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IMMUNOMODULATING PROPERTIES OF A NOVEL SYNBIOTIC ON HEALTHY PERSONS

Tsvetelina Velikova, Ventsislav Nakov*, Ralitsa Georgieva, Kalina Toumangelova-Yuzeir, Ekaterina Ivanova-Todorova, Radislav Nakov*, Elena Karaivanova**, Borislav Vladimirov*, Dobroslav Kyurkchiev**

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Abstract

Probiotics combined with prebiotics, namely synbiotics, may have beneficial effects on human health, since the flora plays an important role in the maintenance of the intestinal immune homeostasis. Twenty healthy persons were enrolled in the study. Blood and stool samples were collected from them before and after 21-day-administration of a novel synbiotic containing five Lactobacillus strains, arabinogalactan and colostrum. The blood samples were tested for total and activated NK cells by flow cytometry and the stool samples – for the cytokines IL-6, TNFα, IFNγ, IL-10, IL-17A and faecal IgA by ELISA. The percentage of activated NK cells after synbiotic supplementation was higher than the percentage before ($p = 0.03$), although we did not find significant alteration in total NK cells number. Two of the investigated cytokines in stool samples – IL-6 and IFNγ, showed significant decrease after synbiotic application ($p < 0.001$). Taken together our results demonstrated immunomodulating effect of oral administration of synbiotic containing Lactobacillus strains, arabinogalactan and colostrum in direction of increased percentage of activated NK cells in the peripheral blood, as well as decreased levels of IL-6 and IFNγ in stool samples. Thus, the synbiotic could be implemented with beneficial effect in prophylaxis with its system anti-viral effect but could induce local immune tolerance in the gut where it is most needed.

Key words: synbiotic, probiotic, Lactobacillus spp, arabinogalactan, colostrum, NK cells, cytokines, Imnunoglobulin A

Introduction. The microbiota in human gastrointestinal tract co-evolved with humans to achieve a symbiotic relationship that leads to physiological and immunological homeostasis [1]. Even chaotic in the early life, the composition of

the human gut microbiota remains globally stable over time in healthy conditions and absence of disturbance, such as antibiotic treatment or "Western diet" [1]. The gut microbiota is also crucial for the development of a proper immune system in the intestinal lumen – bacterial colonization is a prerequisite for immunoglobulin A (IgA) production [2], as well as the innate immune response which controls bacterial growth near the intestinal epithelium by the secretion of antimicrobial peptides and cytokines, in response to microbial-associated molecular patterns and other microbiota metabolites [1]. Thus the flora plays an important role in the maintenance of the intestinal immune homeostasis. However the modern lifestyle may favour a shift in the microbial composition thereby leading to perturbation of this delicate balance and causing chronic dysregulation referred to as dysbiosis [1]. Since the Noble Prize laureate METCHNIKOFF introduced the term probiotics around 1900 [3], a number of definitions have been used. One of the recent but not the last definition is "live microorganisms, that may comprise different bacterial strains, which when consumed in adequate amounts, confer a health effect on the host" [4]. Probiotics exert an influence on the intestinal mucosa and its barrier function by interfering with local and systemic immune reactions [5], while prebiotics are food components which encourage the growth of beneficial bacteria. Mixing pre- and probiotic, namely a synbiotic, can combine synergic effect and may increase the effectiveness of probiotics [1]. There are sound theoretical reasons that probiotics may be beneficial when used for maintenance of health in individuals [6] and thus need to be further assessed. That may be performed at mucosal surfaces where this microbiome is in constant interaction with its host, and thus the products of this interaction could be measured in stool samples [1]. Although probiotic usage remained in the non-academic shadow of alternative medicine for many years, recently an amount of knowledge of the biological and therapeutic effectivity of probiotics has been collected [5, 7]. However, these effects are strain specific, sometimes debatable and further work is still required to confirm their healthy benefits [1].

The aim of the study was to examine the effect of synbiotic containing Lactobacillus strains, larch arabinogalactan and colostrum on some components of the immune system – Natural Killer (NK) cells in peripheral circulation and on some cytokines and IgA in stool samples after 21-day-administration in healthy individuals.

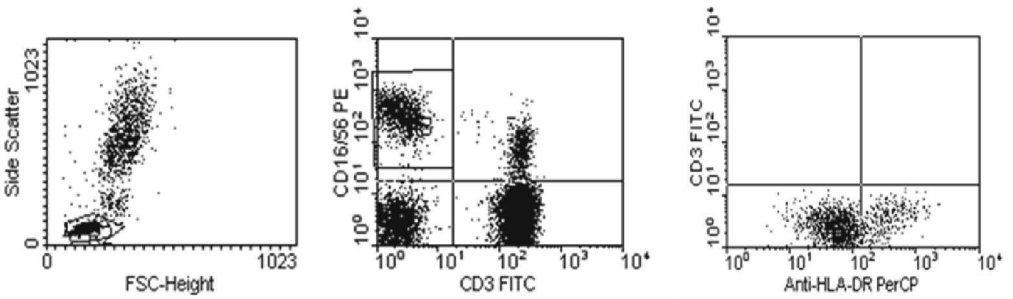
Material and methods. ***Synbiotic blend.*** The synbiotic blend included: five freeze-dried Lactobacillus strains (from Lactina Ltd., Bankya, Bulgaria); larch arabinogalactan (Ametis JSC, Russia) and skim colostrum APS60-20I (Aps-Biogroup, Phoenix, AZ, USA). The blend was partitioned in Gastro-resistant capsules (DRcaps^{TM}capsules, Capsugel) for oral administration. One capsule contains: 400 mg Blend of: Lactobacillus acidophilus LLA-10, NBIMCC 8242 (56 mg), Lactobacillus helveticus LLH-108; NBIMCC 8760 (56 mg), Lactobacillus rhamnosus LLR-L1, NBIMCC 8769 (56 mg), Lactobacillus fermentum LLF-

01, NBIMCC 8781 (56 mg), Lactobacillus bulgaricus LLB-06; NBIMCC 287 (25 mg) up to 250 mg (67.5%) and Colostrum (Bovine, min.20% IgG) (50 mg) and Larch arabinogalactan 100 mg (37.5%). Each capsule contains 250 millions viable organisms at the time of manufacture.

Persons and methods. Twenty healthy persons (7 males and 13 females) at mean age 35 ± 2 years were enrolled in the study. Blood and stool samples were collected before and after 21-day-administration of the previously described synbiotic. The blood samples were tested for total and activated NK cells by flow cytometry (FACSCalibur, BD, USA) using monoclonal antibodies (anti-CD3 FITC, anti-CD16/56 PE, anti-HLA-DR PE Cy5, BD, USA). The stool samples were homogenized (1 g stool in 1000 ml of phosphate buffered saline) and centrifuged at 20 000g for 15 min at 4 °C. The supernatants of the stool extracts were tested by enzyme immunoassay for the cytokines IL-6, TNFa, IFNg, IL-10, IL-17A (each with Human ELISA kit, Gen-probe Diaclone SAS, France) and faecal IgA (Secretory IgA ELISA, Immuchrom GMBH, Germany).

Statistical methods. The row data were statistically evaluated by parametric and non-parametric tests (Software package for statistical analysis SPSS®, IBM 2009, vs. 19). Differences were considered significant when the p value was less than 0.05.

Results. ***NK cells in peripheral blood.*** NK cells were defined after selecting the cells on electronic gate depending on their size (FSC-H) and granularity (Side scatter) (Fig. 1*A*). From the selected cells we analyzed as percentage only these cells which were positive for CD16/56 but negative for CD3. These cells were then selected in second electron gate (Fig. 1*B*). There were two subpopulations of NK cells depending on activation marker HLA-DR expression (Fig. 1*C*) – HLA-DR positive (right) and HLA-DR negative (left). We did not find significant difference between total NK cells percentage before and after synbi-



A *B* *C*

Fig. 1. Total and activated NK cells in peripheral blood. *A* – Electronic gate according to cell size (FSC-H) and granularity (Side scatter); *B* – Positive for CD16/56 but negative for CD3 cells selected in second electron gate; *C* – HLA-DR positive (right) and HLA-DR negative (left) subpopulation of NK cells

Table 1

Total and activated NK cells before and after oral administration of synbiotic in healthy controls. The results are presented as percentage ± SD (range)

	Before synbiotic administration	After synbiotic administration	Significance, p
Total NK cells	16.1 ± 1.2 (8.1–25.5)	14.6 ± 1.2 (5.1–25.7)	0.073
Activated NK cells (HLA-DR+)	4.9 ± 0.6 (1.3–9.9)	7.1 ± 0.7 (2.6–13.5)	0.03

Table 2

Cytokines and faecal IgA levels in stool samples of healthy persons before and after administration of synbiotic. The results are presented as concentration (pg/ml or μg/ml) ± SD (range). n.s. (non-significant)

	Before synbiotic administration	After synbiotic administration	Significance, p
IL-6, pg/ml	5.6 ± 0.2 (3.9–6.75)	3.1 ± 0.1 (2.3–5.5)	0.000
IFNγ, pg/ml	73.2 ± 2.6 (55.5–92.5)	43.6 ± 3.9 (28.3–99)	0.000
TNFα, pg/ml	36.8 ± 9.6 (0–116.5)	25.6 ± 7.5 (0–103.3)	n.s.
IL-10, pg/ml	35.4 ± 8.4 (2.5–178)	24 ± 5 (7.8–108.8)	n.s.
IL-17A, pg/ml	70.6 ± 25.5 (0–387)	69.9 ± 23 (0–100.1)	n.s.
Secretory IgA, μg/ml	3001.5 ± 599 (207.5–8355.63)	2806.4 ± 622.3 (183.75–8818)	n.s.

otic supplementation (Table 1). At the same time after synbiotic treatment the percentage of activated NK cells was higher than percentage before ($p = 0.03$) (Table 1).

Cytokines and IgA in stool samples. Two of the investigated cytokines in stool samples – IL-6 and IFNγ, showed significant decrease after synbiotic application ($p < 0.001$) (Table 2). However, two of the cytokines were also presented with lower levels than in the beginning of the study – TNFα and IL-10, as well as faecal IgA, but not significantly. Interleukin-17A levels were not changed during the synbiotic using (Table 2).

Discussion. In recent years much effort has been made for understanding the gut-microbiota-host interactions and it has been shown that the microbiota has a great impact on gut immune system development and activity. Some bacterial genera or their metabolites have been identified as drivers of immune tolerance, particularly by their ability to stimulate/activate regulatory responses in the gut [8, 9]. On the probiotic side, Lactobacillus strains have been the most

studied which have shown beneficial effects on gut health [9]. We used synbiotic blend including five Lactobacillus strains previously selected on the basis of their antimicrobial activity and antibiotic susceptibility [10]. The number of total NK cells in peripheral blood did not alter after synbiotic supplementation, but the percentage of the activated fraction (HLA-DR positive cells) did increase significantly. Overall, our results demonstrated systemic effect of the synbiotic leading to augmentation of the percentage of activated NK cells. We could comment the synbiotic activity as enhancer of antiviral defense since the antiviral properties of NK cells in the human body were well known. Analysis of the investigated cytokines showed significant reduction of two of them – IL-6 and IFNγ (Table 2). If we take together their complex and controversial properties, both cytokines have been shown as "pro-inflammatory" on condition this is a relative term. Generally IL-6 enhances immunoglobulin synthesis in the body, including in the gut mucosa, where IFNγ influences enterocytes to present antigens atypically in context of HLA-II molecules, induces Th1 responses and cytotoxicity [11, 12]. Both cytokines stimulate phagocytosis. By reducing the levels of both cytokines, the investigated symbiotic favours local immunosuppression – a default state in gastrointestinal mucosa which is repeatedly exposed to vast number of food and microbial antigens. Prebiotics, as non-digestible part of a synbiotic, have been shown to change specifically the composition and activity of beneficial gut. Mainly oligosaccharides, prebiotics are thought to selectively stimulate butyrate-producing bacteria such as Bifidobacteria and Lactobacilli [1, 13, 14]. Prebiotic ingredient of the investigated synbiotic – arabinogalactan – provides dietetic fibres for the beneficial microbial strains, enhances cell turnover and epithelial proliferation, secretion of mucins, improves the barrier function of the mucosa, and modulates the immune responses in the gut [15, 16]. Besides its effects on mucosal immune system, arabinogalactan was found to stimulate NK cytotoxicity via potentiating the cytokine network, primarily via an increase in the release of IFNγ [17, 18]. We did observe increase of activated NK cells in the peripheral blood, but did not investigate their cytotoxicity. Otherwise in our study the level of IFNγ was decreased after synbiotic supplementation. However, we obtained the result for IFNγ reduction in stool samples but not in blood ones. Skim colostrum is another component of the investigated synbiotic which has also been shown to increase the cytotoxicity of NK cells [19], but also confers with the mucosal immune system by supplying it with growth and nutritious factors, as well as immunoglobulins, lactoferrin, cytokines, prolin-rich polypeptides, etc., mainly with anti-microbial and anti-inflammatory properties [19]. According to the most expressed immunoglobulin of the gastrointestinal tract – IgA, we did not find significant alteration of IgA levels in stool samples after symbiotic supplementation.

Conclusion. Taken together our results demonstrated immunomodulating effect of oral administration of synbiotic with Lactobacillus strains, arabinogalac-

tan and colostrum in direction of increased percentage of activated NK cells in the peripheral blood, as well as decreased levels of IL-6 and IFNγ in stool samples. Thus, the proper balance between the luminal flora and the mucosa as a permanently dynamic, sensitive and host-specific relationship may be influenced by synbiotic in prophylaxis like inducing not only system anti-viral effect but also local immune tolerance in the gut where it is most needed.

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Laboratory of Clinical Immunology
"St. Ivan Rilski" University Hospital
Medical University – Sofia
15, Acad. Iv. Evst. Geshov Blvd
1431 Sofia, Bulgaria
e-mail: ts_velikova@abv.bg

[*] *Clinic of Gastroenterology*
"Tsaritsa Ioanna – ISUL" University Hospital
Medical University – Sofia
8, Byalo more" St
1527 Sofia, Bulgaria

[**] *Lactina Ltd.*
101, Sofia St
1720 Bankya, Bulgaria