changed in response to Colostro Noni and depended on the pathology of the Digestive System (TABLE 25).

Analysis of anti-LPS-IgA in response to the medication under investigation showed that its average amount decreased approximately by 1.5% regardless of GIT pathology. Opposite dynamics was registered for anti-LPS-IgA amounts. There was an increase of this amount by 6.35% in patients with asthma and digestive system disorders. However, the average amount of anti-LPS-IgM reduced by 3.83% in patients with concomitant diseases of GIT compared to the initial level. One should note the fact that with Digestive System disorders the amount of this index remained accurately ($p<0.05$) below control level, but where there are concomitant pathologies this amount surpassed the norm by 7%. In response to the medication under investigation the anti-LPS-IgG levels increased in both patients with or without GIT pathology. In the patients with the pathology there was an increase of anti-LPS-IgG by 15.38%. In the patients without it - by 25.53%.

The therapy that we conducted influenced ambiguously on the dynamics of AEAs (DIAGRAMS 13, 14).

The sanatorium-resort treatment and therapy by means of the medication led to the decline of anti-LPS-IgA and anti-LPS-IgM levels in patients with asthma, but without GIT disorders. Notably, in response to the combined therapy, the decrease of anti-LPS-IgA level passed 2.9 times slower, whereas anti-LPS-IgM level passed 5.3 times slower. The level of anti-LPS-IgG declined in response to sanatorium-resort treatment, but it increased under the influence of Colostro Noni.

Multidirectional dynamics of reviewed indices was registered in the patients with GIT disorders. Sanatorium-resort treatment contributed to the decrease of the average anti-LPS-IgA level 5 times faster

TAB. 24

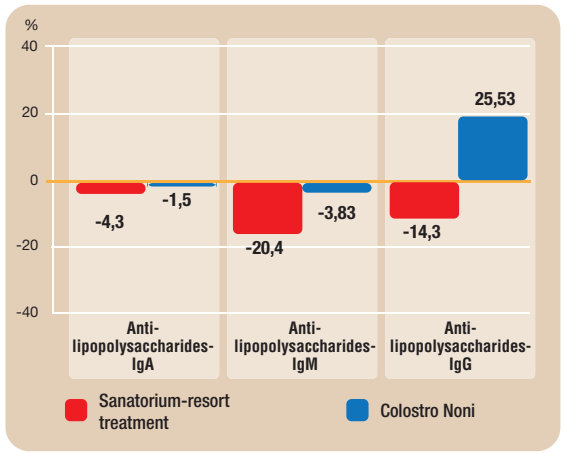
Dynamics of specific antibodies to LPSs of *E. coli* in boys and girls with asthma in response to Colostro Noni (M±m).

N	Indices	n	Anti-LPS-IgA, nominal units of optic density		Anti-LPS- IgM, nominal units of optic density		Anti-LPS-IgG, nominal units of optic density	
			Before	After	Before	After	Before	After
1	Boys (with asthma)	24	0.124 ±0.004	0.123 ±0.003	0.203 ±0.007	0.256 ±0.005	0.090 ±0.003	0.100 ±0.004
	p before/after			-		<0.001		-
	p with control amount			-		-		<0.01
2	Girls (with asthma)	21	0.142 ±0.008	0.152 ±0.006	0.237 ±0.010	0.311 ±0.007	0.092 ±0.004	0.111 ±0.004
	p before/after			-		<0.001		-
	p with control amount			-		-		-
3	Boys (control group)	8	0.132 ±0.012		0.310 ±0.034		0.132 ±0.009	
4	Girls (control group)	8	0.138 ±0.015		0.362 ±0.053		0.107 ±0.008	

TAB. 25

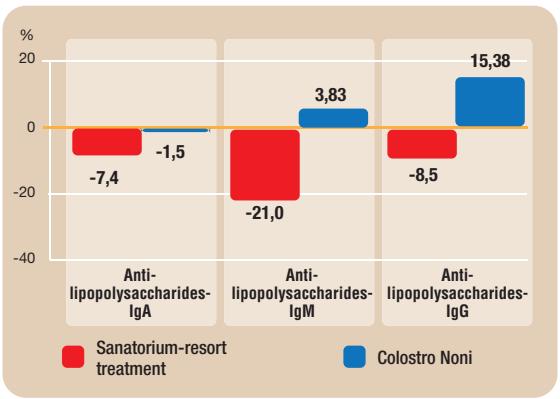
Dynamics of specific antibodies to LPSs of *E. coli* in children with asthma in response to MCL depending on concomitant GIT diseases (M±m).

N	Groups of children	n	Anti-LPS-IgA, nominal units of optic density		Anti-LPS- IgM, nominal units of optic density		Anti-LPS-IgG, nominal units of optic density	
			Before	After	Before	After	Before	After
1	Asthma and GIT diseases	25	0.136 ±0.005	0.134 ±0.005	0.252 ±0.012	0.268 ±0.009	0.091 ±0.003	0.105 ±0.002
	p before/after			-		-		-
	p with contr.			-		<0.05		-
2	Asthma without GIT diseases	20	0.138 ±0.012	0.136 ±0.010	0.366 ±0.043	0.352 ±0.016	0.094 ±0.010	0.118 ±0.005
	p before/after			-		-		-
	p with contr.			-		-		-
3	Control group	16	0.135 ±0.009		0.329 ±0.029		0.123 ±0.007	



DIAG. 13

Influence of sanatorium-resort treatment and Colostro Noni on the level of AEAs in the patients with asthma, without concomitant GIT pathology.



DIAG. 14

Influence of sanatorium-resort treatment and Colostro Noni on the level of AEAs in the patients with asthma and concomitant GIT pathology.

than the therapy with Colostro Noni.

Standard rehabilitation complex led also to the decline of anti-LPS-IgM and anti-LPS-IgG levels. Whereas the application of Colostro Noni provoked the increase of the reviewed indexes.

In our work we conducted a correlation analysis of interrelation between immune factors and endointoxication indexes, so the results are the following: initially heightened anti-LPS-IgA level have direct linear corerlation to PI (r=0.917; p<0.05), inverse week relation to IgE (r=-0.339; p<0.05), i.e. have contributed to the increase of one of the phagocytic links as well as decline of allergization.

Owing to sanatorium-resort treatment its amount almost reached the control level, and linear relation between it and phagocytosis completion index (r=0.974; p<0.001) was set up. Consequently, the increase of anti-LPS-IgA level was a compensatory enhancement of immune response and contributed to improvement of some phagocytic immune links and decrease of IgE.

Initially reduced immune response from anti-LPS-IgM correlated with the indexes of immune B-link: linear correlation with IgM (r=0.510;p<0.01) and inverse one with IgG (r= -0.558;p<0.001), also one should take into account the direct correlation with CD20+ (r=0.600; p<0.1).

Strong correlation with T-link of immunity was set up: inverse one with CD8+ (r= -0.826; p<0.05) and direct one with immune regulatory index (r=0.917; p<0.05).

Towards the end of sanatorium-resort treatment the number of correlative relations between accurately reduced anti-LPS-IgM level and all immune links decreased. We proved the direct linear correlation with CD20+ (r=0.900;p<0.001), strong relation with phagocytic index (r=0.708;p<0.05), inverse, linear correlation with circulating immune complexes (r=- 1.000;p <0.001), CD3+ (r= - 0.963; p<0.001), CD8+ (r= - 0.966; p<0.001), inverse correlation with IgE (r= - 0.446; p<0.01) and phagocytic number (r= - 0.447;p<0.05).

Great number of correlative relations with the indexes of all immune links denotes negative influence of LPS and decompensated immune response from anti-LPS-IgM to general immune *status* before and after treatment.

Accurately reduced anti-LPS-IgM level (p<0.05) before treatment had strong inverse correlation with CD8+ (r= - 0.704; p<0.05), CD3+ (r= - 0.730;p<0.05) and week inverse correlation with IgE (r= - 0.404;p<0.05).

After sanatorium-resort treatment, given trends to further decrease of anti-LPS-IgG level, 9 of 15 indices dealing with all immune links were focused on.

We proved a strong direct correlation with CD20+ (r=0.718;p<0.05), immune regulatory index (r=0.914;p<0.001), phagocytosis completion index (r=0.766; p<0.01), inverse one with CD8+ (r= - 0.748;p<0.05), CD4+ (r= - 0.823; p< 0.001), CD3+(r= - 0.746;p<0.05), phagocytic number (r= - 0.802;p<0.05) and week inverse correlation with IgE (r= - 0.391;p<0.05).

Therefore, accurately reduced anti-LPS-IgG level before and after sanatorium-resort treatment contributed to sustainability and formation of immune defects during the disease remission. The structure of the correlative relations with immune reactivity indexes bore a record to the influence of the antibody system defects specific to LPS of *E. coli* on these relations before as well as after sanatorium-resort treatment.

In response to the therapy with Colostro Noni we proved the direct relation with IgE (r=0.425;p=0.05) taking into account the level change of anti-LPS-IgA, a week relation with IgG (r=0.379; p<0.05) and relevant relation with IgM (r=0.215; p<0.1).

The therapy with Colostro Noni led to accurate (p<0.05) increase of anti-LPS-IgM and formation of week direct relations with IgM (r=0.436p<0.05), phagocytosis completion index (r=0.409;p<0.05), these indices almost reached the control amounts.

In children of this group we didn't find out any correlative correlations between AEAs with AWM level.

Structure of correlative relations with the indexes of all immune links bore a record to negative influence of LPS with accurately reduced anti-LPS-IgM level on general immune *status* before and after treatment.

In children of this group the indexes of immune reactivity correlated accurately with neither initially reduced anti-LPS-IgG level, nor physiologic level after application of the medication under investigation. However, there is a week direct correlation with immune regulatory index (r=0.288p<0.1) and inverse relation with IgG (r= -0.265;p<0.1) before treatment.

Such relation structure doesn't exclude the participation of LPS and defects of immune response from anti-LPS-IgG to general immune *status*; it also highlights the complexity of interrelations between antiendotoxic immunity and immune reactivity.

Therefore, the therapy with Colostro Noni took a multidirectional effect on antiendotoxic immunity factors.

Dynamics of AEAs depended on asthma severity and duration as well as on gender of the patients and concomitant pathology of digestive system. The treatment with Colostro Noni provoked positive changes of humoral antiendotoxic immunity, namely the number of anti-LPS-IgM, anti-LPS-IgG increased; their protective effect against LPS was also intensified.

Integrated effect of the medication was directed to improvement of barrier functions of respiratory passage and GIT mucous membranes.

In conclusion, the therapy with Colostro Noni led to the decrease of endotoxic burden on children with asthma.

Therefore, the results of this study testify that Colostro Noni produces an immunomodulatory effect on cellular, humoral, and phagocytic immune links. Moreover, Colostro Noni influences positively the antiendotoxic immunity of the patients with asthma. Colostro Noni is well tolerated by the patients: we didn't find out any negative side effect.

CONCLUSIONS

1. The patients treated with Colostro Noni developed far less gastroenterological complaints than they did it before.
   In **100%** of cases there was an improvement of appetite in the children; in **50%** of cases they stopped suffering from periodic sickness, vomit and belch after meal; in **63.6%** of cases - they stopped suffering abdominal pain; in **62.5%** of cases gaseous distention was reduced; in **57.1%** of cases intestinal habit was recovered.
2. Immunomodulatory effect of Colostro Noni on cellular immune link consisted in accurate ($p<0.05$-0.001) **increase** of average number of **CD3+**, **CD4+** cells, and significant rise (by 2 %) of lymphocytes carrying on their surface CD8+ receptors.
   – These changes took place against the **decline of CD20+** number; this fact bore a record to normalization of immune response of the child with asthma.
3. The changes in humoral immune link with the administration of Colostro Noni consisted in an accurate ($p<0.001$) **decline of IgE** and **serum IgA** as well as slight **increase** of **IgM**. This fact bears the record to the decrease of allergization of the ill children.
4. Colostro Noni **raised phagocytic activity** of Immune System.
   This fact proved the increase of phagocytic index, phagocytic number ($p<0.001$), and phagocytosis completion index ($p<0.001$).
5. In asthmatic patients treated with Colostro Noni there was a **decrease** of average amount of **circulating immune complexes** by 35.5% ($p<0.01$). This fact bore a record to the decline of antigenic burden on the children with asthma.
6. As part of the study it was found out that Colostro Noni adjusted the **level of AEAs** to *E. coli* endotoxin. This led to the increase of anti-LPS-IgM ($p<0.05$) and anti-LPS-IgG ($p<0.01$) against the decline of anti-LPS-IgA. The dynamics of these indexes depended on asthma severity and duration, patient's gender and concomitant GIT pathology.
7. Colostro Noni was well tolerated by all the patients with asthma and did not show any negative side effect. ■

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