RELATIONSHIP BETWEEN PRO-INFLAMMATORY CYTOKINE LEVELS AND BLOOD BACTERIAL DNA COMPOSITION IN OBESE CHILDREN

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Adipose tissue, being a source of chronic low-grade inflammation, activates cells of the immune system by producing cytokines and chemokines. The balance between pro- and anti-inflammatory molecules and their relationship with blood bacterial DNA in obese children and adolescents has not been studied sufficiently. This study aimed to find patterns of interaction between fractions of bacterial families in healthy and obese children, analyze cytokine levels and their relationship with blood bacterial DNA content, evaluate alpha diversity of blood microbiome and similarities of blood and fecal microbiomes. We examined 163 individuals (children and adolescents), who were divided into 2 groups, obese (n = 80, obesity classes I through III) and healthy (n = 83). The material sampled and studied was venous blood. Only individuals that have not been taking antibiotics, pro- and prebiotics for at least 3 months before the study were included. The methods employed were multiplex ELISA (enzyme immunoassay) and 16S rRNA gene sequencing (region V3–V4). From the angle of bacterial families, we found differences in their content (fractions) in blood microbiome and the frequency of isolation of their DNA therein. Nineteen families accounted for over three quarters of all bacterial DNA identified in the blood. In obese children, one of the dominating roles was played by *Ruminococcaceae*, with their DNA a key part of the microbiome's alpha diversity, while in healthy participants this could be said about *Bacteroidaceae*. Analyzing beta diversity, we found that in obese children, fecal and blood microbiomes differed significantly, which indicates, mainly, extra-intestinal translocation of bacterial DNA. Obese children exhibited increased content of IL17A (p = 0.017) and PD-L1 (p = 0.021); there were differences in blood microbiome between groups. We identified the patterns of interaction between bacterial DNA fractions, and assessed cytokine levels.

Keywords: obesity, cytokines, bacterial DNA, blood microbiome, children, inflammation, PD-L1

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Compliance with ethical standards: the study was approved by the Local Ethics Committee of N.I. Pirogov Russian National Research Medical University (Minutes #186 of June 26, 2019) and Local Ethics Committee of Rostov State Medical University (Minutes #20/19 of December 12, 2019). To be included in the study, all patients and their parents mandatorily signed voluntary informed consent forms.

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ВЗАИМОСВЯЗЬ УРОВНЕЙ ПРОВОСПАЛИТЕЛЬНЫХ ЦИТОКИНОВ С СОСТАВОМ БАКТЕРИАЛЬНОЙ ДНК КРОВИ У ДЕТЕЙ С ОЖИРЕНИЕМ

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Жировая ткань как источник развития хронического низкоинтенсивного воспаления активизирует клетки иммунной системы путем выработки цитокинов и хемокинов. Баланс между про- и противовоспалительными молекулами и их связь с бактериальной ДНК крови при ожирении у детей и подростков недостаточно исследованы. Цель исследования — нахождение паттернов взаимодействия между долями семейств бактериальной ДНК у здоровых детей и с ожирением, анализ уровней цитокинов и их связь с бактериальной ДНК крови, оценка альфа-разнообразия микробиома крови и сходства микробиомов крови и кала. Обследовано 163 человека (дети и подростки), которых разделили на 2 группы: 80 человек с ожирением I–III степени и 83 здоровых. Материал исследования — венозная кровь. Критерии включения в группы — отсутствие приема антибиотиков, про- и пребиотиков в течение трех месяцев. Методы — мультиплексный ИФА (иммуноферментный анализ); секвенирование участка v3-v4 гена 16S рРНК. Выявлены различия в микробиоме крови на уровне семейств как по доле, так и по частоте выделения бактериальной ДНК. Более 3/4 ДНК крови в обеих группах приходилось на 19 семейств. Значимо больший вклад в альфа-разнообразие у детей с ожирением принадлежал бактериальной ДНК *Ruminococcaceae*, однако в группе здоровых детей эта роль принадлежала *Bacteroidaceae*. Анализ бета-разнообразия крови позволил выявить у детей с ожирением значимую отдаленность микробиома крови и кала, что свидетельствует о преимущественно внекишечной транслокации бактериальной ДНК. Выявлено увеличение содержания ИЛ-17А (p = 0,017) и PD-L1 (p = 0,021) у детей с ожирением, а также различия в микробиоме крови в группах. Определены паттерны взаимодействия между долями бактериальной ДНК, оценены уровни цитокинов.

Ключевые слова: ожирение, цитокины, бактериальная ДНК, микробиом крови, дети, воспаление, PD-L1

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Abnormal changes in human microbiota alter metabolism and promote growth of adipose tissue, gradually contributing to the development of obesity. This diagnosis is growing more and more common among both adults and children and adolescents, which substantiates the urgency of search for the yet unknown mechanisms behind this pathology. It is important to timely detect predisposition to obesity in children and adolescents, since it is possible to delay, or, at best, prevent progression of this disease, which leads to type II diabetes mellitus, dyslipidemia, disorders of cardiovascular, reproductive systems, liver, microvascular pathologies, etc. Obesity triggers morphological and functional changes in white adipose tissue, attracting immune cells such as macrophages, T cells, B cells, which infiltrate the tissue and start the inflammatory process. Such inflammation is smoldering and progressive, unlike acute inflammation aimed at eliminating the pathogen and restoring homeostasis. The inflammatory response can be conditioned by both endogenous (DAMP, Damage Associated Molecular Patterns) and exogenous (PAMP, Pathogen Associated Molecular Patterns) ligands (including bacterial DNA) recognized by PRR, pattern-recognizing receptors [1, 2]. Such receptors are expressed, inter alia, by adipocytes. Various pattern-recognizing receptors, such as Toll-like receptors (TLRs), ply an active part in antiviral and antibacterial protection of the body. PAMP boost expression of adipocyte receptors and secretion of proinflammatory cytokines and adipokines. Activation of TLR-3 triggers a cascade of intracellular events accompanied by activation of immune cells and production of chemokines and proinflammatory cytokines like TNFα, IL1β, IL6, IL8, which are associated with expansion of inflammation in adipose tissue that supports development of insulin resistance [3].

At the same time, TLRs are involved in adipogenic differentiation: they are expressed together with PPARs (Peroxisome proliferator-activated receptors) at different stages of adipocyte differentiation [4, 5].

Currently, however, there are no descriptions of changes of blood microbiomecomposition and taxonomic diversity, nor its connections with the inflammatory factors.

Thus, the purpose of this work was to compare the levels of pro- and anti-inflammatory cytokines in blood of obese and healthy children, to reveal their relationship with blood microbiome's taxonomic diversity, and to identify the contribution of bacterial DNA (various families) to the formation of blood microbiome alpha diversity and its connections to the gut microbiome.

METHODS

The study was conducted in 2019–2020. We examined 163 people (children and adolescents) who applied to the children's city polyclinic № 1 in Rostov-on-Don seeking regular checkups. Of these, 80 individuals (aged 10-18 years, mean age 13.25 ± 2.00 years) comprised the study group: with alimentary constitutional obesity of varying degree, they have been diagnosed accordingly earlier and, therefore, followed-up (Table 1). The control group included 83 healthy children and adolescents (aged 10-18 years, mean age 12.92 ± 2.21 years) who did not have obesity nor other metabolic disorders (Table 1).

The inclusion criteria for both groups were abstaining from antibiotics, probiotic and prebiotic drugs for at least 3 months before the study, and availability of a signed informed consent to participate in the study. The exclusion criteria for both groups were severe somatic diseases (chronic renal failure, chronic liver failure, chronic heart failure), intestinal diseases (ulcerative colitis, Crohn's disease), acute stage of any disease. To be included in the study group, the participants also had to have been diagnosed with alimentary constitutional obesity of class I through III and have the body mass index of SDS > +2.0. The study was single-center, single-stage, with a random sample.

Blood of microbiome taxonomic composition was studied at the Center for Digital and Translational Biomedicine of Center for Molecular Health; Rostov Region Children's City Hospital № 1; Pirogov Russian National Research Medical University; Kazan (Volga Region) Federal University. All participants donated blood and feces for laboratory studies, and had their anthropometric parameters registered.

We isolated microbial DNA in blood samples using a QIAamp BiOstic Bacteremia DNA Kit (Qiagen; Germany) in accordance with the manufacturer's protocol. DNA quality control, its quantitative and qualitative composition were evaluated by spectrophotometry (Qubit, Thermo Fisher Scientific; USA) and gel electrophoresis (1% agarose).

MiSeq benchtop sequencer (Illumina; USA) enabled library preparation and sequencing of the V3–V4 variable region of 16S rRNA gene. We analyzed the resulting 16S rRNA gene sequences (reads) with the help of QIIME software (version 1.9.1) [6] and Greengenes v.13.8 reference database [7], the sequence similarity threshold was put at 97%.

Concentrations of cytokines IL6, 8, 10, 17, TNFa were determined with commercially available kits: Milliplex (Merck; Germany) — Human Cytokine/Chemokine Magnetic Bead Panel; fractalkine — Human Myokine Magnetic Bead Panel; PD-L1 (programmed cell death-ligand 1) — Human Immuno-Oncology Checkpoint Protein Panel Magnetic Bead Panel (ELISA), on a Magpix analyzer (Bio-Rad Laboratories; USA).

For statistical processing of the data, we used MedCalc® Statistical Software version 20.110 (MedCalc Software Ltd; Belgium). The normality of distribution in all datasets was checked with the Shapiro–Wilk test. Since no distribution was normal, we used the median and its [25–75] percentiles the measure of central tendency. Mann–Whitney U test enabled comparison of the values of beta diversity indices (Euclidean distance, Manhattan distance, Bray–Curtis dissimilarity), cytokine levels, and analysis of differences in blood bacterial DNA. Chi-squared test was used to establish the frequency of detection of DNA of different bacterial families in blood samples donated by the participants (both groups). Regardless of the test, the differences were considered significant at $p \leq 0.05$. To identify the relationship between the content of individual families (provided they were isolated in the blood samples) and the characteristics of diversity of blood microbiome, we calculated Spearman's rank correlation coefficient. Correlation

Table 1. Anthropometric indicators of groups

Group	Control group	Study group	*p*-value
BMI, kg/m^2, Me [Q_1; Q_3]	20.1 [19.4; 21.2]	27.0 [25.9; 28.8]	< 0.001
Weight, kg, Me [Q_1; Q_3]	49.0 [45.0 ;55.5]	71.0 [61.0;78.0]	< 0.001
Height, cm, Me [Q_1; Q_3]	157.0 [150.0; 165.0]	159.0 [154.0; 164.0]	0.619
Age, years, Me [Q_1; Q_3]	13.0 [11.0; 15.0]	13.0 [11.8; 15.0]	0.237
Gender (m/f)	57.8%/42.2%	52.5%/47.5%	0.187

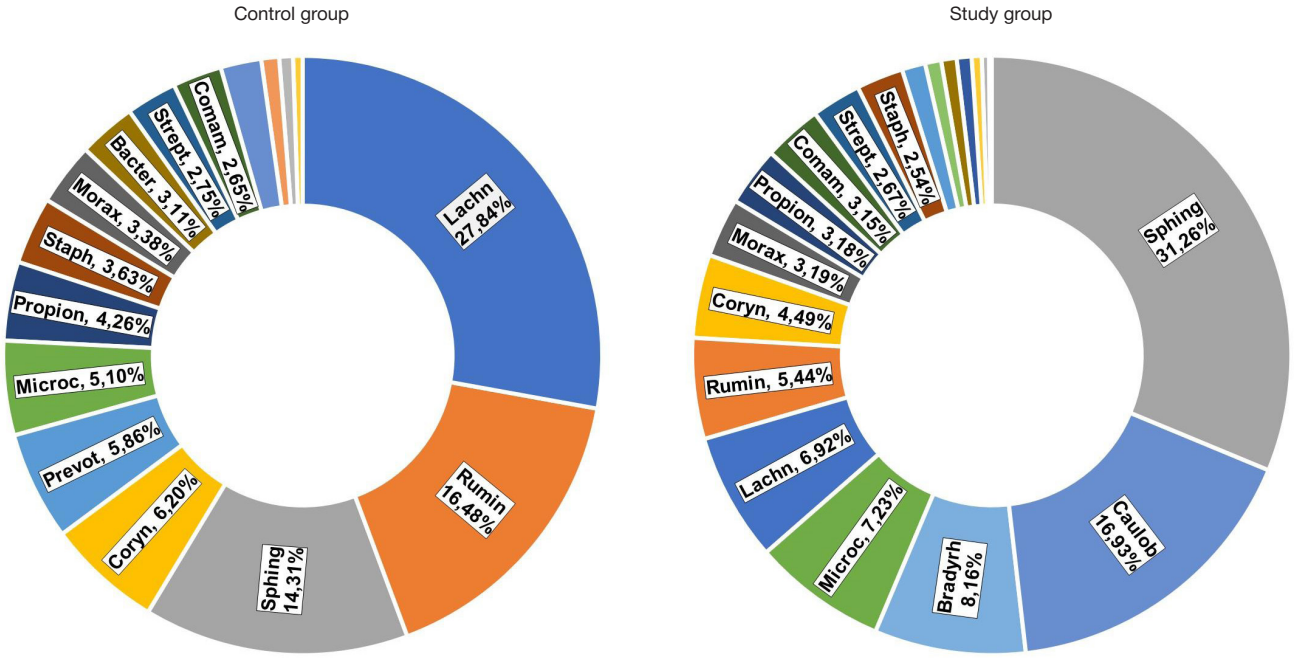

Fig. 1. Distribution of DNA of bacterial families identified the blood samples, both groups, %. *Sphing — Sphingomonadaceae*, *Caulob — Caulobacteraceae*, *Bradyrh — Bradyrhizobiaceae*, *Microc — Micrococcaceae*, *Lachn — Lachnospiraceae, Rumin — Ruminococcaceae, Coryn — Corynebacteriaceae*, *Morax — Moraxellaceae*, *Propion — Propionibacteriaceae*, *Comam — Comamonadaceae*, *Strept — Streptococcaceae*, *Staph — Staphylococcaceae*, *Prevot — Prevotellaceae*, *Bacter — Bacteroidaceae*

factors (rho) were considered provided that |rho| ≥ 0.3, the constraint force was moderate on Chaddock scale (with $p \leq 0.05$).

RESULTS

In the blood of healthy children, we identified bacterial DNA from 29.0 [24.5–37.0] families, while samples donated by obese participants presented DNA belonging to 34.0 [28.0–42.0] bacterial families. In both groups, 19 families contributed over 75% of all the bacterial DNA registered: *Sphingomonadaceae*, *Caulobacteraceae*, *Bradyrhizobiaceae*, *Micrococcaceae*, *Lachnospiraceae*, *Ruminococcaceae*, *Corynebacteriaceae*, *Moraxellaceae*, *Propionibacteriaceae*, *Comamonadaceae*, *Streptococcaceae*, *Staphylococcaceae*, *Prevotellaceae*, *Chitinophagaceae*, *Bacteroidaceae*, *Porphyromonadaceae*, *Veillonellaceae*, *Enterobacteriaceae*, *Methylobacteriaceae* (Fig. 1).

Compared to the control group, blood donated by obese participants significantly more often contained DNA of the following bacteria: *Chitinophagaceae* ($p < 0.001$), *Caulobacteraceae* ($p < 0.001$), *Bradyrhizobiaceae* ($p = 0.004$), Porphyromonadaceae ($p = 0.091$), and *Sphingomonadaceae* ($p < 0.001$); the situation was quite the contrary for *Prevotellaceae* ($p < 0.001$) and *Lachnospiraceae* ($p < 0.001$) (Table 2).

Statistically more common in obese children were bacterial DNA of: *Sphingomonadaceae* ($p = 0.026$), *Ruminococcaceae* ($p = 0.085$), *Caulobacteraceae* ($p = 0.020$), *Bradyrhizobiaceae* ($p < 0.001$), *Porphyromonadaceae* ($p = 0.033$), *Chitinophagaceae* ($p < 0.001$), *Pasteurellaceae* ($p = 0.061$); less frequently, we registered *Prevotellaceae* ($p = 0.044$), and *Thermaceae* ($p = 0.084$) (Table 2).

The analysis of taxonomic affiliation of blood bacterial DNA in the study group revealed a greater diversity thereof in obese children [8]. On the level of families, we analyzed relationships between indicators of alpha diversity and taxonomic composition of bacterial DNA, and, in both groups, found negative correlations between alpha diversity and DNA of the following families: *Corynebacteriaceae*, *Micrococcaceae*,

Table 2. Comparison of the frequency of occurrence of DNA from individual bacterial families and their shares in the total pool of blood bacterial DNA

Family	Frequency of occurrence in participants, %		Share of the family in the total bacterial DNA pool	
	Control group	Obese children	Control group	Obese children
Lachnospiraceae	85.5	86.3	0.1043 [0.0116–0.1954]	0.0314 [0.0074–0.0737]***
Prevotellaceae	84.3	71.3**	0.0220 [0.0053–0.0379]	0.0057 [0–0.0167]***
Sphingomonadaceae	84.3	95.0**	0.0536 [0.0115–0.1444]	0.1418 [0.0675–0.1946]***
Comamonadaceae	84.3	92.5	0.0100 [0.0029–0.0252]	0.0143 [0.0051–0.0371]*
Ruminococcaceae	71.1	82.5*	0.0618 [0–0.1281]	0.0247 [0.0035–0.0753]
Caulobacteraceae	65.1	81.3**	0.0082 [0–0.0750]	0.0768 [0.0148–0.1052]***
Bradyrhizobiaceae	49.4	78.8***	0 [0–0.3148]	0.0370 [0.0031–0.3191]**
Porphyromonadaceae	44.6	61.3**	0 [0–0.0097]	0.0036 [0; 0.0113]*
Chitinophagaceae	32.5	65.0***	0 [0–0.0039]	0.0039 [0–0.0118]***
Pasteurellaceae	22.9	36.3*	0 [0–0]	0 [0–0.0025]
Thermaceae	36.1	23.8*	0 [0–0.0053]	0 [0–0]*

Note: the differences are significant compared to the control group: * — $p < 0.1$; ** — $p < 0.05$; *** — $p < 0.001$.

Table 3. The relationship between blood microbiome's alpha diversity and blood bacterial DNA at the taxonomic level

	Control group	Obese children
	Bacteroidaceae family	
Shannon Index	rho = 0.669. $p < 0.001$. $n = 57$	**rho = 0.358*. $p = 0.015$. $n = 46$**
Simpson Index	rho = 0.666. $p < 0.001$. $n = 57$	**rho = 0.356*. $p = 0.015$. $n = 46$**
Chao1 Index	rho = 0.533. $p < 0.001$. $n = 57$	
PD Index	rho = 0.586. $p < 0.001$. $n = 57$	
OTUs	rho = 0.575. $p < 0.001$. $n = 57$	
	Prevotellaceae family	
Shannon Index	rho = 0.680. $p < 0.001$. $n = 70$	rho = 0.540. $p < 0.001$. $n = 56$
Simpson Index	rho = 0.678. $p < 0.001$. $n = 70$	rho = 0.537. $p < 0.001$. $n = 56$
Chao1 Index	rho = 0.320. $p < 0.001$. $n = 70$	rho = 0.358. $p = 0.007$. $n = 56$
PD Index	rho = 0.535. $p < 0.001$. $n = 70$	rho = 0.329. $p = 0.013$. $n = 56$
OTUs	rho = 0.445. $p < 0.001$. $n = 70$	rho = 0.333. $p = 0.012$. $n = 56$
	Lachnospiraceae family	
Shannon Index	rho = 0.593. $p < 0.001$. $n = 71$	rho = 0.638. $p < 0.001$. $n = 68$
Simpson Index	rho = 0.614. $p < 0.001$. $n = 71$	rho = 0.627. $p < 0.001$. $n = 68$
Chao1 Index	rho = 0.348. $p = 0.003$. $n = 71$	rho = 0.368. $p = 0.002$. $n = 68$
PD Index	rho = 0.369. $p = 0.002$. $n = 71$	rho = 0.365. $p = 0.002$. $n = 68$
OTUs	rho = 0.345. $p = 0.003$. $n = 71$	rho = 0.321. $p = 0.008$. $n = 68$
	Ruminococcaceae family	
Shannon Index	rho = 0.308. $p = 0.018$. $n = 59$	**rho = 0.714*. $p < 0.001$. $n = 65$**
Simpson Index	rho = 0.357. $p = 0.006$. $n = 59$	**rho = 0.706*. $p < 0.001$. $n = 65$**
Chao1 Index		rho = 0.394. $p = 0.001$. $n = 65$
PD Index		rho = 0.461. $p < 0.001$. $n = 65$
OTUs		rho = 0.390. $p = 0.001$. $n = 65$

Note: * — reliability of differences in correlation coefficients between the groups.

Propionibacteriaceae, *Streptococcaceae*, *Staphylococcaceae*, *Caulobacteraceae*, *Bradyrhizobiaceae*, *Sphingomonadaceae*, *Comamonadaceae*, *Moraxellaceae*.

Only for families exhibited positive correlations with blood microbiome's diversity indicators, and, predominantly, their biotopes are large intestine (*Bacteroidaceae*, *Lachnospiraceae*, *Ruminococcaceae*) and oral cavity (*Prevotellaceae*) (Table 3).

To identify the biotopes that shape blood's microbiome, we analyzed correlations between the characteristics of the alpha diversity and shares of the various bacterial families that contributed the detected DNA. Positive correlations were established for *Bacteroidaceae*, *Prevotellaceae*, *Lachnospiraceae*, and *Ruminococcaceae*. Also, the correlations were positive for DNA of the microorganisms inhabiting mainly 2 biotopes, the large intestine and the oral cavity, as well as the upper respiratory tract, and the genitourinary system; as for negative correlations, such were registered for DNA of bacteria found in the large intestine, on the skin, oropharynx, nasopharynx, esophagus, stomach, water, soil.

A noteworthy fact: in obese children, we registered a multidirectional change in the degree of positive correlations of alpha diversity indicators and bacterial families inhabiting the same biotope, as in the case of *Bacteroidaceae* and *Ruminococcaceae* families, with the said degree becoming milder for the former and stronger for the latter.

Obese participants exhibited fewer extreme negative correlations between alpha diversity indicators and DNA of the *Caulobacteraceae* and *Bradyrhizobiaceae* families, while also showing negative correlations between the said indicators and DNA of the *Porphyromonadaceae* family; both of these observations are noteworthy in light of the fact that these families grow significantly more common in the total pool of blood bacterial DNA. Bacteria of the *Caulobacteraceae* family inhabit soil and water, and those belonging to the *Bradyrhizobiaceae* family are part of the core of the breast milk's microbiome.

The analysis of correlations in the groups revealed differences in the structure of relationships between DNA of different taxonomies: there were identified patterns common to both groups of participants and those unique to either study or control group.

It should be noted that correlations revolve around DNA of the *Propionibacteriaceae* family, which establishes links through two correlation patterns, one involving *Lachnospiraceae*, *Prevotellaceae*, *Ruminococcaceae*, *Bacteroidaceae*, the other — *Enterobacteriaceae*, *Corynebacteriaceae*, *Streptococcaceae*, *Staphylococcaceae*, *Comamonadaceae*, *Moraxellaceae*, *Micrococcaceae*, *Caulobacteraceae*, *Sphingomonadaceae* (Fig. 2, 3).

A noteworthy fact: in the study group, there are more unique correlations, and they mainly revolve around DNA of microbiome inhabiting extracellular biotopes (skin, oral cavity, soil, water).

In the context of analysis of beta diversity, we compared blood and fecal microbiomes. This comparison revealed a significantly more advanced beta diversity in the study group (Euclidean distance $p = 0.03$, Manhattan distance $p = 0.07$, Bray-Curtis dissimilarity $p = 0.07$), which indicates a greater difference between the microbiomes of blood and feces in obese children, and, in turn, reflects the contributions of intestinal and extra-intestinal microbiomes in the formation of blood microbiome, with the the part played by the former smaller than that of the latter (Fig. 4).

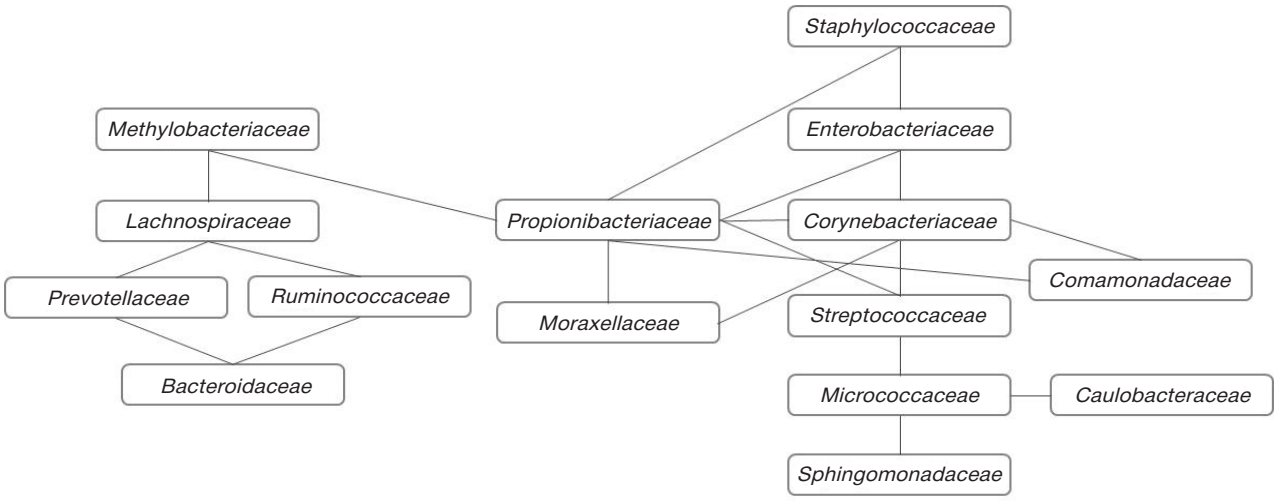

Fig. 2. Positive correlations between bacterial DNA, control group (healthy children)

Previously, we have shown that in the study group, blood microbiome's alpha diversity is insignificantly increased, which translates into a statistically significant growth of the number of operational taxonomic units (OTUs) and phylogenetic diversity (PD) index acquiring an upward trend. Given the above, these findings are complemented by the data on beta diversity and correlations between DNA of different families, with alpha diversity of obese children's blood microbiome enriched with DNA from extra-intestinal microbiomes and not intestinal microbiomes [8].

The study of cytokine levels revealed that obese children have the content of IL17A and PD-L1 significantly greater than their healthy peers (Table 4).

Based on the correlation analysis, we established statistically significant positive relationships in the study group (Table 5).

In the control group, correlation analysis allowed establishing the following relationships between cytokines and bacterial DNA (at the family level):

IL10 had moderate positive correlation with bacterial DNA of *Erysipelotrichaceae* (rho = 0.438, $p = 0.005$, $n = 40$); IL8 — with *Bacteroidaceae* (rho = 0.370, $p = 0.005$, $n = 57$); IL6 — with *Pseudomonadaceae* (rho = 0.528, $p = 0.012$, $n = 22$); IL17A — with *Microbacteriaceae* (rho = 0.544, $p = 0.006$, $n = 23$), *Bacillaceae* (rho = 0.796, $p < 0.001$, $n = 18$), *Fusobacteriaceae* (rho = 0.506, $p = 0.032$, $n = 18$); PD-L1 — with [*Tissierellaceae*] (rho = 0.353, $p = 0.044$, $n = 33$), *Pseudomonadaceae* (rho = 0.528, $p = 0.012$, $n = 22$).

In the study group, we identified that IL10 had a moderate positive correlation with *Lactobacillaceae* (rho = 0.395, $p = 0.034$, $n = 29$); IL8 — with *Veillonellaceae* (rho = 0.354, $p = 0.017$, $n = 45$); IL6 — with *Nocardiaceae* (rho = 0.605, $p = 0.010$, $n = 17$), *Lactobacillaceae* (rho = 0.380, $p = 0.042$, $n = 29$), *Veillonellaceae* (rho = 0.459, $p = 0.002$, $n = 45$); IL17A — with *Nocardiaceae* (rho = 0.521, $p = 0.032$, $n = 17$), TNFα — with *Nocardiaceae* (rho = 0.495, $p = 0.043$, $n = 17$), fractalkine — with *Bacillaceae* (rho = 0.705, $p = 0.010$, $n = 12$).

The correlations between bacterial DNA and cytokine content revealed in the groups were associated with different families. In the group of healthy children, the systemic pro-inflammatory cytokine IL8 correlated with bacterial DNA of *Bacteroidaceae*, which act as protectors in the intestine; in the group of obese children, we registered no such correlation. It should also be noted that in the control group, we found relationships with families belonging to five phyla: *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Fusobacteria*; in the study group, there were only two phyla involved, *Firmicutes* and *Actinobacteria*.

DISCUSSION

Our study has shown that obese children and adolescents have significantly elevated levels of IL17A and PD-L1. Normally, IL17A participates in maintaining the integrity of the epithelial barrier of mucous membranes through regulation of occludin,

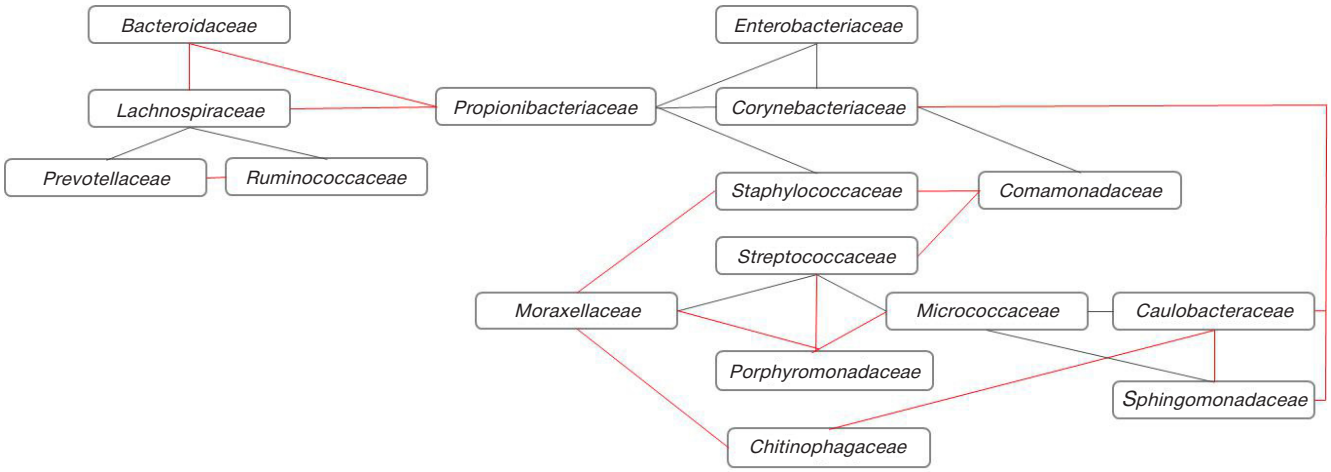

Fig. 3. Positive correlations between bacterial DNA, study group (obese children) Red lines show unique correlations in the group of obese children, black lines — correlations common with the control group

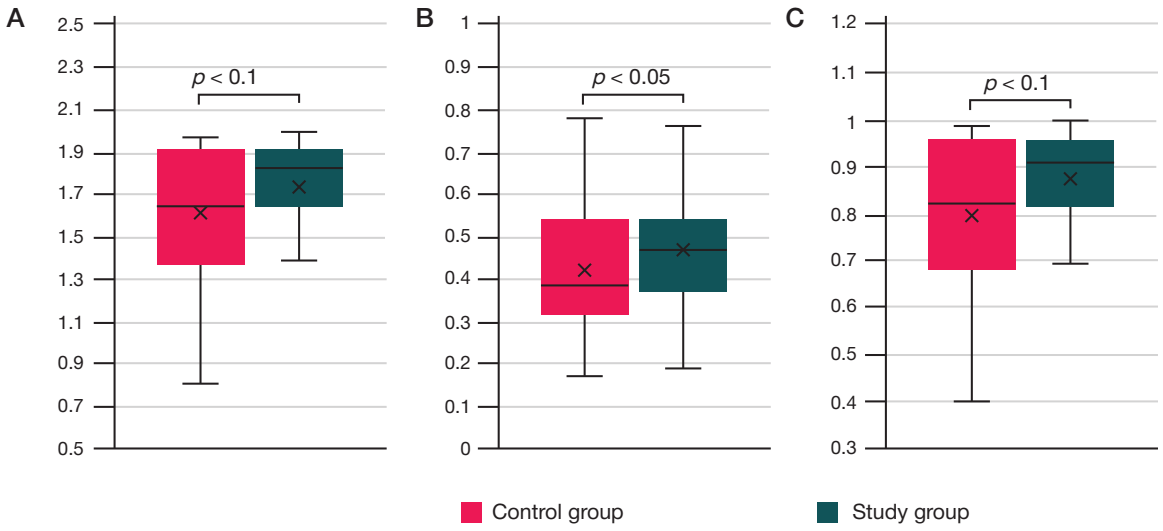

Fig. 4. Beta diversity in the groups. **A**. Manhattan distance. **B**. Euclidean distance. **C**. Bray-Curtis dissimilarity

but its main function is to recruit neutrophils into the intestine when there is a threat of excessive permeability and with the aim of maintaining homeostasis. In one study, it was shown that inhibition of IL17A or IL17RA translated into a serious deterioration of the epithelial barrier [9]. Moreover, IL17A is the main regulator of the host-microbiota interaction at the physiological level and in the context of immuno-mediated inflammatory diseases [10]. In mice experiments, knockout of the IL17 gene induced intestinal inflammation [11]. However, experiments on the transgenic mice model of multiple sclerosis (HLA-DR3) have shown that IL17A deficiency (HLA-DR3. IL17A-/- mice) promotes breeding of Treg-inducing intestinal bacteria, such as *Prevotella*, *Parabacteroides* and *Bacteroides*, which translates into a lighter course of the disease. It has also been shown that IL17A can regulate Treg activity and influence the course of the disease by modulating gut microbiota and establishing a new mechanism that allows immunomediators to affect that microbiota. Interestingly, DR3 mice with sufficient content of IL17A have the disease in a lighter form when kept together with the mice deficient in IL17A, which further emphasizes the dominant role of the microbiota in inducing Treg and alleviating the disease [12]. IL17A is involved in the pathogenesis of autoimmune diseases, such as rheumatoid arthritis, psoriasis, systemic lupus erythematosus, bronchial asthma, etc. [13]. The protocols of treatment of spondyloarthritis and psoriatic arthritis that relied on inhibition of IL17 caused the numbers of *Clostridiales* to shrink and those of *Bacteroidales* to grow. Thus, it can be assumed that, having elevated levels of IL17A, obese children are at risk of developing autoimmune diseases.

PD-L1 is a ligand that plays an important role in inhibiting the T cell-mediated immune response [14]. Binding of PD-L1 to PD-1 causes apoptosis of effector T cells and allows tumor cells to remain unaffected by the immune system, which makes the prognosis in the respective cases unfavorable. Several studies have shown that inhibition of interaction between PD-L1 and PD-1 enhances the adaptive immune response and increases antitumor activity [15–17]; it was also revealed that PD-L1 is expressed on white and brown adipocytes in mice [18]. PD-L1 is a rather complicated molecule: its low expression or knockout on adipocytes increases antitumor immunity in mice with a neoplasm, and in the absence of a tumor, ablation of PD-L1 adipocytes exacerbates diet-induced weight gain, infiltration of pro-inflammatory macrophages into adipose tissue and insulin resistance [19]. PD-L1 has a twofold effect: on the one hand, when it is expressed at a slower rate, the antitumor immunity becomes stronger and suppresses tumor growth, and on the other hand, its boosted expression in adipocytes allows maintaining adipose tissue homeostasis and mitigating smoldering inflammation associated with proliferation of the adipose tissue and its infiltration by macrophages [20]. The gut microbiome may modulate the response of melanoma patients

Table 4. Content of pro- and anti-inflammatory cytokines in children's blood serum

Analyzed indicator	Healthy children (n = 83)	Obese children (n = 80)	p-value
IL6, pg/ml, Me [Q_1;Q_3]	1.09 [0.28; 6.58]	1.19 [0.52; 8.24]	0.561
IL8, pg/ml, Me [Q_1;Q_3]	8.65 [4.05; 16.3]	11.5 [6.08; 21.4]	0.103
IL10, pg/ml, Me [Q_1;Q_3]	1.93 [0.67; 4.08]	2.30 [0.94; 6.02]	0.357
IL17A, pg/ml, Me [Q_1;Q_3]	0.69 [0.13; 4.88]	4.22 [0.39; 9.56]**	0.017
TNFa, pg/ml, Me [Q_1;Q_3]	12.8 [7.74; 21.8]	13.2 [9.00; 20.7]	0.856
Fractalkine, pg/ml, Me [Q_1;Q_3]	63.1 [47.1; 228]	90.8 [34.5; 209]	0.188
PD-L1, pg/ml Me [Q_1;Q_3]	25.8 [9.10; 53.1]	35.7 [19.5; 55.0]**	0.021

Note: различия достоверны по сравнению с контрольной группой: *p < 0,1; ** p < 0,05; *** p < 0,001.

Table 5. Statistical parameters of the cytokines correlation analysis

	Obese children				Healthy children		
	IL6	IL8	IL10	IL17A	IL6	IL8	IL17A
IL8	rho = 0.583 $p < 0.001$ $n = 80$				rho = 0.439 $p < 0.001$ $n = 83$		
IL17A	rho = 0.505 $p < 0.001$ $n = 80$	rho = 0.391 $p < 0.001$ $n = 80$	rho = 0.349 $p = 0.001$ $n = 80$		rho = 0.344 $p = 0.001$ $n = 83$	rho = 0.446 $p < 0.001$ $n = 83$	
IL10	rho = 0.383 $p < 0.001$ $n = 80$						
TNFa		rho = 0.441 $p < 0.001$ $n = 80$	rho = 0.400 $p < 0.001$ $n = 80$	rho = 0.427 $p < 0.001$ $n = 80$			rho = 0.316 $p = 0.004$ $n = 83$

to PD-1 immunotherapy. A group of researchers suggested that patients with a "favorable" intestinal microbiome (e.g., with a high diversity and abundance of *Ruminococcaceae* and *Faecalibacterium*) have an enhanced systemic and antitumor immune response mediated by an improved antigen presentation and effector T cell function at the periphery of and inside tumor's microenvironment. Patients with "unfavorable" intestinal microbiome (e.g., with poor diversity and high relative content of *Bacteroidales*), on the contrary, have impaired systemic and antitumor immune responses mediated by limited intracellular lymphoid and myeloid infiltration and impaired ability to present antigens. The results of the respective study underscores therapeutic potential of modulation of the gut microbiome in patients receiving immunotherapy with checkpoint blockade [21].

The composition of dominant families of blood microbiome was similar in both group. Despite this we have registered significant differences in the frequency. This allows an assumption that children suffer significant negative changes in the permeability of intestine and other biotopes, mainly the skin. Alteration of the microbiota affects the quality and quantity of the wall microbiota, the processes of production of mucus by goblet cells that prevents penetration of pathogens. In the group of obese children, we have shown that DNA of the *Bacteroidaceae* family makes a significantly smaller contribution to the alpha diversity of the blood microbiome, which is consistent with the data from other researchers that investigated stool microbiome [8]. Other researchers have proven that a growing share of Proteobacteria against the background of obesity, non-alcoholic fatty liver disease and non-alcoholic steatohepatitis [22–24] indicates predisposition to impairments of functions of liver and the entire gastrointestinal tract. A statistically significant decrease of the share of *Lachnospiraceae* in the study group entails deceleration of butyrate synthesis, which means poorer supply of energy to the intestinal epithelial cells and thinning of the intestinal barrier due to a slower activation of the claudine-1 protein [25]. Studies show that *Ruminococcaceae* are associated with the development of inflammatory bowel diseases, such as ulcerative colitis and Crohn's disease.

The main biotope for *Ruminococcaceae* is the large intestine, for *Bacteroidaceae* — large intestine, oral cavity, upper respiratory tract, and genitourinary system, and for *Prevotellaceae* — oral cavity, which suggests that translocation of bacterial DNA therefrom slows down.

According to the published papers, obese children exhibit positive correlations between the content of trefoil factors, namely, TFF3, and the intestinal microbiota, which indicates a violation of tolerance of the mucosal barrier to the microbial community [26].

Thus, it is likely that in obese children, due to the impaired peripheral tolerance and permeability of mucosal barriers, bacterial DNA translocates not only from the intestine, but also from the oral cavity and skin. As a result, blood microbiome grows more diverse. Bacterial DNA activates TLR9 receptors and triggers the secretion of pro-inflammatory cytokines, IL17A in particular, thus forcing the body to maintain balance and deploy anti-inflammatory mechanisms by activating PD-L1 synthesis.

CONCLUSIONS

In the group of obese children, alpha diversity is enriched with bacterial DNA of the *Lachnospiraceae*, *Prevotellaceae*, and *Ruminococcaceae* families. In that group, beta diversity becomes more advanced, too, which indicates a more intensive translocation of bacterial DNA from extra-intestinal microbiomes. Compared to the control group, there appear new patterns of correlations. In obese children, increased levels of IL17A and PD-L1 point to activation of pro- and anti-inflammatory mechanisms and the development of smoldering inflammation. Obesity is characterized by the appearance of significant correlations of DNA of the *Nocardiaceae* family with pro-inflammatory cytokines, which highlights the role of translocation of DNA of this taxon in the development of smoldering inflammation and the possibility of developing approaches to reduce this translocation as part of an obesity treatment protocol.

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