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The influence of oligosaccharide-containing formula feeding on establishment of intestinal biocenosis and protective functions of child's organism

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Dietary modulation of the intestinal microflora and immune response is one of the important problems in the nutritional sciences today. Human milk oligosaccharides play an important role in postnatal development of the intestinal flora and protective functions of child's organism. The present review summarizes the data of experimental research and clinical studies concerning the possible effects of probiotic mixture of galacto-oligosaccharides and fructo-oligosaccharides in Ukraine. The data demonstrate that prebiotic oligosaccharides such as studied mixture provide beneficial effects for formula fed infants. The results from several studies in Ukraine demonstrate that probiotic oligosaccharides stimulate the growth of bifidobacteria and lactobacilli, reduce the growth of pathogens, decrease faecal pH, normalize the stool consistency and modulate immune system as human milk does.

Key words: *Prebiotics, FOS, GOS, oligosaccharides, intestinal flora, immunonutrition, immune system, infants*

Wpływ pokarmu zawierającego ologisacharady na powstanie biocenozy jelitowej oraz funkcji ochronnej organizmu dziecka

Żywnościowe kształtowanie się mikroflory przewodu pokarmowego i odpowiedzi immunologicznej jest jednym z najważniejszych problemów dzisiejszych nauk żywieniowych. Ludzkie mleko oligosacharydowe (mleko matki) odgrywa ważną rolę w rozwoju poporodowym mikroflory przewodu pokarmowego, jak i funkcji obronnych organizmu dziecka. Prezentowana analiza streszcza dane eksperymentów i badań klinicznych dotyczących potencjalnych efektów mieszanek probiotycznych galaktooligosacharydowych i fruktooligosacharydowych na Ukrainie. Dane dowodzą, że probiotyki oligosacharydowe, takie jak przebadana mieszanka, mają korzystny wpływ na niemowlęta karmione mieszankami. Rezultaty z kilku przeprowadzonych badań na Ukrainie dowodzą, że probiotyki oligosacharydowe pobudzają wzrost bifidobacterii i lactobacillum, redukują wzrost czynników patogennych, obniżają pH kału, normalizują konsystencję stolca i kształtują system odpornościowy człowieka tak jak robi to ludzkie mleko.

Słowa kluczowe: *probiotyki, FOS, GOS, oligosacharydy, mikroflora przewodu pokarmowego, żywienie zawierające środki wpływające na odporność, system immunologiczny, niemowlęta*

It is already well known that an optimum feeding behaviour belongs to the leading factors which determine the health of children and adults, their physical and psychic development. In many researches it was observed that correlation exists between nutrition and frequency of appearance and severity of infectious diseases [10, 11, 22, 26, 30, 36, 49]. Yet in 1968 in the monograph of R.K. Chandra "The relation between immunology, nutrition and disease in elderly people" it was confirmed that malnourished people are more susceptible to infections. An attempt to explain this dependence was done in the monograph; however the only progress of immunology during the last decades clarified the pathogenetic mechanisms of these processes [11].

For the first time the term “immunonutrition” was used in 1992 by J. Daly in the article devoted to the metabolic and immunological aspects of enteral nutrition enriched with arginin, nucleinic acids and ω-6-fatty acids in patients after surgical interventions [14]. Later it was found that specially adopted nutrition causes changes of phagocytosis and neutrophil bactericidal activity, total amount of neutrophils, lymphocytes, activated T-lymphocytes, NK-cells, immunoglobulins A, M, G and γ-interferon production in adults and children with various diseases and pathological states [6, 13, 17, 26, 31, 34].

Accumulation of new data confirms a thesis that infants' nutrition has long-lasting outcomes for health and plays an important role in prevention of a group of chronic non-infectious diseases in adults and influences the function of physiologic systems of child in the future. Children fed inappropriately to their physiological needs have increased frequency and severity of intestinal and other infections and high risk of premature death. Besides that, the lack of nutrients or their disproportion causes failure to thrive, decrease of cognitive function, immune disorders, appearance of allergic reaction, improper realization of genetic potential for child development, change of structure of cellular membranes and receptors, appearance of pathologic intestinal biocenosis [3, 4, 7, 8, 30, 32, 37, 45].

Immunity is an important and dynamic system which constantly synthesizes new molecules and compounds, provides high level of cell proliferation and differentiation, maintains a tissue uniqueness, protects our organism from penetration of extraneous proteins and biopolymers, keeps under control humans genetic program by destruction of transformed and old cells, determines the course of infectious, inflammatory, autoimmune and allergic processes [17].

More often immune system is referred as a system of endogenous nutrition. One of the basic function of immunity is disintegration of highly-molecular organic compounds to the primary molecules (amino acids, monosaccharides, fatty acids, lipids, nucleic acids) and providing of effective and complete reutilization of nutriments, appeared in the course of vital processes (dead cells and products of their decomposition, microorganisms, incompletely digested food) [10, 17].

From this position, it could be defined that the unique system of cells nutrition in the organism includes 3 subsystems: exogenic digestive system, immune system and intracellular system of disintegration. Their concordance is provided by signal molecules: molecules of immunoglobulins, molecules of major histocompatibility complex and heat shock molecules [17, 36].

The immune system of stomach and bowels has got the name of gut associated lymphoid tissue (GALT). It is localised along the surface of stomach and bowels in the lamina propria and includes isolated and grouped lymphoid follicles (Peyer's patches), lymphoid tissue of appendix, tonsils, mesenteric lymph nodes. From the last data, 60–70% of immune cells in child’s organism are localised in the gastro-intestinal tract [17, 22].

Immunomodulative effect of nutrients in the GALT carries out at subcellular, cellular and intercellular levels of cooperation. It is realised by the change of intestinal flora, properties of cellular membrane and activation of membrane's enzymes, adjusting of receptors expression and affinity, activation of receptor-dependent signal systems or initiation of additional signals in a cell, modulation of transcription factors and cellular cycle, change of gene expression, immunoglobulins, cytokines, regulation of apoptosis [6, 17, 13, 22].

A total surface of mucosal membrane of gastro-intestinal tract is approximately 300 m^2. It is an important barrier, which protects our organism from penetration of pathogenic microorganism from contaminated food and water. Mucous membrane of gastrointestinal tract is an organ with complicated structure, metabolic and regulatory functions which consists of glycomucoproteins, immune cells, immunoglobulins, lysozyme et al. Mucosa is covered by adhesive microorganisms which not only take part in the processes of food detoxication, immunomodulation and fermentation but also influence on mucus quality and amount. Unspecific protective factors which help to maintain homeostasis of the organism include: the components of saliva, acid of gastric juice, bile, mucus and adequate bowel's peristalsis. The main defence of mucous membranes is considered an antigen-specific secretory IgA [17, 22].

Isolated lymphoid follicles contain mainly B-lymphocytes, some T-helpers, and T-suppressors. Peyer's patches are divided into three areas: dome (consists of lymphocytes, macrophages, plasma cells and M-cells, responsible for antigen absorption and transport to the inside of lymphoid follicle), B-cellular area (localized under a dome, contains plenty of B-cells – precursors of IgA producers) and T-cellular area (contains mainly subpopulations of T-lymphocytes). An antigen

delivered to the dome of lymphoid follicle by M-cells is released, caught by antigenpresenting cells, disintegrated and presented to T-lymphocytes. As a result there is recognition of antigen. B-lymphocytes which carry superficial IgM switch to the synthesis of IgA. In inductive part of mucosa there are immunocompetent cells which provide initial immune answer. After migration to effector areas (first of all – Lamina propria of mucous membrane) specific T- and B-lymphocytes provide an accumulation of effector cells, responsible in the future for cellular and humoral forms of immune answer. Appendix also contains lymphoid follicles covered by M-cells. Mesenteric lymph nodes contain mainly B-lymphocytes, which are precursors of IgA producers. There is recognition of antigens, caught in bowels, in the mesenteric lymph nodes [17].

In the GALT antigen contacts with T-cells and B-lymphocytes – precursors of plasma cells, which produce IgA. Lymphokines secreted by T-cells switch B-lymphocytes from IgM to IgA production, following by clonal B-cells proliferation and maturation to plasma cells. It is complicated process with involvement of Th2-lymphocytes subclass, interleukins (IL-4, IL-5, IL-6 et al), transforming growth factor β. Mature and prepared to the IgA production B-lymphocytes, through lymphatic vessels and ductus thoracicus get into systemic circulation and spleen. After that they settle in the mucous membranes of different organs, mainly in the place of cells sensibilisation ("homing" effect). Such settling apart is important for the young children and provides protective effect in condition of weaker immune defence. Lamina propria is a place for the synthesis of antigenspecific IgA and polipeptide (joined-chain or j-chain) which is the connecting chain of these immunoglobulins. Then 2 molecules of IgA connected by j-chain (dimer) unite with glicoprotein, secretory component, which stabilizes secretory IgA and reliably protects it from the action of proteolytic enzymes of intestinal juice. IgA complex is packed in cytoplasm vesiculae, transported and released on apical part of mucosa epithelium [17].

Polimeric secretory IgA (sIgA) is able to neutralize effectively viruses, bacteria and their toxins, enzymes. It can partly block the processes of viral and bacterial adhesion to the epithelial cells of mucosa, viral attachment to cellular membranes and inhibit intracellular replication of viruses. It is proved that sIgA enhances activity of phagocytes and lymphocytes, induces significant cytotoxic effect to pathogenic bacteria. The basic role of sIgA is fastening of food and other allergens and infectious agents which can pass epithelium barrier and cause allergic reactions or infectious disease [17, 36].

Besides that, in mucosa there are localized intraepithelial gamma- and delta-T-lymphocytes which are considered to be the first link of defence. After stimulation these lymphocytes can differentiate into T-helper (CD4+) or T-killer (CD8+) cells. After antigen stimulation gamma- and delta-T-lymphocytes produce various cytokines, which stimulate growth of epithelial cells, destroy harmful germs (including intracellular) and own unviable epithelial cells. Probably, chronic course of some diseases is related to the primary lack of gamma- and delta-T-lymphocytes [17, 22].

Intestinal immune system performs 2 important and antagonistic functions: defence against pathogenic microorganisms (IgA-antibodies and cellular-mediated response) and suppression of immune reaction against food proteins and bacterial components of intestinal microflora [22].

For today several factors have been considered responsible for child's immune response: genetic predisposition to immune disorders (carriers of antigens HLA-DW), transfer of antibodies in the prenatal period and with breast milk, duration of breastfeeding. Important are also the nature of antigen, its dose, frequency of introduction, chemical structure, absorption, pressing, age of child at the first contact with an antigen, penetration of gastrointestinal tract mucosa, state of local immunity and intestinal microflora [17, 22].

Maternal milk is an optimum natural regulator of these processes. For today various immunoactive components of breast milk are known which can be divided into several groups according to their action. The first group is presented with antibacterial compounds: secretory immunoglobulins, lactoferrin, lysozyme, lactoperoxidase, nucleotides, antibodies, k-casein and α-lactalbumin, haptocorrin, mucins, lactadherin, free secretory component, oligosaccharides and prebiotics, fatty acids, maternal leucocytes and cytokines, sCD14, complement and complement receptors, β-defensins, toll-like receptors, bifidus factor, tolerance/priming compounds (cytokines: IL 10 and TGFβ; anti-idiotypic antibodies). The second group contains immune development compounds: macrophages, neutrophils, lymphocytes, cytokines, growth factors, hormones, milk peptides, long-chain polyunsaturated fatty acids, nuckleo-

tides and adhesion molecules. The next group includes anti-inflammatory compounds: cytokines (IL 10 and TGFβ), IL-1 receptor antagonist TNFα and IL-6 receptors, sCD14, adhesion molecules, long-chain polyunsaturated fatty acids, hormones and growth factors, osteoprotegerin [1, 5, 19, 28, 33, 35, 42]. And it yet not complete list of immunoactive components of breast milk! Normal microflora of gastrointestinal tract is an important part of human ecosystem which plays an important role in the immunophysiologic regulation of many processes, directed to the maintenance of immunologic homeostasis. Intestinal microflora has received more and more attention over the last few years 45, [3, 9, 12, 20, 24, 25, 29, 43, 45, 46]. Evidence has clearly demonstrated that the establishment of indigenous microflora is fundamental for [33, 38]:

- generation of immunophysiological regulation in terms of both protection against infection agents and acquisition of immune tolerance;
- the non-immunological protective function of the intestinal system – gatekeeper;
- a variety of nutritive and metabolic activities of the gastrointestinal system [23, 26, 30]

Development of the microbiota in the newborn GI tract depends on the original inoculum, the immediate living environment and early feeding practices. Before birth the infant is sterile. During vaginal delivery the natural colonization of the infant starts with bacteria mainly from the vaginal and intestinal flora of the mother [3, 33, 49].

For the further development of infant's intestinal flora the diet plays a very important role. Bifidobacteria dominate in the intestinal flora of breastfed infants shortly after birth. During artificial feeding without prebiotics the intestinal flora considerably change: the quantity of bifidobacteria and lactobacilli decreases meantime the numbers of opportunistic microorganisms and various bacterial associations increase, what is typical for dysbiosis and mature intestinal microbiota. As a consequence, within a few weeks the intestinal flora becomes different in breastfed and formula fed infants [5, 20, 21, 37].

The species and concentration of bacteria vary from the stomach to the intestine and colon. In the proximal small bowie and stomach, most of the bacteria are aerobic and gram-positive, and the concentration is low, about 10^3 to 10^4 colony-forming units (CFUs)/mL of luminal content. In contrast, within the colon, bacterial concentration increases sharply, reaching 10^{11} to 10^{12} CFUs/mL of luminal content. More then 400 different species of bacteria reside there, the dominant species are anaerobes [24, 31, 45, 49]. The resulting mature intestinal microbiota harbors 10 times more cells than the host.

Yet in 1900 Tissier proved that bifidobacteria are predominant in intestinal flora of breastfed infants [47]. The prebiotic concept, developed by the Gibson and Roberfoid in 1995 [23], is now firmly established. Human milk is a true prebiotic, and its neutral oligosaccharides are known as the main "bifidus factor" [7]. Besides 7% lactose, human milk contains approximately 1% neutral oligosaccharides. Therefore, these oligosaccharides make up a large part of human milk composition, similar to the proteins level [5]. Prebiotic oligosaccharides are configured in such a way that the small intestinal enzymes cannot hydrolyses them for absorption. Accordingly, they enter the colon intact and provide the "preferential food" for certain colonizing bacteria through the process of fermentation [19].

Prebiotics which can be supplied naturally (brest milk) or be used as food additives (galactooligosaccharides) are non-digestible oligosaccharides which enter the colon and are fermented to change the colonic environment and stimulate the increased proliferation of certain commensal bacteria, bifidobacteria and lactobacillus, which function as probiotics to stimulate intestinal host defences [22, 32, 33]. This indirect effect of prebiotics, e.g. an altered colonic milieu leading to stimulus of bifidobacteria and lactobacillus proliferation, has been considered as the primary role for prebiotics as a health-promoting dietary supplement. However, more recently several studies have suggested that prebiotics can also have a direct effect on the GULT that does not require the proliferation of commensal probiotic [22].

A great clinical experience of using of oligosaccharide-containing formulas produced by Nutricia company (Nutrilon, Nutrilon Hypoallergic, Nutrilon Comfort for children before 6 months of life and senior) has been accumulated in Ukraine. These formulas contain mixture of oligosaccharides (galactooligosaccharides [GOS] and fructooligosaccharides [FOS] with 9:1 ratio) in the concentration 0,8 g/100 ml [37].

In the study of Prof. V.D.Ott and co-authors positive effects in infants fed with formula "Nutrilon-1" (34 children) were established in comparison with the control group (22 children): improved child's behaviour, frequency of defecation, faecal pH, metabolic processes in bowels mucosa,

multiplied bifidobacteria and lactobacilli in faeces. Follow-up examination of children biochemical tests allowed making conclusion that this formula not only provides all essential nutrients, but also has positive influence on the functional condition of gastrointestinal tract, normalizes intestinal microbiota and metabolic processes in intestinal mucosa. That is why it could be defined as formula for functional nutrition [39].

Research of Prof. S.L. Nyankovskyy and O.S.Ivahnenko was devoted to the investigation of influence of oligosaccharide-containing formulas on establishment of intestinal biocenosis in infants of the first year of life. The study included 40 children: 14 children (average age – 3,2 ± 1,1 months) fed with "Nutrilon-1" formula, 16 children (average age – 7,1 ± 1,4 months) fed with "Nutrilon-2" formula, and 10 children fed with a standard formula without oligosaccharides. The authors found that artificial feeding is followed by dysbiotic changes and progressive diminishing of the quantity of bifidobacteria, lactobacillus, normal E.Coli and multiplying the numbers of aerobic and opportunistic bacteria. Administration of formulas with oligosaccharides during 3 months allowed attaining the reliable increase in numbers of bifidobacteria and lactobacilli, positively influenced establishment of intestinal microbiota making formula feeding as effective as breastfeeding. The results of stool analysis in these children approached the same results as in breastfed infants [36].

Prof. V.A. Tishchenko and co-authors in their research which included 20 new-born infants demonstrated that breastfed children and infants who received the formula enriched with oligosaccharides had the same qualitative, quantitative and functional tolerance and also quantitative characteristic of intestinal microbiota [48].

In their study with involvement of 23 children of the first 2 months of life, Prof. T.M. Klimenko and co-authors demonstrated that feeding with "Nutrilon-1" formula results in the decline of faces pH promotes the growth of acidophilic bacteria and warns surplus growthof pathogenic flora [27]. In the research performed by G.D. Dorofeyeva and co-authors in 20 children of the first year of life it was established that using of formula with oligosaccharides effectively ceases dyspepsia, meteorism, peristaltic disorders, promotes body weight increase, normalizes the number of bifidobacteria, however insufficiently inhibits the growth of hemolitic E.Coli [15]. In another research these authors observed 32 children fed with oligosaccharide-containing formulas. The aim of the study was to further investigate the influence of formula feeding with prebiotics on intestinal microbiota in children of the first year of life suffering from intestinal dysbiosis. The researches showed that during observation period the incidences of dyspepsia, meteorism, and peristaltic disorders reduced, body weight increased, sensibilization to food and bacterial allergens and allergic symptoms diminished, quantity of bifidobacteria increased and amount of opportunistic flora decreased (Proteus, Klebsiella, Enterobacter) [16].

Prof. L.N. Arayev and co-authors studied an efficiency of oligosaccharide-containing formula in 20 children of the first year of life. It was proved that in comparison with the control group infants fed with this formula had positive dynamics of physical development, stool improvement, augmentation of bifidobacteria and lactobacilli, decrease of lactosonegative and hemolytic species of E.Coli and quantity of opportunistic bacteria (Staphylococcus, Proteus, Citrobacter, Enterobacter) [2].

The data of S.G. Zolotareva and co-authors suggested that the use of formula with oligosaccharides in 11 children aged 6 months resulted in substantial increase of quantity of bifidobacteria, lactobcilli, nornal E.Coli and decline of amount of opportunistic flora, comparatively with children fed with the standard formula [50].

The research of Prof. O.E. Fedortsiv was devoted to the study of efficiency of formula „Nutrilon" for infants with malnutrition of I-II stages in a period of transitional and optimum feeding. There were involved 57 children at the age of 2–12 months from Ternopil Child's House. The results of dynamic study of body weight increase showed advantage of the formulas with oligosaccharides comparatively with formulas without oligosaccharides which were used in the control group [21].

The aim of research of Prof. K.D.Duka and S.I.Ilchenko was to evaluate the influence of oligosaccharide-containing formula on nutritional status and mucosal immunity of 52 infants from an ecologically unfavourable area. It was found that for artificial-fed children is typical suppressive orientation of immune reactions, lymphopenia, tense humoral immunity with relatively increased levels of IgG, IgA and IgE, low capacity for interferon synthesis, intestinal dysbiosis. According to researchers' data the use of mentioned

formula during 3 months allowed to correct significantly insufficiency of immune defence [18].

Prof. E.V. Prohorov and co-authors studied the efficiency of formula „Nutrilon-1" in children with functional disorders of gastrointestinal system. The research involved 19 children of 2–6 months with different functional disorders of gastrointestinal tract: regurgitation syndrome, intestinal cramps, and functional constipations. The authors showed that the use of formula with oligosaccharide allows liquidating dysbiosis and improving immune resistance of children [40].

36 children of the first year of life fed with oligosaccharide-containing formula were included into research of Prof. Yu.G. Reznichenko and co-authors. 132 infants from the ecologically unfavourable city district were fed with a standard formula (control group). The results suggested that in comparison with the control group children fed with an enriched formula had 25–45% lower morbidity (malnutrition, acute respiratory infections, rickets, anaemia and intestinal dysbiosis) what had positive influence on baby's health [41].

The interesting results were obtained by Prof. O.M. Mukvich and V.D. Ott in the research comparing the levels of blood cytokines, glycosaminoglycans, fucose and hexose in coprofiltrates in children with intestinal dysbiosis. 25 healthy breastfed infants of 1 to 5 months (control group), 85 children of the same age with stage II–III dysbiosis including 45 breastfed infants (group 2), and 40 children fed with prebiotic-containing formula "Nutrilon-1" (group 3) were under observation. The authors demonstrated some positive changes in children from the group 3 after a period of 2–3 weeks of enriched formula feeding: they became more quiet; meteorism, bubbling and abdominal pain on palpation reduced; sicky urges to defecation disappeared; faeces became soft and homogeneous; steatorea and aminorea diminished in 67,5% of infants. At the same time the reliable increase of bifidobacteria and lactobacillus concentration and tendency to decrease of opportunistic flora were noted. Data of this research suggested that children with intestinal dysbiosis develop immune response with macrophages involvement: the levels of proinflammative cytokines (IL-6, IL-6R, IL-8) rose and the concentration of antiinflammative cytokine (IL-4) declined as an evidence of Th1-lymphocytes activation. Children fed with oligosaccharide-containing formula has decreased level of proinflamative cytokines (IL-6, IL-6R, TNF) and increased level of IL-4, that means activation of Th2-lymphocites function, stimulation of humoral immunity and immunoglobulin synthesis. Despite cytokines level did not attain a norm, concentration of their active form (IL-6/IL-6R) after the feeding with formula "Nutrilon-1" didn't differ from control. The synthesis of protective fucoglycoproteins in formula-fed children with oligosaccharide and breastfed children was the same [34].

The analysis of mentioned above researches testify that the level of reliability of considerable part of them is insufficient. That is why the multicentral study aimed to estimate the effectiveness of formula "Nutrilon-1" with oligosaccharides in children of 1–3 months has been started in Ukraine. There are 6 centres participating in the study: Lviv (2 centres), Kyiv (2 centres), Donetsk (1 centre), Odesa (1 centre). 270 term babies are planned to be involved into the study. All participated children are randomized into 3 groups: 90 breastfed infants, 90 infants fed with oligosaccharide-containing formula starting from the first 2 weeks of life; and 90 infants fed with a standard formula without oligosaccharides starting from the first 2 weeks of life. The study has been designed to evaluate children's physical development, nutritional tolerance, levels of SIgA and Defensin in saliva, lysozyme in faeces and intestinal microbiota composition. The mentioned indexes and values are planned to be obtained at achievement of children's age of 1 month, 3 months and 1 year. On the basis of the achieved results the conclusion will be drawn about the possible influence of formula with oligosaccharides on baby's immunity, physical development and intestinal microbiota formation.

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