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Clinical trial / experimental study (consort compliant): Optimal time period to achieve the effects on synbiotic-controlled wheezing and respiratory infections in young children

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SUMMARY

Introduction Urban life is often followed by immune dysfunction and loss of immune tolerance in the youngest children.

Objective The study aimed to determine optimal time efficiency of a synbiotic (5×10^9 *Lactobacillus acidophilus* Rosell-52, *Bifidobacterium infantis* Rosell-33, *Bifidobacterium bifidum* Rosell-71) in controlling respiratory infections and wheezing disease.

Methods We randomly selected a group of children younger than five years, hospitalized earlier, and classified them into three groups.

Results The incidence of respiratory infection before the study was once a month, while after a three-month supplementation with the synbiotic children rarely suffered from respiratory infections, and the state was maintained after six-month and nine-month supplementations with the synbiotic. The decreased incidence of respiratory infections was followed by a falling incidence of concomitant wheezing. A significant increase in tIgA serum was observed in all groups for only three months, the increase being the highest in children with recurrent respiratory infections accompanied by wheezing. After a nine-month administration of the synbiotic, total IgE serum was lower in all groups of patients.

Conclusion The optimal duration of administration of the synbiotic containing three probiotic cultures to provide effective control of the frequency of respiratory infections was three months, and six months were required to establish control of the frequency of wheezing. This synbiotic is useful for immunomodulation in children and is well-tolerated in young children.

Keywords: biodiversity; synbiotics; wheezing; respiratory tract infections; children

INTRODUCTION

Urban life along with high exposure to certain chemical substances, limited green space and abandoned traditional diet and habits have caused loss of diversity among plants, animals, their habitats and microbial world, followed by immune dysfunction and loss of immune tolerance in humans, primarily in the youngest children [1]. The concept of biodiversity loss may explain the rapid increase in the incidence of allergic diseases since 1960 to the present day, following the so-called "hygiene hypothesis", also referred to as "microbial hypothesis" [2, 3, 4]. Over the last 50 years the number of patients with asthma in the world has increased three and one-half times, i.e. the currently growing incidence of asthma in children is 0.18% per year [3]. The World Allergy Organization published guidelines for prevention of allergies involving the application of probiotic bacteria in fermented food or in encapsulated form [1, 5].

In recent surveys by Greek and American authors it is argued that immunological mechanism of probiotics has not been clearly established and the same is true for synbiotics [6, 7]. It is known that microbiome in one- to three-year-olds may achieve consistent balance that is maintained in adults, with the intestinal microbiome playing a key role in maintaining "mucosal health," with this dynamic ecosystem being responsible for the initiation and progression of many chronic inflammatory diseases [7, 8].

Overall, probiotic bacteria stimulate immune maturation and the establishment of oral and immune tolerance as preconditions for health. The effect of probiotics and prebiotics in the form of synbiotics are subject to the quality of biofilm formation in the gastrointestinal tract, depending on the type and level of probiotic bacteria in a supplement [9]. Presently, there is a large number of mixed probiotic cultures blended with various prebiotics, and accordingly, different effects have been identified.

OBJECTIVE

The study aimed to determine optimal time efficiency of the synbiotic in control of respiratory infections, as well as of allergic diseases (primarily wheezing illness), to make comparison between the groups involved, and comparison with optimal time of increase in total IgA (tIgA) serum in children younger than five years of age.

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METHODS

We were interested in whether our synbiotic containing three probiotic cultures would stimulate the growth of tIgA, and in the time required for the serum growth in children with frequent respiratory infections and/or wheezing illness; whether it would lead to the "without health problems" state, which is in immunological sense comparable to the state of tolerance due to previous modulation, and whether it would provide prolonged control of infection and allergic diseases [10, 11]. The results of standardized protocol were retrospectively analyzed.

Randomization and masking

We randomly selected a group of children under five years of age who were admitted to the hospital over the previous year, and classified them into the following three main groups: G-I (group I, with respiratory infection and wheezing), G-II (group II, with respiratory infection without wheezing), and G-III (group III, with wheezing without accompanying respiratory infections). Children in the group with wheezing-associated respiratory infection (G-I) manifested wheezing once or a few times a month. Wheezing as a symptom of bronchial obstruction was the decisive criterion and we identified it in children with wheezing bronchitis and asthma, predominantly in G-III.

The children were given dietary supplement synbiotic containing 5×10^9 colony forming units, in the form of lyophilized microcapsules containing viable probiotic bacteria *Lactobacillus* (L.) *acidophilus* Rosell-52, *Bifidobacterium* (B.) *infantis* Rosell-33, *B. bifidum* Rosell-71, labeled as LABIBB [12, 13, 14]. Synbiotic LABIBB also contains a prebiotic in the form of 750 mg of fructooligosaccharides from asparagus, garlic, onion, wheat, oats, and soybeans, stimulating growth of probiotic bacteria, and excipients (potato starch, vanilla flavor). A single dose of LABIBB synbiotic has an energy value of 6.03 kcal. It only contains 1.43 g of carbohydrates and 0.035 g of protein, but no fats. LABIBB synbiotic is a powder soluble in water, or tea.

Before the supplementation with LABIBB synbiotic, we identified the absence of anemia in patients and we excluded anemia as a risk factor for recurrent infections. We put the emphasis on correlation between the clinical manifestation and atopic status, on the one hand, and the levels of immunoglobulin in affected children, on the other.

The immune status was assessed using the value of C-reactive protein (CRP), leucocyte count, lymphocyte count, the percentage of virocytes, levels of immunoglobulin (tIgA, tIgG, tIgM), serum level of vitamin D, and serum level of zinc. The LABIBB synbiotic was prescribed to patients whose condition had been monitored and compared every three months over a nine-month period. The patients who entered the study had not been administered any probiotic supplementation, which was an excluding criterion.

Atopic status was assessed using skin prick test (SPT) determining susceptibility toward total IgE (tIgE) and 30 common respiratory and food allergens. High atopic status was determined in children with induration ≥3 mm compared to the negative control SPT, low atopic status with induration 1–2 mm and non-atopic status was connected to the absence of induration and erythema [15]. Criteria for the group of 'sensitized children' were personal medical history including one or more allergic diseases (eczema, food allergy, other types of urticaria, wheezing bronchitis, and asthma), positive SPT results, and tIgE ≥17 IU/l. Criteria for the group of 'non-sensitized children' were absence of allergic diseases, negative SPT results toward 30 allergens and tIgE ≤17 IU/l.

Value of tIgA ≤0.15 g/l measured by Makler® ILAB (Biokit, Barcelona, Spain) system suggested selective deficiency of tIgA.

Some authors were often faced with parents' failure to take their children to scheduled medical check-ups [12]. In our case, the problem was overcome by scheduling check-ups through day hospital care, where we obtained complete laboratory analyses and findings of clinical examination within an hour, without hospitalization, which was motivating for parents to attend regularly. By the end of the study, additional motivation for the parents to bring their children for check-ups was their impression that the children suffered markedly less from respiratory infections and wheezing after only a few weeks of synbiotic administration.

Statistical analysis

We calculated descriptive statistics – central tendency and dispersion parameters of the investigated variables. Mean value and standard deviation were calculated for each variable, wherein particular attention was paid to significance >95%, with the corresponding p-value. To determine the effects of application of synbiotic compared to the initial state, we applied one-factor repeated measures (ANOVA). To calculate specific variable correlation we computed the Pearson's correlation coefficient and Pearson's chi square test ($P\chi^2$) with contingency coefficient. Complete data processing was performed using SPSS statistical software v.12.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The present study is an observational, cohort 'case-control type' approach analyzing the three groups of 78 hospitalized children: 50 in G-I, 17 in G-II, and 11 in G-III. Data on age, sex, diet during infancy, on wheezing illness from personal medical history, eczema, allergy to cow's milk and other urticaria, allergic rhinitis, family medical history data, the incidence of wheezing and respiratory infections, reason for hospitalization, C-reactive protein value, and hematological status of patients are presented in Table 1.

The incidence of respiratory infections and wheezing during the nine-month supplementation with LABIBB synbiotic is shown in Tables 2 and 3. Diagnostic tests with accompanying charts for IgA and IgE are shown in Table 4, and Graphs 1 and 2.

Table 1. Characteristics of investigated groups

Characteristics			Group of patients		
			I (n = 50)	II (n = 17)	III (n = 11)
Gender, n (%)	Male		33 (66.0)*	10 (59.0)*	4 (36.0)*
	Female		17 (34.0)	7 (41.0)	7 (64.0)
Age (years)	Mean±SD		15.89±11.36**	26.15±15.00**	28.36±12.69**
History, n (%)	W/A diseases before the study		35 (70.0)	8 (46.0)	11 (100.0)
	Wheezing bronchitis		22 (44.0)	5 (29.0)	3 (27.0)
	Asthma		11 (22.0)	2 (12.0)	5 (46.0)
	Eczema		7 (14.0)	0	2 (18.0)
	Cow's milk allergy		1 (2.0)	0	1 (9.0)
	Other urticaria		2 (4.0)	0	1 (9.0)
	Allergic rhinitis		1 (2.0)	2 (12.0)	3 (27.0)
	W/A diseases in first-degree relatives		26 (52.0)	4 (23.5)	7 (63.6)
Frequency of wheezing, n (%)	Before synbiotics		49 (98.0)	7 (41.2)	11 (100.0)
	After three months of synbiotic		18 (35.3)***	1 (5.9)**	6 (54.5)**
	After six months of synbiotic		0***	0**	0**
	After nine months of synbiotic		0***	0**	0**
Frequency of respiratory infection, n (%)	Before synbiotics		31 (62.7)	12 (70.6)	4 (36.4)
	After three months of synbiotic		4 (7.8)***	1 (5.9)***	1 (9.1)*
	After six months of synbiotic		0***	0***	0*
	After nine months of synbiotic		0***	0***	0*
Main reasons for hospitalization before synbiotics, n (%)	Pneumonia		49 (98.0)	7 (41.0)	0
	W/A diseases	Asthma	0	0	5 (46.0)
		Cow's milk allergy	0	0	3 (27.0)
		Eczema	0	0	2 (18.0)
		Allergic rhinitis	0	0	1 (9.0)
Hematology findings at admission, mean±SD	CRP (mg/l)		17.49±28.54	46.52±66.75	6.82±12.66
	Leukocytes ($\times 10^9$/l)		13.65±4.24	13.69±6.38	9.57±3.48
	Lymphocytes		0.41±0.2	0.4±0.21	0.43±0.08
	Virocytes		0.36±0.49	0.53±0.51	0.09±0.165
	Erythrocytes ($\times 10^{12}$/l)		4.39±0.60	4.38±0.71	4.48±0.45
	Hemoglobin (g/l)		119.48±20.77	116±15.40	125.55±8.97
	Platelets		348.4±116.21	308.53±107.95	284.91±60.22
Other diseases, alone or associated with pneumonia on admission, n (%)	Otitis		8 (16.0)	7 (41.0)	0
	Bronchiolitis		2 (4.0)	0	0
	Pharyngitis		0	1 (6.0)	2 (18.0)
	Laryngitis		1 (2.0)	0	0
	Sepsis		1 (2.0)	3 (18.0)	0
	Gastroenteritis		1 (2.0)	0	0
	Angina		1 (2.0)	0	0
	Flu		1 (2.0)	0	0
	Severe respiratory insufficiency		1 (2.0)	0	0
	Pancreatitis		0	1 (6.0)	0
	Urinary infection		0	1 (6.0)	0
Other conditions on admission, n (%)	Enlarged adenoids		0	1 (6.0)	0
	Hearing loss		0	1 (6.0)	0
	Contact with tuberculosis		0	1 (6.0)	0
	Bronchiectasis		0	1 (6.0)	0
	Joint pain		0	1 (6.0)	0
	Diabetes		0	0	1 (9.0)
	Hypotrophy		1 (2.0)	0	0
	Obesity		0	0	1 (9.0)
	Constipation		0	0	1 (9.0)

* p<0.05; ** p<0.01; *** p<0.001

SD – standard deviation; W/A – wheezing/allergy (diseases); CRP – C-reactive protein

Table 2. Frequency of respiratory infections in children during synbiotic supplementation

Period	Group of patients		
	I	II	III
Baseline	62.7%	70.6%	36.4%
After three months	7.8%	5.9%	9.1%
After six months	0.0%	0.0%	0.0%
After nine months	0.0%	0.0%	0.0%

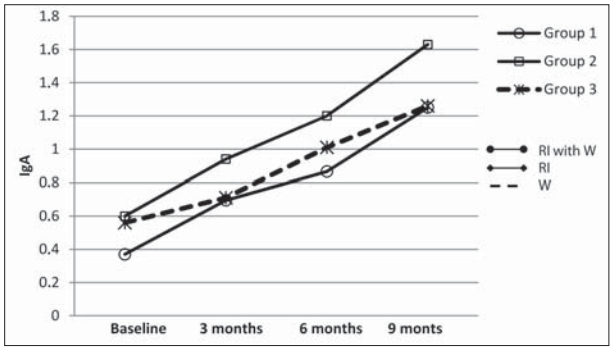
Table 3. Frequency of wheezing in children during synbiotic supplementation

Period	Group of patients		
	I	II	III
Baseline	92.0%	41.2%	100.0%
After three months	35.3%	5.9%	54.5%
After six months	0.0%	0.0%	0.0%
After nine months	0.0%	0.0%	0.0%

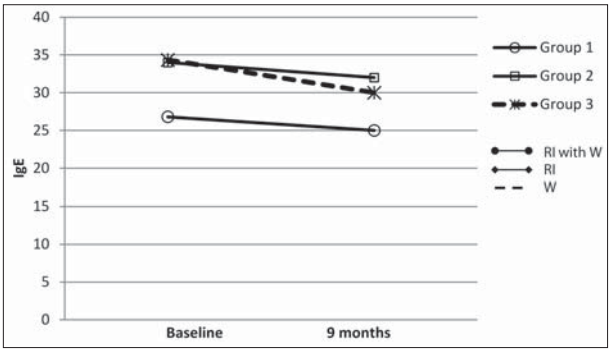
No side effects of LABIBB synbiotic were identified in the examined children and it was well tolerated in children aged month and one-half to five years. This result was consistent with findings of other authors who also assessed the safety profile and tolerance of probiotics in newborn children and infants [16, 17, 18].

DISCUSSION

Previous studies on probiotics have rarely focused on children of the youngest age and meta-analyses have not provided sufficient evidence for probiotic- or synbiotic-controlled incidence of respiratory infections [8, 16, 19]. A conclusion of Taylor et al. [17] that postnatal administration of probiotic strains of *L. acidophilus* was not effective in con-



Graph 1. IgA serum levels in children with respiratory infections (RI) and wheezing (W) during nine months of supplementation of LABIBB synbiotic



Graph 2. IgE serum levels in children with respiratory infections (RI) and wheezing (W) during nine months of supplementation of LABIBB synbiotic

Table 4. Diagnostic tests

Characteristics		Group of patients		
		I (n = 50)	II (n = 17)	III (n = 11)
Allergy Skin Prick Test	Wheal (mm), mean±SD	9.74±9.63	6.33±6.78	12.64±10.07
	n (%)	34 (68%)	12 (71%)	8 (73%)
	Wheal (mm), mean±SD	2	2	2
	n (%)	1 (2%)	1 (6%)	1 (9%)
	Wheal (mm), mean±SD	0	0	0
	n (%)	15 (30%)	1 (6%)	2 (18%)
Serum tIgE (IU/L), median (range)	Before synbiotics	26.80 (9.62–47.16)	34 (11.7–154)	34.33 (8.28–118.11)
	After nine months of synbiotic	25.00 (9–46.00)***	32 (11–150)**	30 (5–111.75)**
	IgE median change	1.17 (0.515–1.83)***	1 (0.805–1.765)***	4.635 (2.28–6.08)***
Serum tIgA (g/L), median (range)	Before synbiotics	0.37 (0.2 – 0.82)	0.6 (0.21 - 1.575)	0.56 (0.11 - 1.12)
	After three months of synbiotic	0.695 (0.323-1.14) ***	0.94 (0.385 - 1.61)	0.71 (0.37 - 1.34)
	After six months of synbiotic	0.865 (0.673-1.41) ***	1.2 (0.74-1.875) ***	1.01 (0.78-1.4)**
	After nine months of synbiotic	1.25 (0.980-1.723)***	1.63(0.990-2.15)***	1.26 (1.09-1.99)**
Changes in tIgA versus changes in tIgE		-0.039	0.629**	-0.285
tIgG serum (g/l), mean±SD	Before synbiotics	6.64±2.97	7.61±2.91	7.68±4.15
	After three months of synbiotic	10.37±3.24***	11.54±3.03**	11.34±4.26**
tIgM serum (g/l), mean±SD	Before synbiotics	1.24±0.57	1.29±0.49	0.96±0.25
	After three months of synbiotic	1.64±0.53***	1.66±0.36***	1.38±0.44**
Vitamin D in serum (ng/ml), mean±SD	Before synbiotics	26.87±11.63	16.35±2.3	25.96±25.29
Zinc in serum (µmol/l), mean±SD	Before synbiotics	13.13±2.98	12.25±1.48	12.45±1.21

* p<0.05; ** p<0.01; *** p<0.001

t – total; Ig – immunoglobulin in serum

tIgA normal range – 0.7–4 g/l; tIgG normal range – 7–16 g/l; tIgM normal range – 0.4–2.3 g/l

trolling atopic eczema suggested that we check the efficiency of our mixture of three probiotic strains to control wheezing bronchitis and asthma. The study by Kukkonen et al. [16] motivated us to determine the impact of our three-strain probiotic blend on the control of respiratory infections and an increase in total IgA serum in the youngest children [8].

We found that boys were more likely to have suffered from frequent respiratory infections or respiratory infection with striking clinical features than girls (Phi=3.325, p=0.19) (Table 1). The average age of children who suffered from frequent respiratory infections or respiratory infection with striking clinical features was 16 months, of which 44% were younger than 12 months. In all children under 12 months of age, recurrent respiratory infections were accompanied by wheezing. In two- to five-year-olds, respiratory infections were more rarely accompanied by wheezing if compared to younger age. Wheezing bronchitis and asthma were identified more often in girls than in boys, and the average age of wheezers was 28 months (G-I vs. G-II p=0.013; G-I vs. G-III p=0.01; G-II vs. G-III p>0.99, by Bolan Medical College test).

The children in G-I and G-II usually suffered from pneumonia. In these children, the incidence of respiratory infection before the study was once a month, while after a three-month supplementation with LABIBB synbiotic the children rarely suffered from respiratory infections (G-I 7.8%, p<0.0005 by Wilcoxon signed rank-test [WT]; G-II 5.9%, p=0.001; G-III 9.1%, p=0.046), and the state was maintained after a six-month supplementation (G-I p<0.0005; G-II p=0.001; G-III p=0.046 [WT]), and after nine-month supplementation with LABIBB synbiotic (G-I p<0.0005; G-II p=0.001; G-III p=0.046 [WT]) (Tables 1 and 2). This finding is consistent with the results of Cazzola et al. [12] obtained in children aged three to seven years, and Kukkonen et al. [16], who investigated pregnant women and children of the youngest age, confirming the immunomodulatory effect of certain types of synbiotics.

Simultaneously with the implementation of LABIBB synbiotic, the decreased incidence of respiratory infections was followed by falling incidence of concomitant wheezing (G-I 35.3%, p=0.0005; G-II 5.9%, p=0.014; G-III 54.5%, p=0.003 [WT]) (Table 1). Concomitant wheezing was found to be atopic in 68% of children in G-I (Tables 3 and 4). Britton and Versalović [20] and Moller and de Vrese [21] detected restitution of balance between Th1 and Th2 responses influenced by cytokines (TNF, IL6, IL10), released by monocytes in a patient supplemented with *B. lactis* and *L. acidophilus* blend over a period of eight weeks. This was in contrast to the findings presented by de Vrese et al. [22]. By clinical assessment, we determined controlled frequency of respiratory infections with or without wheezing (G-I, G-II) in children supplemented with LABIBB synbiotic within three months, which may suggest restoration of the immune balance.

Before administration of LABIBB synbiotic, we found selective deficit of tIgA serum (Table 4) in every fifth child (18%), manifested by recurrent respiratory infections or serious clinical symptoms of respiratory infection, with or without wheezing (G-I, G-II), while other patients in these two groups had slightly lower values of tIgA serum (>0.16 g/l). Before the introduction of LABIBB synbiotic, the values of tIgA serum were significantly lower in children suffering from wheezing-associated respiratory infections (median 0.37 [0.2–0.82]) than in children with respiratory infection without wheezing (median 0.6 [0.21–1.575]), and this relationship (Pearson correlation, r=-0.534, p=0.027) was maintained throughout the period of application of LABIBB synbiotic, i.e. after three months (G-I, p<0.000; G-II, p=0.092, G-III p=0.062 [WT]); after six months (G-I p<0.000; G-II p<0.000; G-III p=0.050 [WT]); and after nine months (G-I, p<0.000; G-II, p<0.000; G-III p=0.050 [WT]) (Table 4). Due to LABIBB synbiotic, a significant increase in tIgA serum was observed in all groups for only three months, the increase being the highest in children with recurrent respiratory infections accompanied by wheezing (median 0.695 [0.323-1.14], p<0.000 [WT]) (Table 4, Graph 1). The tIgA serum values were constantly growing in all groups (Table 2). The increase in tIgA serum in children from G-I and G-II was followed by clinical improvement, i.e. the absence of infection and concomitant wheezing after only three months of supplementation with LABIBB synbiotic, which can be related to clinical immune maturation [8, 23, 24, 25].

Immunological maturation was due to an increase in the number and diversity of the gastrointestinal tract microbiota after the administration of our blend of three probiotic strains [2, 21, 26]. A number of authors have shown particular potential of *Lactobacillus acidophilus* Rosell-52 to increase the number of immune cells, to stimulate the release of IL6 and clonal expansion of B-lymphocytes supposed to produce IgA, to participate in competitive exclusion of pathogens, to stimulate macrophages and cytokines that coordinate the activity of the entire immune system, primarily in Th-1 response and thus promote immune recovery [20, 22-25, 27, 28]. Kechagia et al. [27] argued that probiotic bacteria are able to regulate maturation of myeloid dendritic cells and release of cytokines determining the activity of T-cells toward the one of the following immune responses: Th1, Th2, or Treg.

Atopic wheezing in 68% of children in G-I and 71% of children in G-II is associated with significant family medical history of wheezing and/or allergic diseases (Table 1) in mother, father, brother or sister (p=0.039), but not necessarily associated with actual degree of sensitivity in these children (size of induration obtained SPT, tIgE). Finally, nine-month LABIBB synbiotic supplementation in children with recurrent respiratory infections with or without wheezing (G-I, G-II) appeared to have timely immune maturation accompanied by an increase in tIgA serum, complete clinical picture of "not-catching respiratory infections" and "absence of wheezing symptoms," and decline of the tIgE.

Before LABIBB synbiotic supplementation, selective deficit in tIgA serum was detected in every third child suffering from wheezing in G-III (36%). Median tIgA serum in G-III was 0.56 (0.11–1.12). As in other groups, TIgA serum in G-III showed growth, but it happened slowly, and only within six to nine months of supplementation with LABIBB did synbiotic tIgA serum appear to rise quickly (three months

p=0.062, six months p=0.05, nine months p=0.05) (Table 4, Graph 1). Children suffering from wheezing had symptoms of the disease twice a month before supplementation with LABIBB synbiotic; after three months of supplementation the symptoms manifested once in two months; after six months of supplementation with LABIBB synbiotic, no symptoms were detected in the previous three months and such a state was maintained until the ninth month of supplementation with LABIBB synbiotic. In children with wheezing bronchitis or asthma (G-III), the increase in tIgA serum was accompanied by the absence of wheezing only after six months of supplementation. It should be noted that atopic type of wheezing was detected in 73% of children with wheezing (G-III) (Table 1), with SPT induration 12.64 (10.07 mm, elevated levels of total IgE79.19 [111.22 IU/l [median 34.33 [8.28–118.11]]) (Table 4, Graph 2).

Children's parents were mainly familiar (64%) with the fact that there were first degree relatives suffering from wheezing and/or an allergic disease (Table 1). Approximately 36% of parents in G-III reported respiratory infection within six months or more before the start of the study. After six months of application of LABIBB synbiotic, these children did not display any symptoms of respiratory infection or concomitant wheezing (Tables 2 and 3).

From the clinician's perspective, children with predominantly allergic wheezing diseases achieved immunological maturity relatively slowly – within six months, induced by LABIBB synbiotics, while children with respiratory infections with or without wheezing achieved immune maturity somewhat faster – within three months (IgA for three months: G-I p=0.000; G-II p=0.092; IgA for six months: G-I p=0.000; G-II p=0.000 [WT]). Presumed immune mechanism in atopic children is the following: the mixture of three probiotic strains (LABIBB) first elicited subtle intestinal inflammation, followed by an increase of fecal IgA and tIgA serum, with simultaneous controlled release of tIgE. Similar effects were obtained by several authors in their research by using a mixture of four probiotic strains (*L. rhamnosus* GG, *L. rhamnosus* LC705, *B. breve* Bb99, *Propionibacterium freudenreichii* ssp. *Shermanii*) or a single strain of lactic acid bacteria [8, 23, 24, 27]. De Vrese et al. [22] found that lymphocytes producing IgA, and thus providing protection against inhaled pathogens, came from the gastrointestinal tract, where they were cloned over a long period of time under the influence of probiotic bacteria [25]. Product fermented by *B. breve* differentially activated kinase affecting modulation of biological function of dendritic cells and establishment of Th1/Th2 balance of immune response, and probiotic mechanisms of action require a six- to nine-month period, as clinically confirmed by our present study [26, 29].

After nine months of administration of LABIBB synbiotic, tIgE serum was lower in all groups of patients (G-I p<0.000; G-II p=0.005; G-III p=0.005 [WT]; and Kruskal–Wallis test p=0.001). Slight decrease in tIgE serum was detected along with increase in tIgA serum, after nine months of LABIBB synbiotic supplementation in children with respiratory infection without wheezing (Spearman's rho test p=0.802 in G-I, p=0.038 in G-II; p=0.425 in G-III). Taylor et al. [17] and Kuitunen et al. [18] did not detect a decrease in tIgE serum influenced by a four-probiotic strain synbiotic, which is contrary to the findings of Kalliomaki et al. [24].

We did not identify a significant change in reciprocal relationship between tIgE and tIgA after a nine-month supplementation with LABIBB synbiotic in children with wheezing-associated respiratory infection (Spearman's rho test p=0.802 in G-I). As Kuitunen et al. [18] found, we also explained such finding by paradox Th2 stimulation associated with T-regulatory lymphocytes activation caused by probiotic mixture. It is clear that the potential of probiotic blend is determined by types and number of probiotic strains, and strength and duration of effect on immune maturation and immunomodulation. To achieve good health, probiotic mixture "has to" control immune responses determined by epigenetic changes and atopic and infectious diatheses.

The selective deficit of IgA in children with wheezing-associated respiratory infection (G-I) before the application of LABIBB synbiotic was accompanied by slightly reduced values of tIgG in 66% of subjects, and a significant increase in tIgG up to reference values (G-I p=0.000; G-II p=0.005; G-III p=0.006 [WT]) appeared during three months of LABIBB synbiotic application, along with an increase in IgA and IgM (G-I p=0.000; G-II p=0.001; G-III p=0.029 [WT]). This is also clinical evidence of immune maturation influenced by LABIBB synbiotic in children of the youngest age, which has also been discussed by Vyas and Ranganathan [11], Famularo et al. [26] and Takahashi et al. [30].

CONCLUSION

We may conclude that optimal duration of administration of a synbiotic containing three probiotic cultures (LABIBB) to provide effective control of the frequency of respiratory infections was three months, and six months were required to establish control of the frequency of wheezing. Clinical features changed along with simultaneous increase in total IgA serum and the changes maintained during the period of application of LABIBB synbiotic. The increase in IgA was accompanied by an increase in the level of IgG and IgM kinds of immunoglobulins and a decrease in IgE. LABIBB synbiotic is useful for immunomodulation in children and well tolerated in young children. We have planned to check-up our patients in two years after the termination of synbiotic supplementation to identify possible delayed effects of LABIBB synbiotic on children's health. Strategy of implementation of LABIBB synbiotic presumes safe supplementation for children and effectively controlled disorders caused by the loss of biodiversity in the environment.

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Клиничко испитивање/експериментално истраживање (стандардизовано приказивање): оптимално време потребно да синбиотик постигне ефикасну контролу шиштања у грудима и респираторне инфекције код мале деце

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КРАТАК САДРЖАЈ

Увод Градски живот је често праћен дисфункцијом имунског система и губитком имунске толеранције код најмлађе деце.

Циљ рада Истраживање је усмерено на одређивање оптималног времена деловања синбиотика (5×10^9 *Lactobacillus acidophilus Rosell-52*, *Bifidobacterium infantis Rosell-33*, *Bifidobacterium bifidum Rosell-71*) у контроли респираторних инфекција и шиштања у грудима (тзв. визинга).

Методе рада Методом случајног избора издвојена су деца млађа од пет година која су била раније болнички лечена, те потом сврстана у три групе.

Резултати Респираторна инфекција се пре истраживања јављала једном месечно, док су после три месеца суплементације синбиотиком деца ретко боловала од инфекција дисајних органа. Ово стање се одржавало и после шест и после девет месеци суплементације синбиотиком. Смањена учесталост респираторних инфекција била је праћена смањењем инциденције пратећег визинга. Значајан пораст нивоа укупног *IgA* у серуму забележен је у свим групама за само три месеца. Повећање нивоа је било највеће код деце с рекурентним респираторним инфекцијама праћеним визингом. После девет месеци примене синбиотика ниво укупног *IgE* у серуму је био нижи у свим групама болесника.

Закључак Оптимално време примене синбиотика са три пробиотске културе потребно да обезбеди ефикасну контролу учесталости респираторних инфекција је три месеца, а шест месеци је потребно да се успостави контрола учесталости визинга. Овај синбиотик је користан за имуномодулацију код деце и добро га подносе деца раног узраста.

Кључне речи: биоразноврсност; синбиотик; визинг; респираторне инфекције; деца

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