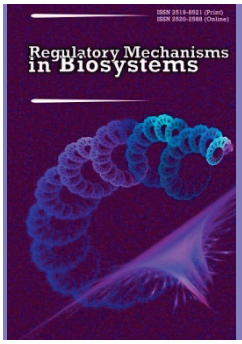

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Gut microbiota and changes in cytokine profile in animals with experimental acute disseminated peritonitis on the background of diabetes

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In the pathogenesis of acute widespread peritonitis and accompanying diabetes, a vital link is an endogenous intoxication caused by the translocation of microorganisms and their toxins from the intestine into the blood, metabolic changes, and immunological reactivity of the body. Our work aimed to investigate the microbial composition in the parietal intestinal biotope and the features of the blood cytokine profile in animals with acute disseminated peritonitis on the background of streptozotocin-induced diabetes. The study was conducted on 56 sexually mature non-linear white male rats. Diabetes mellitus was modeled by a single intraperitoneal injection of streptozotocin (60 mg/kg). On the 14th day of the development of streptozotocin-induced diabetes mellitus, a 10% filtered fecal suspension (0.5 mL) was injected into the abdominal cavity of animals, and acute generalized peritonitis was initiated. Sowing on nutrient media was carried out for bacteriological research to isolate a pure culture of microorganisms and their identification. The concentration of TNF-α, IL-1β, and IL-6 was studied by solid-phase enzyme immunoassay. The research results demonstrate an imbalance of cytokines in the dynamics of experimental acute disseminated peritonitis against the background of diabetes and quantitative and qualitative changes in the microbiota of the parietal intestinal biotope. A decrease in the number of *Escherichia coli* strains isolated in monoculture and an increase in the number of two-component and three-component microbial associations were revealed, among which *Enterobacter aerogenes*, *Escherichia coli*, *Bacteroides* spp., *Proteus mirabilis*, *Klebsiella* spp. and *Candida* species prevailed.

Keywords: microbiological monitoring; pathogens; interleukins; peritonitis; hyperglycemia.

Introduction

Treatment of acute disseminated peritonitis (ADP) is an urgent problem of surgery due to the mortality from this disease, which ranges from 12.5% to 39.2% (Kim et al., 2017; Afuwape et al., 2020; Lykhman et al., 2020; Tai et al., 2020; Zaporozhan et al., 2020). One of the reasons for the high mortality rates in ADP is the appearance in recent years of highly virulent antibiotic-resistant microflora, which determines the pathogenetic features of the course, clinical manifestations of the inflammatory intra-abdominal process (Sharma et al., 2017; Kotsar & Kochnieva, 2021). At the same time, the qualitative and quantitative composition of the microflora plays a significant prognostic value. Until recently, the severity of the infectious process of the peritoneum was mainly associated with *Escherichia coli* or staphylococci. Bacteriological studies conducted in recent years have demonstrated the polymicrobial nature of acute inflammation of the peritoneum with the participation of various aerobic and anaerobic microorganisms (Prasad et al., 2014; Clements et al., 2021).

Most patients with ADP have a comorbid pathology, among which diabetes mellitus (DM) makes up 7.5% to 14.0% (Raeeszadeh et al., 2017; Ross et al., 2018; Bashchenko et al., 2020; Grotelüschen et al., 2020; Karpenko et al., 2022). Intestinal dysbiosis in patients with diabetes mellitus predicts the severity of abdominal sepsis (Popejoy et al., 2017; Toniolo et al., 2019). A decrease in the number of neutrophil leukocytes and a violation of phagocytosis (Tessaro et al., 2017; Qiu et al., 2020; Sameliuk et al., 2022; Kukhtyn et al., 2022), and as a result, hyperglycemia leads to an increase in the number and virulence of microorganisms. Translocation of bacteria and their toxins from the lumen of the gastrointestinal tract into the blood contributes to the transformation of the intestine into a source of endotoxicosis (Peregudov & Khanevich, 1996), which leads to the generalization of the infection with subsequent dysfunction of the target organs and determines the further course of ADP.

The ability to translocate is characteristic of enterobacteria, *Escherichia coli*, *Proteus* sp., and Gram-positive aerobes, while obligate anaerobes have the most challenging time overcoming the intestinal barrier. The initiation and main stages of the development of the inflammatory response are mainly controlled by proinflammatory cytokines, which are synthesized by macrophages, neutrophilic leukocytes, and T-lymphocytes in response to stimulation by bacterial antigens. Several studies indicate that the endotoxin of gram-negative bacteria stimulates macrophages and neutrophils to produce TNF-α, IL-1, IL-6, IL-8, and IL-12 (Arango Duque & Descoteaux, 2014; Vergadi et al., 2018; Kaneko et al., 2019; Riga et al., 2019; Fu & Harrison, 2021). Exotoxins activate T-cells and monocytes to produce IL-1, IL-2, and TNF-α (Giesbrecht et al., 2019). At the same time, the peculiarities of the quantitative and qualitative composition of the microorganisms of the small intestine and the dynamics of changes in interleukins in the blood during acute inflammation of the peritoneum under the conditions of concomitant diabetes are not sufficiently elucidated and require a more thorough study.

This work aims to study the microbial composition in the parietal intestinal biotope and the features of the cytokine profile in the blood of

animals with experimental acute disseminated peritonitis on the background of streptozotocin-induced diabetes.

Materials and methods

The study was conducted on white non-linear rats (n = 56), which were kept in a vivarium following the "Standard rules for organizing, equipping, and maintaining experimental biological clinics (vivariums)". The experiments were conducted following the provisions of the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986); Directives of the Council of Europe 86/609/EEC (1986); Law of Ukraine No. 3447-IV "On the protection of animals from cruelty"; General ethical principles of experiments on animals, adopted by the First National Congress of Bioethics of Ukraine (2001), which was confirmed by the conclusion of the members of the commission on bioethics of the Danylo Halytskyi LNMU (protocol No. 8 of November 23, 2020, protocol No. 6 of June 22, 2021).

For microbiological monitoring of the parietal intestinal biotope and the study of the level of cytokines in the blood in the dynamics of the development of ADP against the background of diabetes, the animals were divided into the following groups:

– rats with simulated acute disseminated peritonitis (ADP);

– rats with simulated acute disseminated peritonitis on the background of streptozotocin-induced diabetes mellitus (SID);

– a control group of animals injected subcutaneously with 0.9% NaCl.

Animals with ADP and concomitant SID and rats with acute peritonitis without accompanying endocrine pathology were divided into subgroups (eight rats each). The division into subgroups was carried out depending on the timing of the withdrawal of the animals from the experiment (the first, third, and seventh days from the moment of introduction of the fecal suspension). These terms correspond to the reactive, toxemic, and terminal stages of peritonitis, according to K. S. Simonyan. The experimental animals of the experimental groups were removed from the experiment by overdose of sodium thiopental (at the rate of 100 mg/kg of weight).

The insulin-dependent form of diabetes was reproduced according to the method (Ramos-Lobo et al., 2015) by a single injection of streptozotocin (Sigma) into the abdominal cavity on an empty stomach in a dose of 60 mg/kg, previously dissolved in a sodium-citrate buffer solution (pH = 4.5). Before the injection, the right subclavian area was treated three times with a 10% betadine solution. The needle was inserted at an angle of 45° to the surface of the anterior abdominal wall until it "collapsed". After the injection, the animals received per os glucose solution during the first 24 h to prevent transient hypoglycemia. Glucose content during the experiment was studied using the glucose oxidase method. On the 14th day of the development of SID, the level of glucose in the blood of the rats was determined, and further simulation of acute inflammation of the peritoneum (combined pathology) was carried out in the animals with a level of hyperglycemia, which prevailed at 11.1 mmol/L.

Acute disseminated peritonitis was modeled by injecting 10% filtered fecal suspension into the abdominal cavity at a dose of 0.5 mL per 100 g of animal weight, according to Lazarenko et al. (2008). Fecal suspension was obtained by mixing the isotonic solution and cecal contents of three intact animals and filtering it twice through a double layer of gauze. Fecal suspension was administered no later than 20 minutes after its preparation. To prevent damage to internal organs, the animals were kept in a vertical position, with the caudal end up. Using the method of puncturing the ventral wall in the center of the midline of the anterior abdominal wall, directing the end of the needle alternately into the area of the right and left hypochondrium, into the right and left pubic areas, the necessary amount of fecal suspension was injected.

Bacteriological studies included sampling of the parietal intestinal biotope in a sterile test tube, inoculation on a nutrient medium, selection of a pure culture, and identification of aerobic and anaerobic microorganisms using highly selective media. For the cultivation of streptococci, we used meat-peptone agar with the addition of whole blood, staphylococci – yolk-salt agar, enterococci – a medium for enterococci, bacteria of the Enterobacteriaceae family – Endo and Ploskirev's medium, bacteroids – Kitta-Tarotsi medium, yeast-like fungi of the genus *Candida* – medium Saburo. Aerobic and facultatively anaerobic microorganisms were counted after 24–48 hours of incubation in a thermostat at 37 °C, and fungi of the genus *Candida* – after 3–5 days of cultivation in a thermostat at 20–22 °C. Identification of the obtained pure culture was carried out by morphological (microscopy, Gram staining), cultural (specific signs of growth on solid and liquid nutrient media), and biochemical properties (growth on media with carbohydrates and other particular nutrient media). Based on the obtained data, the frequency with which specific pathogens and their associations were encountered (%) was calculated. To determine the level of interleukins (IL), namely IL-1α, IL-10, and TNF-α in the blood serum of rats, the enzyme immunoassay method was used.

The analysis of research results was carried out using the Statistica 7.0 software package (StatSoft Inc., USA). Data are presented in the table as x ± SD (mean ± standard deviation). To compare the difference in mean parameters between control and experimental groups, we used Tukey's test, where differences were considered statistically significant at $P < 0.05$ for all data.

Results

The frequency of detection of microorganisms isolated from the parietal intestinal biotope of rats with ADP is shown in Table 1. Representatives of the genera *Escherichia coli* (75.0%), *Enterobacter* sp. (16.7%), and *Enterococcus* sp. (16.7%) dominated among the detected microorganisms. Monoinfection was identified in 41.7% of cases, and an association of pathogens was identified in 58.3%. Two-component microbial associations were isolated in 54.2%, and three-component microbial associations were isolated in 4.2% of cases. Among all isolated cultures, gram-negative microorganisms accounted for 69.2%, gram-positive microorganisms – 23.1%, and fungi of the genus *Candida* – 7.7%.

To analyze the dynamics of changes in the microbial composition in the parietal intestinal biotope, animals were studied 1, 3, and 7 days after the introduction of the fecal suspension (Table 1). On the first day of development of experimental ADP, monoculture was found in 62.5% of cases, with two-component microbial associations – in 37.5%. The priority pathogen was *Escherichia coli*, which is isolated in all monoculture crops and associated with *Staphylococcus* spp. (12.5%) and *Enterococcus* spp. (12.5%). In one case, the association was represented by *Enterobacter aerogenes* and *Streptococcus* spp.

Table 1

The composition of the isolated microflora of the parietal intestinal biotope of animals with acute disseminated peritonitis (%, n = 8)

Type of microorganism	The frequency of isolation of strains of microorganisms		
	first day	third day	seventh day
Escherichia coli	87.5	–	62.5
Enterobacter aerogenes	12.5	–	25.0
Klebsiella spp.	–	–	12.5
Staphylococcus spp.	12.5	–	–
Streptococcus spp.	12.5	12.5	12.5
Enterococcus spp.	12.5	12.5	25.0
Candida species	–	12.5	25.0
Bacteroides spp.	–	12.5	12.5
Proteus mirabilis	–	12.5	12.5

On the third day of the development of experimental ADP, monoculture was identified in 50.0% of cases. Two-component microbial associations mainly comprised *Escherichia coli* and cocci (75.0%). In one case, the association of microorganisms consisted of *Enterobacter aerogenes* and *Proteus mirabilis*.

The lowest frequency of detection of *Escherichia coli* monocultures (25.0%) was established on the seventh day of the experimental ADP. Two-component associations were sown in 62.5% of cases, and three-component microbial associations were sown in 12.5%. Associations of microorganisms consisted of gram-negative rods, among which *Escherichia coli* and *Enterobacter aerogenes* prevailed, and gram-positive cocci, among which *Enterococcus* spp. prevailed. Associations of *Candida* species and *Escherichia coli* were sown in 25.0% of cases. The three-component microbial association consisted of *Candida* species + *Escherichia coli* + *Enterobacter aerogenes*. Associations of *Proteus mirabilis* and *Bacteroides* spp. were found only in 12.5% of cases.

Bacteriological examination of the parietal intestinal biotope of animals with ADP on the background of SID revealed a predominance of gram-negative microorganisms, including *Escherichia coli* (50.0%), *Enterobacter aerogenes* (29.2%) and *Candida* species (29.2%). Monoinfection was identified in only 25.0% of cases. Two-component microbial associations were isolated in 58.3% of cases, and three-component microbial associations in 16.7%. Among all isolated cultures, gram-negative microorganisms accounted for 67.4%, gram-positive microorganisms – 17.4%, and fungi of the genus *Candida* species – 15.2%.

Microbiological monitoring of pathogens of the parietal intestinal biotope on the first day of the development of ADP against the background of SID revealed the dominance of *Escherichia coli* in 62.5% of cases (Table 2). *Escherichia coli* was found in monoculture in 37.5% of observations. Cocci were cultured in two cases, and *Staphylococcus* spp. was predominant among them. In five cases, two-component associations were observed, which included *Escherichia coli*, *Staphylococcus* spp., and *Candida* species.

Table 2

The composition of the isolated microflora of the parietal intestinal biotope of animals with acute disseminated peritonitis against the background of streptozotocin-induced diabetes (%, n = 8)

Type of microorganism	The frequency of isolation of strains of microorganisms		
	first day	third day	seventh day
Escherichia coli	62.5	50.0	37.5
Enterobacter aerogenes	12.5	25.0	50.0
Klebsiella spp.	–	12.5	25.0
Staphylococcus spp.	25.0	12.5	–
Streptococcus spp.	–	12.5	12.5
Enterococcus spp.	12.5	12.5	12.5
Candida species	25.0	25.0	37.5
Bacteroides spp.	12.5	25.0	25.0
Proteus mirabilis	12.5	12.5	25.0

On the third day of the development of acute peritonitis on the background of DM, monoculture was identified only in 25.0% of cases, two-component microbial associations – in 62.5%, and three-component microbial associations – in 12.5%. Associations of microorganisms consisted of gram-negative rods, among which a specific share was *Escherichia coli*, *Enterobacter aerogenes* and *Bacteroides* spp., gram-positive cocci, and *Candida* species. On the seventh day of ADP against the background of SID, monoculture of *Escherichia coli* was detected in only 12.5% of cases. In comparison, two-component microbial associations were identified in 50.0% and three-component microbial associations in 37.5%. *Enterobacter aerogenes* and *Escherichia coli* prevailed in two-component associations. Three-component associations of microorganisms consisted of gram-negative rods, among which *Proteus mirabilis* and *Klebsiella* spp., gram-positive cocci (*Streptococcus* spp.), and *Candida* fungi were the priority. Associations of microorganisms isolated in the parietal intestinal biotope are presented in Table 3.

Analysis of the results of the bacteriological study of the parietal intestinal biotope of animals with ADP and animals with ADP on the background of SID revealed a decrease in the share of *Escherichia coli* and the appearance of *Proteus mirabilis*, *Bacteroides* spp. and fungi of the genus *Candida* already on the first day after the introduction of fecal suspension in animals with combined pathology. On the third day, we also monitored a decrease in the number of *Escherichia coli* against the background of an increase in the microflora of representatives of the genus *Enterobacter aerogenes*, *Bacteroides* spp., *Klebsiella* spp., and *Candida* fungi compared to animals with acute disseminated peritonitis. In addition, the growth of the number of two-component associations of microorganisms was monitored. On the seventh day of the development of ADP against the background of SID, an increase in the microflora of representatives of the genus *Enterobacter aerogenes*, *Bacteroides* spp., *Proteus mirabilis*, and fungi of the genus *Candida* was found at the same time as a decrease in the number of *Escherichia coli* compared to microbiological findings in animals with experimental acute disseminated peritonitis without accompanying diabetes. There was a significant decrease in the number of *Escherichia coli* strains isolated in monoculture and an increase in the number of three-component associations dominated by strains of *Proteus mirabilis*, *Klebsiella* spp., and *Candida* fungi.

Table 3

Associations of microorganisms isolated in the parietal intestinal biotope (%)

Associations of microorganisms	Groups of animals	
	ADP	ADP on the background of SID
two-component associations		
Escherichia coli+*Staphylococcus* spp.	8.3	4.2
Escherichia coli+*Enterococcus* spp.	8.3	4.2
Escherichia coli+*Bacteroides* spp.	4.2	–
Escherichia coli+*Candida species*	4.2	–
Escherichia coli+*Enterobacter aerogenes*	4.2	12.5
Enterobacter aerogenes+*Streptococcus spp.*	4.2	–
Enterobacter aerogenes+*Proteus mirabilis*	4.2	–
Enterobacter aerogenes+*Bacteroides* spp.	–	4.2
Enterobacter aerogenes+*Candida species*	–	4.2
Streptococcus spp.+*Candida species*	4.2	–
Streptococcus spp.+*Enterococcus* spp.	4.2	4.2
Staphylococcus spp.+*Enterococcus* spp.	–	4.2
Bacteroides spp.+*Proteus mirabilis*	4.2	–
Klebsiella spp.+*Staphylococcus* spp.	–	4.2
Klebsiella spp.+*Enterococcus* spp.	4.2	–
Candida species+*Proteus mirabilis*	–	8.3
Candida species+*Bacteroides* spp.	–	8.3
three-component associations		
Escherichia coli+*Enterobacter aerogenes*+ *Bacteroides* spp.	–	4.2
Klebsiella spp.+*Candida species*+*Proteus mirabilis*	–	8.3
Enterobacter aerogenes+*Streptococcus* spp.+ *Bacteroides* spp.	–	4.2

In the process of destruction of microorganisms in the abdominal cavity, toxins, products of destruction of cellular structures, and lysosomal and proteolytic enzymes are released, which are chemoattractants for polymorphonuclear leukocytes. Neutrophils and monocytes migrating to the focus of inflammation during phagocytosis release cytokines. Cytokines are triggers and regulators of the severity of inflammatory reactions and immunological reactivity and determine the nature and spread of the inflammatory process in the abdominal cavity. Disturbance of the balance between proinflammatory and anti-inflammatory interleukins is a recognized factor in the severity of the inflammatory process.

As a result of the study of proinflammatory cytokines in animals with experimental ADP on the first day of the disease, a statistically significant increase in the content of IL-1β by 86.0% and TNF-α by 217.9% was found compared to the data of the control group of animals (Fig. 1). The level of anti-inflammatory IL-10 was reduced by 11.2%, respectively.

On the third day after the introduction of the fecal suspension, we observed a significant increase in the level of IL-1β by 103.4% and TNF-α by 233.3% ($P < 0.001$) and a decrease in IL-10 by 13.2% ($P < 0.01$) compared to the values of the corresponding cytokines in the control group.

The maximum values of IL-1β and TNF-α were recorded on the seventh day of ADP, which exceeded the control values by 106.7% and 235.3%, respectively ($P < 0.001$), at a time when the level of IL-10 decreased by 99.2% ($P < 0.001$).

We found that on the first day after the introduction of fecal suspension in animals with experimental ADP on the background of SID, the level of IL-1β in the blood serum of animals increased by 94.4% ($P < 0.001$) compared to control animals and by 4.5%, respectively to the value in animals with ADP without concomitant pathology. IL-1β activates T- and B-lymphocytes enhances their cytotoxic properties, and initiates the synthesis of TNF-α. We established an increase in TNF-α by 196.8% ($P < 0.001$) compared to the data of the control group and its decrease by 6.7% from the value of animals with GPP without concomitant pathology.

On the third day of ADP development, under conditions of presimulated SID in rats, an increase in the concentration of IL-1β – by 114.6% and TNF-α – by 220.5% was observed ($P < 0.001$) compared to the control. IL-1β prevailed by 5.2% of the value in animals with GPP, and TNF-α was reduced by 3.8%.

On the seventh day after the introduction of fecal suspension in animals with combined pathology, a probable increase in the level of IL-1β by 121.3% and TNF-α – by 235.3% compared to the control group was found ($P < 0.001$). Concerning the group of animals with ADP, the difference in IL-1β values was 7.1%.

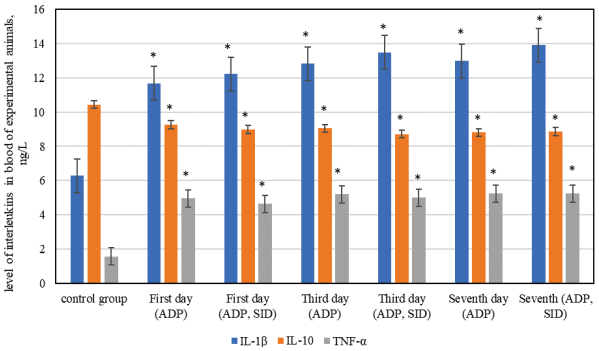

Fig. 1. Dynamics of interleukins in the blood of experimental animals (ng/L): statistically significant changes were taken into account compared with the control (* – $P < 0.05$)

The level of anti-inflammatory IL-10 in animals with ADP on the background of SID was reduced by 13.9% on the first day, by 16.6% on the third day, and by 15.1% on the seventh day compared to control values ($P < 0.01$). Compared with the value in the group of animals with ADP without concomitant pathology, we found that IL-10 on the first and third days was lower by 3.0% and 3.9% and higher by 0.7% on the seventh day.

Therefore, the results of the conducted studies demonstrate an imbalance of cytokines in the dynamics of the course of ADP against the background of diabetes and quantitative and qualitative changes in the microbiota of the parietal intestinal biotope, namely a decrease in the number of *Escherichia coli* strains isolated in monoculture and an increase in the number of two-component and three-component microbial associations, among which predominated *Enterobacter aerogenes*, *Escherichia coli*, *Bacteroides* spp., *Proteus mirabilis*, *Klebsiella* spp. and *Candida* species.

Discussion

The gut ecosystem is represented by the core microbiome, which consists of the microbiome present in all people, or at least in large part in healthy people. In this line, Hugon et al. (2015) isolated 2,172 different species in the gut, divided into 12 different phyla, observing that Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes accounted for up to 93.5% of the total microbial population. The Proteobacteria group mainly consists of the genera *Escherichia* and *Enterobacter*, while *Bifidobacterium* predominates in the phylum Actinobacter (Huttenhower et al., 2012; Zhou et al., 2022).

Several studies have been conducted on the microbiota in diabetes associated with developing acute peritonitis (Ortega et al., 2020; Craciun et al., 2022; Piccioni et al., 2023). Toniolo et al. (2019) associated bacteremia and sepsis with this disorder of carbohydrate metabolism with *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, Enterobacteriaceae, Enterococci, *Pseudomonas aeruginosa*, *Candida albicans*. Popejoy et al. (2017), analyzing the microbiota of patients with diabetes complicated by intra-abdominal infection, found strains of Enterobacteriaceae, *Escherichia coli*, *Enterococcus faecalis*, *Bacteroides* spp. We observed a decrease in the number of *Escherichia coli* strains isolated in monoculture and an increase in the number of microbial associations, among which *Enterobacter aerogenes*, *Escherichia coli*, *Bacteroides* spp., *Proteus mirabilis*, *Klebsiella* spp. – dominated in animals with ADP on the background of SID and *Candida* species.

Bacterial pathogens are triggers for the activation of immunocompetent cells and the launch of a complex cascade of interactions between cytokines. It is known that lipopolysaccharides of Gram-negative bacteria activate the synthesis of TNF-α, IL1-β, IL-6, and IL-8 by macrophages in ADP. Among the polysaccharides of *B. fragilis*, polysaccharide A is the most pronounced and well-characterized molecule with immunomodulatory properties, which contributes both to the establishment of intestinal homeostasis and the development of peritonitis and sepsis (Lobo et al., 2016; Gilmore et al., 2022). *Proteus mirabilis* was found to induce the production of IL-1β, worsening inflammation in the intestine (Seo et al., 2015; Armbruster et al., 2018; Kiani et al., 2021).

In our study, the number of strains of *Bacteroides* spp. and *Proteus mirabilis* increased on the first, third, and seventh days after administration of the fecal suspension compared to animals with ADP. We observed an increase in the content of TNF-α and IL1-β during the development of the combined pathology against the background of an increase in the number of microbial associations in the parietal intestinal biotope, a significant proportion of which was made up of strains of *Bacteroides* spp. and *Proteus mirabilis*. The number of *Escherichia coli* strains isolated in monoculture decreased during all stages of development of ADP on the background of SID; however, this pathogen accounted for a significant share in two-component and three-component associations of microorganisms. This confirms the assumption that intestinal colonization by Enterobacteriaceae bacteria contributes to endotoxemia and hyperproduction of proinflammatory cytokines in the combined pathology.

Hyperglycemia stimulates IL1-β production during macrophage differentiation (Moganti et al., 2017). The higher levels of IL1-β that we found in animals with ADP and concomitant DM can be attributed not only to the activation of macrophages as a result of β-cell damage of the pancreatic islets of Langerhans but also to an increase in the number of two-component and three-component microbial associations, which are IL-1β inducers.

In our study, we observed an imbalance of cytokines, characterized by a sequential intensification of the synthesis of IL-1 β, TNF-α, and a decrease in the level of IL-10 following the control. A decrease in the synthesis of IL-10 is a sign of the development of suppression of the immune response (Grynchuk et al., 2021), which is possibly associated with an increase in the number of strains of *Enterobacter aerogenes*, *Escherichia coli*, *Bacteroides* spp., *Proteus mirabilis*, and *Klebsiella* spp. in the intestinal biotope and *Candida* fungi.

Conclusion

The progression of experimental ADP and accompanying SID is accompanied by quantitative changes in the microbiota of the parietal intestinal biotope, namely an increase in the number of two-component and three-component microbial associations. Qualitative changes in the intestine are characterized by a decrease in the number of *Escherichia coli* strains isolated in monoculture and an increase in the number of *Enterobacter aerogenes*, *Bacteroides* spp., *Proteus mirabilis*, *Klebsiella* spp. and *Candida* species, which is accompanied by an imbalance of IL-1β, TNF-α, and IL-10 in the blood of animals.

The authors declare that there is no conflict of interest.

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