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Role of gut microbiota in pathogenesis of selected chronic diseases

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ABSTRACT

The human digestive system is colonized by a huge number of microorganisms, that are referred to collectively as the gut microbiota. The composition of intestinal microorganisms are shaped from an early life and undergoes constant changes depending on the influence of external factors, such as: type of delivery, feeding the young child, diet in subsequent years of life, pharmaceuticals use, stress, lifestyle or infections and previous inflammation within the digestive tract. Despite transient changes in microbiota composition, the intestinal ecosystem is constantly striving to maintain homeostasis, both qualitative and quantitative, which is fundamental to human health and human development. Microbes present in the intestines are responsible for sealing the intestinal barrier, mucin production, stimulation of the angiogenesis process, supporting digestive processes by fermentation and decomposition of undigested food residues, vitamin production or protection from pathogenic microorganisms. As shown by numerous studies carried out in recent years, intestinal dysbiosis plays a fundamental role in the development of many chronic diseases such as inflammatory bowel disease, diabetes, obesity, celiac disease, connective tissue diseases and others. Insightful understanding of the interactions between microorganisms and the host organisms can provide new information about pathogenesis of diseases as well as new ways to prevent and treat intestinal or systemic disorders. The aim of this work is to review the latest reports on the role of the gastrointestinal microbiome in selected chronic diseases.

***Keywords*:** gut microbiota, autoimmune disease, metabolic disease, immune system response

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1. INTRODUCTION

The human digestive system is colonized by a huge number of microorganisms, that are referred to collectively as the gut microbiota. More than 99% of natural gastrointestinal microbiota are 4 types of microorganisms: Bacteroidetes (24%), Firmicutes (64%), Actinobacteria (3%) and Proteobacteria (8%), among which the following types predominate: *Bifidobacterium, Lactobacillus, Streptococcus, Bacteroides* or *Enterobacteriaceae* [1]. Besides bacteria, the composition of intestinal microbiota including slightly less numerous populations of fungi, viruses, archaea or protozoa. The composition of intestinal microorganisms are shaped from an early life and undergoes constant changes depending on the influence of external factors, such as: type of delivery, feeding the young child, diet in subsequent years of life, pharmaceuticals use, stress, lifestyle or infections and previous inflammation within the digestive tract [2]. Despite transient changes in microbiota composition, the intestinal ecosystem is constantly striving to maintain homeostasis, both qualitative and quantitative, which is fundamental to human health and human development [3].

Microbes present in the intestines are responsible for sealing the intestinal barrier, mucin production, stimulation of the angiogenesis process, supporting digestive processes by fermentation and decomposition of undigested food residues, vitamin production or protection from pathogenic microorganisms [4]. They also play a key role in the modulation of the host's immune response which is mediated through Toll-Like Receptors (TLR) and Nucleotide Oligomerization Domain (NOD). The correct series of interactions between the microorganisms and cells of the gut lymphoid tissue - GALT (Gut Associated Lymphoid Tissue) is the basis for the development of appropriate tolerance to ubiquitous commensal bacteria and food antigens delivered to the body every day [5].

The effects of interactions between humans and their intestinal microbiome are not only related to the functioning of the gastrointestinal tract, but can also influence other processes and organs, therefore the disturbance of microbiota composition called dysbiosis can have serious health implications. When one of the stages of this delicate interaction fails, it may result in the occurrence of autoimmune or inflammatory diseases [5].

As shown by numerous studies carried out in recent years, intestinal dysbiosis plays a fundamental role in the development of many chronic diseases such as inflammatory bowel disease (IBD), diabetes, obesity, connective tissue diseases and others [6-10]. Insightful understanding of the interactions between microorganisms and the host organisms can provide new information about pathogenesis of diseases as well as new ways to prevent and treat intestinal or systemic disorders.

The aim of this work is to review the latest reports about role of the gastrointestinal microbiome in selected chronic diseases.

2. INTESTINAL MICROBIOTA AND OBESITY

In recent years, there have been many studies on the potential link of intestinal microorganisms with the development of obesity [11-16]. There are several mechanisms explaining the interaction between microbiome and host metabolism that may contribute to the development of this disease. Intestinal microbiota plays an important role in intestinal mucosal permeability, and metabolic activity of microorganisms is crucial meaning in obtaining calories

from digested food, absorption of polysaccharides, as well as in the process of fat accumulation in the host's fat tissue, which partly may explain the importance of microorganisms in the development of disorders related to excessive weight [17, 18]. Previous studies conducted with both animals and humans seem to confirm this hypothesis.

Ley et al. showed that the gut of obese mice was characterized by a different bacterial colonization compared to rodents with normal body weight. Intestinal microbiota of overweight mice was characterized by a significantly increased number of *Firmicutes*, while the number of bacteria of the genus *Bacteroides* was reduced compared to rodents siblings with normal body weight. Moreover, the transfer of bacteria from the digestive tract of obese animals to germ-free mouse, caused a significant increase in weight compared to mice which received intestinal bacteria from animals of normal body weight [11].

Similar dependencies about quantitative disorders in the intestinal microbiota within *Bacteroides* and *Firmicutes* are also found among humans [12-14]. It is assumed that this different composition may affect the difference in obtaining energy from digested food. These assumptions seem to confirm the fact that during studies conducted by Ley et al., it was observed that due to weight loss, the ratio of *Bacteroides*/*Firmicutes* increased to a degree depending on the percentage of mass reduction, while independent of the supply of calories [15]. Also Turnbaugh et al. using metagenomic and biochemical analysis, showed that intestinal microbiota of obese individuals was characterized by different metabolic capacity and obtained more energy from the supplied food than the microbiota of lean individuals [16]. Moreover, the transfer of intestinal microorganisms from obese individuals to the digestive system of animals with normal body mass, resulted in a significant body fat increase and weight gain compared to animals colonized with intestinal microbiota obtained from lean individuals [16, 19]. It is interesting to note that the fat content among conventionally reared mice was 40% higher than in germ-free animals, despite reduced food intake. Furthermore, the conventionalisation of germ-free mice with intestinal microbiota from the cecum of conventionally raised rodents, caused an 60% increase in fat content and the development of insulin resistance within 14 days despite a reduction in food intake [17].

These results seem to confirm the hypothesis that the composition of intestinal microbiota affects the amount of energy obtained from digested food, and furthermore it also promotes insulin resistance, an increase blood glucose and leptin levels and hypertrophy of adipocytes, which identifies intestinal microbiota as an important factor in etiology of obesity.

3. THE CONTRIBUTION OF INTESTINAL MICROORGANISMS TO THE DEVELOPMENT OF TYPE 1 AND TYPE 2 DIABETES

Until recently, it was thought that the main reason for the rapid increase in the number of patients suffering from diabetes is a change in eating habits and lifestyle. However, recent reports indicate, that intestinal microbes may play an equally important role in the pathogenesis of this disease [6, 20, 21]. More and more evidence shows that intestinal microorganisms determine the formation of metabolic disorders as a result of an abnormal gut barrier permeability as a results of inducing inflammation of low intensity [22, 23]. For some time now, it has been suggested that in patients with type 2 diabetes (T2DM), chronic low-grade inflammation with abnormal expression and production of many inflammatory mediators, such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) is associated with

lipopolysaccharide, which is an integral component of the Gram-negative cell membrane, whose increased concentration is found in patients with type 2 diabetes (T2DM) [23, 24]. It is supposed that this endotoxin induces excessive proinflammatory cytokine production, which is associated with an increased blood glucose level and connected with suppression of insulin signaling by inhibition of insulin receptor tyrosine kinase activity which contributes to the development of insulin resistance and the development of diabetes [20-22].

A study on mice confirmed a positive relationship between concentration of bacterial lipopolysaccharide in plasma and insulin resistance and type 2 diabetes [24]. The confirmation of this hypothesis may be the fact that the experiment consisting in prebiotic supplementation of leptin-deficient (ob/ob) mice, a model of type 2 diabetes, induced significant changes in microbiota, i.e. the increase number of bacteria of the genus *Lactobacillus* spp., *Bifidobacterium* spp. and *Clostridium coccoides*, which was correlated with improved glucose tolerance and reduction of low-grade inflammation. Lower plasma lipopolysaccharide and decrease level of inflammatory cytokines were also observed. Moreover, prebiotic-treated mice were characterized by a reduced expression of oxidative stress markers and metabolic changes, which was the result of improved tight-junction integrity due to changes in microbiota. Besides, the influence of the change in the composition of microorganisms on the increased production of gastrointestinal peptides: GLP-1, GLP-2, YY peptide, ghrelin (which are of key importance in the regulation of glucose homeostasis) were found [25, 26].

Studies involving people with T1DM and T2DM also demonstrated differences in the composition of their intestinal microbiota compared to healthy people. Larsen et al. showed that patients with T2DM were characterized by a significantly reduced number of *Firmicutes*, while microbes belonging to the class of *Betaproteobacteria* occurred in a significantly higher number. Moreover, number of these bacteria was correlated with blood glucose level. Positive correlation between *Bacteroidetes*/*Firmicutes* ratio and the *Bacteroides*/*Prevotella* proportion to the *C.coccoides* group/*Eubacterium rectale* with plasma glucose concentration was observed [27].

Also Salamon et al. showed that patients with T2DM are characterized by a reduced number of *Firmicutes* and an increased abundance within the group of *Proteobacteria*. Interestingly, the number of *Bacteroidetes* was different depending on the type of diabetes. Patients with type 1 diabetes (T1DM) were characterized by significantly increased colonization of the intestine by *Bacteroidetes* compared to the control group, whereas in group of people with T2DM, an opposite tendency was observed [28]. This is consistent with the studies carried out on the rat's model, in which *has been shown* that the *increase* in the *number* of *Bacteroidetes* is correlated with T1DM [29].

Gosiewski et al. were one of the few, who attempt to show the relationship between fungal dysbiosis and the occurrence of diabetes. As a result, the researchers showed that among people with type 1 diabetes and type 2 diabetes the number of fungi of the genus *Candida* was a significantly higher compared to the healthy people (respectively: T1DM: 4.35×10^{3} T2DM: 9.13×10^{4} vs. control group: 6.72×10^{1}) [30].

While the above results show increased colonization of the intestine by fungi in diabetic patients, there are still too few studies of mycobiome to determine whether these changes are secondary effects of this disease resulting from elevated blood sugar level which create specific conditions for intensive colonization by fungi or maybe dysbiosis within the mycobiome contribute to the pathogenesis of the disease.

4. CHANGES IN GUT MICROBIOTA ASSOCIATED WITH NEUROPSYCHIATRIC DISORDERS

The brain and the gut communicate to each other through the gut–brain axis. Communication between these organs has a two-way course and involves participation of endocrine, neuronal and immunological pathways [31]. Numerous scientific studies show that the composition of intestinal microbiota, through the secretion of abundant amount of neuroactive compounds and immunomodulatory substances, shapes the proper structure as well as functions of the brain regions involved in the regulation of anxiety, pain, cognitive ability or control of emotions and mood [31-33]. In addition, it is suggested that the microbiome is necessary for the normal gross morphology and ultrastructure of the amygdala and hippocampus [34]. Due to the documented impact on intestinal microbiota on the proper functioning and development of mental health, the role of intestinal dysbiosis in the development of neurodevelopmental or neurodegenerative disorders is postulated [35-37].

Previous studies including the analysis of the composition of intestinal microbiota people with neuropsychiatric diseases have shown that patients were characterized by disorders in the composition of the gastrointestinal microbiome. And so, people with autism were characterized by significantly lower number of bacteria of the genus *Bifidobacterium*, *Prevotella*, *Coprococcus* while numer of bacteria of the genus *Lactobacillus*, *Clostridium* and *Bacteroidetes* were increased [38-42]. It was also shown that children with autism were characterized by increased colonization by *Clostridium difficile*, which was positively correlated with the level of 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPHPA), which is the main metabolite produced by numerous *Clostridium* species. It is a metabolite of m-tyrosine (3-hydroxyphenylalanine), which have influence the reduction of the level of brain catecholamines and blocks dopamine beta-hydroxylase, leading to excess dopamine and the development of abnormal behaviors and symptoms characteristic of autism in experimental animals (stereotypical behavior, hyperactivity and hyperreactivity) [43].

The gut microbiota of people with Alzheimer's disease (AD) shows a significantly higher number of *Bacteroidetes* and less *Firmicutes*, *Actinobacteria* and *Bifidobacterium* compared to the control group [44]. These observations have also been confirmed by studies involving the mouse model of Alzheimer's disease, which additionally showed a reduced colonization by *Proteobacteria*, *Verrucomicrobia* and an increased number of *Tenericutes*. Interestingly, the transplantation of intestinal microbiota from mouse model of AD into germ-free rodents, resulted in the formation of a beta-amyloid plaques in the brain, which is a characteristic symptom of AD development [45].

In recent years, there have been papers postulating that the participation in the pathogenesis of AD may have *Porphyromonas gingivalis*, which is listed among the probable etiological factors of periodontitis. The DNA of this bacterium was detected both in human brains among deceased patients who suffered from AD, as well as in the cerebrospinal fluid of living people with suspicion of this disease. In addition, in the samples taken from the brains of patients suffering from AD, the researchers also detected toxic enzymes secreted by these bacteria, known as gingipains. Their presence was associated with two different indicators of AD: abnormal tau protein and ubiquitin. Moreover, in further laboratory studies, oral administration of this pathogen to mice led to the colonization of animal brains by *P. gingivalis* and it was associated with increased production of beta-amyloid [46].

Intestinal dysbiosis was also demonstrated in Parkinson's disease (PD) and it was manifested by increased number of *Bacteroidetes*, *Enterobacteriaceae* and the bacteria of the genus *Ralstonia* belonging to Proteobacteria. Decrease in the number of *Faecalibacterium*, *Blautia*, *Coprococcus* and *Roseburia* has also been documented [47]. Moreover, these dysbiosis have been associated with the induction of inflammation and abnormal folding of α-synuclein (α-syn) which is a presynaptic neuronal protein genetically and neuropathologically related with PD [47, 48].

In studies about pathophysiology of stress and anxiety, it was shown that the development of these disorders is favored by *Campylobacter jejuni* infection which influenced the induction of c-Fos protein - a marker of neuronal activation [49]. Goehler et al. showed that infection of mice (by supplying of 0.2 ml of the solution containing *Campylobacter jejuni*) affected the development of anxiety and behavioral disorders compared to control mice which received *physiological saline* solution [49]. Interestingly, the supply of probiotics containing *Bifidobacterium longum*, *Bifidobacterium infantis*, *Lactobacillus helveticus* and *Lactobacillus rhamnosus* has been shown to alleviate anxiety and normalize animal behavior [50-54].

Dysbiosis occurring in the above disease entities may disturb the integrity and permeability of the intestinal barrier, consequently disrupting communication between the gut and the central nervous system. As a result, there is an increase level of antigens in the blood, which activates the secretion of inflammatory mediators which are able to penetrate blood-*brain barrier* [55, 56].

It has been shown that lipopolysaccharide present in the cell wall of gram-negative bacteria may affect the mood, as well as cognitive ability. It is possible through the present of receptors recognizing molecular bacterial patterns such as NOD or TLR located in the surface of glial cells or with the participation of indirect mechanisms associated with the activation of the immune response [56]. These disorders can initiate apoptosis of nerve cells and also contribute to the development of emotional disorders, cognitive limitations and other mental disorders [3, 35, 56, 57]. Furthermore, the microorganisms present in the gut are involved in the regulation of gene expression associated with myelination of prefrontal cortex, which is a key area involved in the development of disorders such as schizophrenia, depression and autism [58].

One of the first animal experiments regarding the effect of intestinal microbiota in the prevention of depression showed that supplementation of rats with the probiotic strain of *Bifidobacterium infantis* has an impact on the significantly increased production of tryptophan, a precursor of serotonin in the central nervous system (CNS). After supplementation, an increase concentration of kynurenine acid (which plays an important role in the functioning of the CNS) and reduction of the level of proinflammatory cytokines IFN-gamma, TNF-α and IL-6 was also observed. The addition of bifidobacteria also resulted in the reduction of 5-hydroxyindoleacetic acid (5-HIAA) (constituting the end product of transformation of tryptophan - a metabolite formed from deamination of serotonin) in the frontal cortex and reduction of 3,4-dihydroxyphenylacetic acid (DOPAC) in the amygdala. It was also associated with beneficial effect on changes in behavior [59].

Equally promising results of probiotic therapy to combat stress have been demonstrated by Liang et al. [60]. The study used a rat model of depression, in which rodents were subjected to chronic stress for 21 days. One of the tested group received the probiotic strain of *Lactobacillus helveticus* NS8 throughout the stress induction period and next rats were observed during behavioral tests. The results showed that probiotic supplementation reduced

chronic behavioral disorders caused by stress, such as anxiety or depression, and limited the development of cognitive dysfunction compared to the group of rats which did not receive a probiotic. Moreover, the supply of *L. helveticus* NS8 resulted in lower *plasma corticosterone* (CORT) and adrenocorticotropic hormone (ACTH) levels. Increase of plasma anti-inflammatory interleukin-10 (IL-10) concentration, as also as higher hippocampal serotonin (5-HT) and norepinephrine (NE) levels were also observed [60].

Probiotic combination of *Lactobacillus* and *Bifidobacterium* in model of post myocardial infarction (MI) depression has also been studied.

Thus combination therapy with *L. helveticus* and *Bifidobacterium longum* eased depression after MI by reducing the concentration of proinflammatory cytokines, restoring intestinal barrier integrity and as well as by limiting apoptosis of nerve cells in the structures of the limbic system which is responsible for the central functions of emotion [61, 62]

Bravo et al. showed the effect of lactic acid bacteria on changes in the gamma-aminobutyric acid (GABA) receptor involved in the pathogenesis of anxiety and depression [51]. *Mice supplementation* with *L. rhamnosus* (JB-1) induced region-dependent changes in GABAB1b mRNA in the brain with increases in cortical regions and concomitant reductions in expression in the amygdala, hippocampus and locus coeruleus compared to control group.

Moreover, *L. rhamnosus* (JB-1) reduced the expression of GABAAα2 mRNA in the prefrontal cortex and amygdala, and increased GABAAα2 in the hippocampus. In addition, this strain reduced the level of stress-induced corticosterone and limited the development of anxiety and depression-related behavior [51].

Other independent studies have also documented the role of microorganisms in the production and stimulation of the secretion of neurotransmitters and neuroactive compounds. It has been reported that the production of the inhibitory neurotransmitter GABA may be induced by the bacteria of the genus of *Lactobacillus* and *Bifidobacterium*, while *Escherichia, Bacillus* and *Saccharomyces* spp. are able to stimulate noradrenaline production. On the other hand, *Candida*, *Streptococcus, Escherichia* and *Enterococcus* spp. are capable to producing serotonin, and *Bacillus* is able to secrete dopamine. Some bacteria of the genus *Lactobacillus* spp. are also able to produce acetylcholine [63, 64]. These neurotransmitters synthesized by microorganisms are able to penetrate the intestinal mucosa and mediate the physiological functions of the brain and play a role in the pathophysiology of some psychiatric diseases, therefore the above data emphasize how important role in the two-way communication of the gut-brain axis are bacteria, and furthemore, they suggest that some microorganisms may prove to be useful therapeutic supplements in stress-related disorders

5. ASSOCIATION OF INTESTINAL MICROBIOME WITH CELIAC DISEASE

Celiac disease is a serious autoimmune disease that occurs in genetically predisposed people who have DQ2 or DQ8 haplotypes encoding specific antigens in the HLA region. However, the genetic factor itself is insufficient to trigger clinical symptoms, which suggests the key role of environmental factors (including microbiological disorders) in the pathogenesis of this disease [65].

Recently, there have been reports that the composition of intestinal microorganisms in patients with celiac disease is altered compared to healthy people. A reduced number of bacteria of the genus *Lactobacillus* and *Bifidobacterium* has been demonstrated [66, 67]. In addition,

the number of *Verrucomicrobia*, *Parcubacteria* and *Euryarchaeota* was increased and it was spositively correlated with TNF-α level and short-chain fatty acids (SCFA) [68].

Excessive amounts of *Proteobacteria* - in particular *Neisseria flavescens* have also been documented. Moreover, in *ex vivo* studies using CaCo-2 cells (the most widespread cellular system imitating human intestinal epithelium), was shown that this bacterium was able to escape from the lysosomal compartment and to induce an inflammatory response in dendritic cells and in intestinal mucosa explants. In addition, *N.flavescens* affected the secretion of significant amounts of IL-12 and TNF-α [69].

The key question remains whether the dysbiosis found in CD patients is the result of a rigorous diet, or perhaps it is a factor triggering the onset of celiac disease in genetically predisposed individuals. Considering the fact that the dysbiosis of the gastrointestinal microbiome may contribute to the induction of many inflammatory signals, the role of microorganisms as a potential factor initiating the development of the disease can not be neglected.

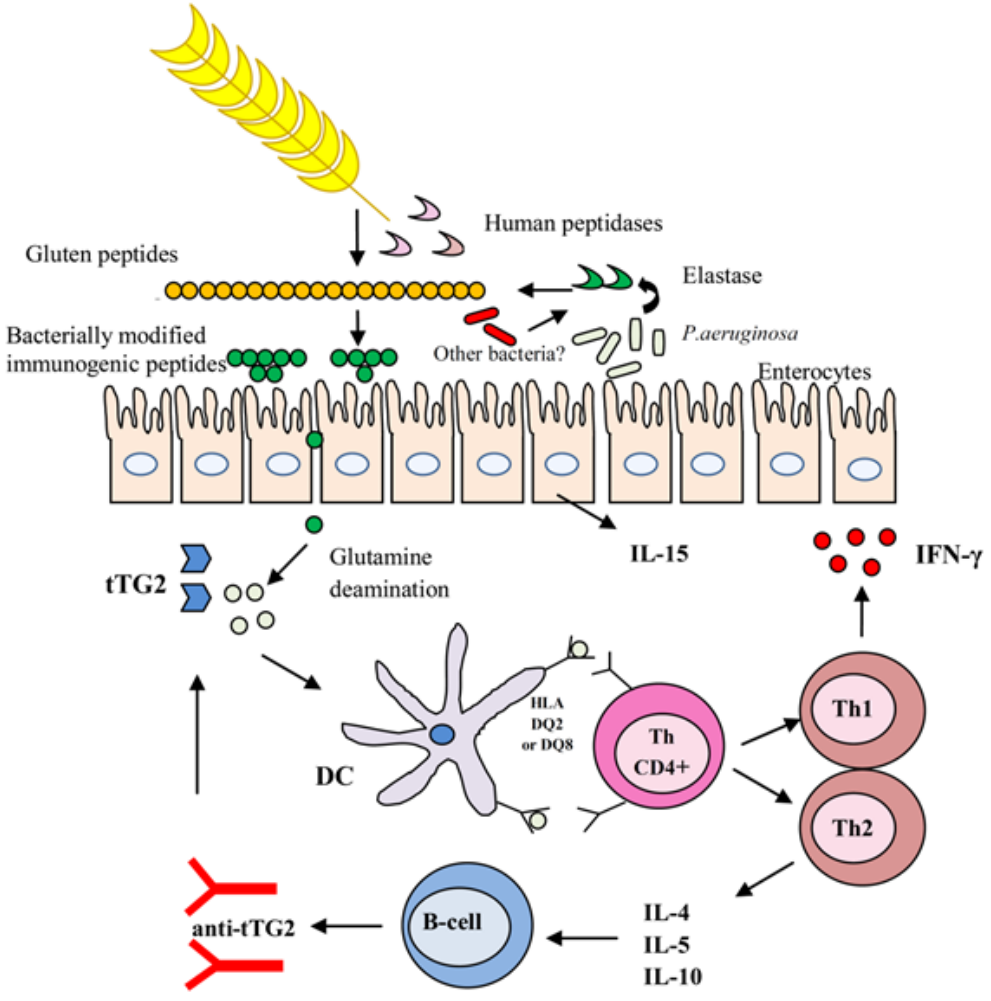

Figure 1. Model depicting how presence of some bacteria in the gut (for example *P. aeruginosa*) can modify gluten peptides which translocate the mucosal barrier more efficiently and in a genetically predisposed host, these bacterially modified immunogenic peptides can interact with antigen-presenting cells expressing HLA-DQ2 or DQ8.

Studies carried out by Caminero et al., seem to confirm this hypothesis. Researchers have shown that some microorganisms, such as *Pseudomonas aeruginosa*, occurring in excessive numbers in patients, interact with gluten to produce peptides which activate a strong immune response that occurs in the course of celiac disease. The study based on colonizing the digestive tract of germ-free C57BL/6 mice with bacteria isolated from the small intestine of patients with celiac disease (group 1) or bacteria from healthy people (group 2). Next, after gluten gavage into the stomach, the amount of gliadin and proteolytic activities were evaluated in intestinal contents. As it turned out, the bacteria isolated from each group of patients, produced distinct gluten-degradation patterns in the mouse small intestine. *Pseudomonas aeruginosa* from patients with celiac disease showed elastase activity and produced peptides which translocated the intestinal barrier and activated gluten-specific T-lymphocytes (Fig. 1). In contrast, *Lactobacillus* spp. from the duodenum of the control group degraded the immunogenic peptides produced by *P. aeruginosa*, reducing their antigenicity [70].

It is interesting to note that Van Beurben et al. Van Beurden et al observed symptom relief and restoration of duodenal villi after fecal transplantation, which could be attributed to a strong microbial compound with celiac disease symptoms [71].

Considering the fact that intestinal microbiota is involved in the digestion of gluten proteins with the formation of toxic or properly tolerated peptides, maintains the intestinal barrier tightness through the release of zonulin and the expression of proteins forming tight junctions, promotes the growth and maturation of the intestinal epithelium and regulates the activity of the immune system by expression of cytokines and pro- and anti-inflammatory proteins, the role of microorganisms in the pathogenesis of celiac disease should not be underestimated.

6. ROLE OF MICROBIOME IN PATHOGENESIS OF INFLAMMATORY BOWEL DISEASE

The pathogenesis of inflammatory bowel disease (IBD) involving two major disease entities such as Crohn's disease (CD) and Ulcerative Colitis (UC) is still unknown. It is only supposed that immunological, genetic and environmental factors decide about the morbidity. In recent years, more and more often, the role of microorganisms in the pathogenesis of IBD has been postulated [72, 73]. The role of microorganisms in formation of IBD is confirmed by many scientific experiments and clinical observations, among others: exacerbation of the disease due to food poisoning or previous stomach infections [74, 75], inability to induce intestinal inflammation in experimental animals cultured in germ-free conditions [76], alleviation of symptoms of the disease after antibiotic therapy, probiotics supplementation or bowel cleansing [76-78] as well as remission induction and alleviation of inflammatory lesions after intestinal microbiota transplantation from healthy people [79].

In recent years, as a result of insightful research on the gastrointestinal microbiome, it has been shown that people suffering from IBD are characterized by an imbalance in the composition, quantity and functioning of individual microorganisms.

Extensive research carried out by Frank et al., including 190 resected intestinal tissues samples, showed that the gastrointestinal microbiota composition of patients with IBD showed significantly reduced colonization by *Firmicutes* within the *Lachnospiraceae* family and

reduced *Bacteroidetes* levels in relation to healthy individuals. In addition, among patients, there was an increase in the number of *Proteobacteria* and *Actinonobacteria* [80].

Heidarian et al. demonstrated similar relationships in their studies. Researchers documented the decreased number of *Bacteroides* spp., *Faecalibacterium prausnitzii*, *Prevotella* spp. and *Methanobrevibacterium*. Moreover, the intestinal dysbiosis among patients with IBD, correlated with the degree of disease activity and higher level of secretion of proinflammatory interleukin 8 (IL-8), which excessive production is necessary in initiating and maintaining inflammation in IBD [81].

Other independent studies have documented that the total contribution of bacteria in inflamed intestinal tissue from patients with IBD was different compared to the control group and moreover, composition of microorganisms varied depending on the disease entity [82]. The tissue from patients with CD was dominated by *Streptococcus,* whose constituted 78.51% of all bacteria, where in the control group the proportion of these microorganisms was negligible and consistuted only 3%. On the other hand, patients with UC were primarily characterized by colonization of *Lactobacillus* constituted *approximately* 90% of the total bacteria, where the participation of these microorganisms in gut from healthy people was less than 8%. It is worth mentioning that in the control group, the dominant genus was *Bifidobacterium,* whose percentage distribution was 78.95%, while their level in patients was below 10% [82].

The situation was similar in case of gut colonization of IBD patients by fungi. Intestinal tissues collected from inflammatory lesions were characterized by significantly higher diversity and richness of gastrointestinal mycobiome than tissues resected from gut without inflammation. A huge disproportion was noted among the species of *Candida* spp., *Malassezia restricta*, *Saccharomyces castelli*, *Cryptococcus neoformans*, *Gibberella moniliformiss* or *Alternaria brassicicola* [83]. It is worth adding that the species diversity and richness of the mucosal mycobiome were associated with the expression of IFN-γ, TNF-α or IL-10. Expression of IFN-γ and TNF-α was significantly increased in the inflammatory lesions, whereas IL-10 showed opposite trend. As well as the diversity of the fecal fungal microbiota positively correlated with serum C-reactive protein (CRP), Crohn's Disease activity index (CDAI) [83]. On the other hand, analyzes of stool samples (describing *gut microbiome*), carried out by our research team, showed that the gut of children with CD was characterized by significantly higer numer of fungi of the genus *Candida* compared to the control group [84, 85]. Moreover, the number of fungal cell was reduced after treatment and it was correlated with a decrease in CDAI (Fig. 2). Interesting are the results of study conducted by Hoarau et al., who showed that increased colonization of the intestine by *Candida tropicalis* positively correlated with degree of inflammation and increased level of antibodies against *Saccharomyces cerevisiae* (ASCA) which are a biomarker of Crohn's disease. In addition, the researchers showed that the presence of these fungi was associated with an excessive number of *Escherichia coli* and *Serratia marcescens*. Further immunological studies showed that these microorganisms interacted together to forming a strongly adhering to the intestinal mucosa biofilm, inducing an overactive inflammatory response which can result in CD symptoms [86]. Besides the commonly found dysbiosis among IBD patients, there are also results suggesting that the pathogenesis of these diseases entity may be caused by individual microorganism which are often isolated from patients and which could participate in the initiation of inflammation of the gastrointestinal tract. Until now participation of *Mycobacterium paratuberculosis* (MAP), *Pseudomonas, Chlamydia, Yersinia pseudotuberculosis*, *Listeria monocytogenes*, *Bacteroides frag*ilis, *Escherichia coli,* or *Bacteroides vulgatus* have beed considered [87-89].

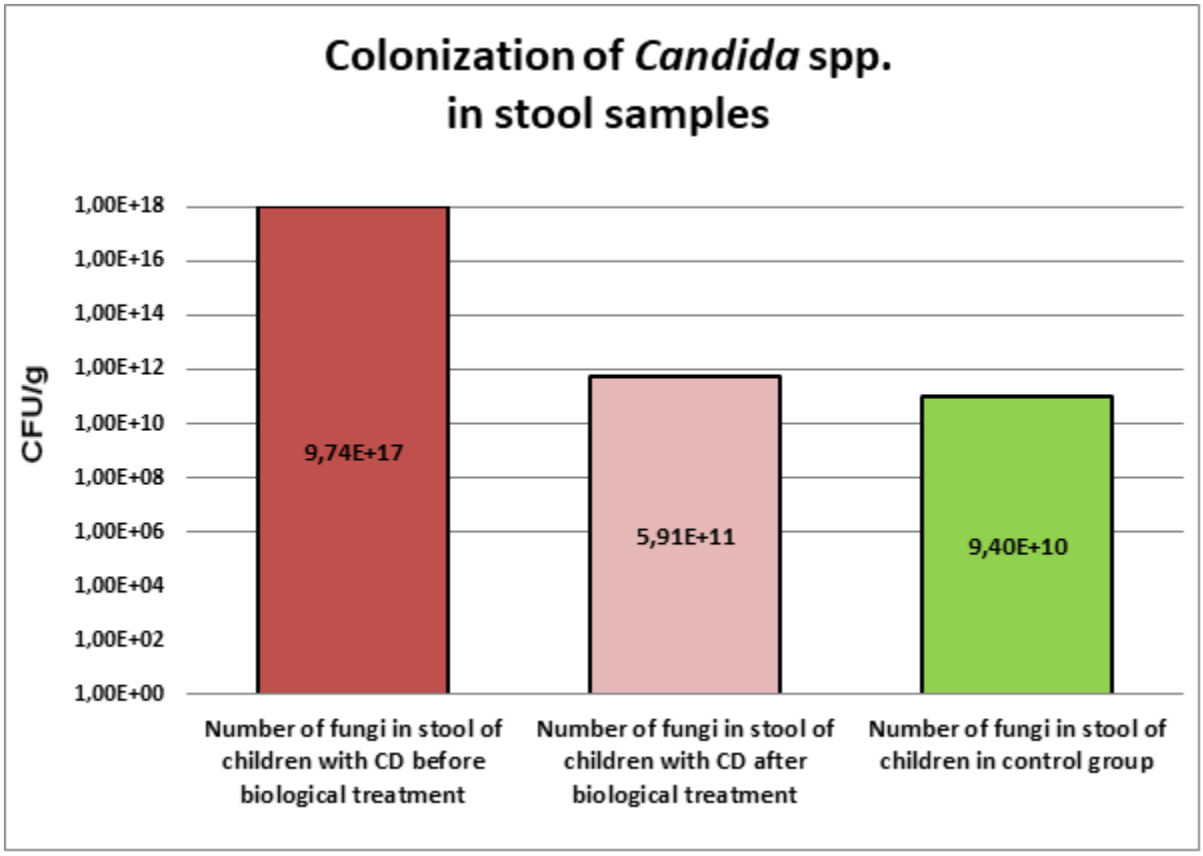

Figure 2. Colonization of fungi of the genus *Candida* in the feces of patients with CD before treatment and after TNF-α therapy compared to control group [84].

In particular, the contribution of MAP was one of the most common research objects [90-92]. This bacterium is significantly more often found among people with IBD compared to healthy population [91-93]. Furthemore, considering the fact that MAP has a specific cell wall composition and structure containing *muramyl dipeptides* or mycolic acids, makes this bacterium able to promote the induction of an overreactive immune response which may initiate the development of inflammatory lesions characteristic of IBD [94]. In addition, mycobacteria are able to invade host cells through: growth inside the cells of the infected organism, the ability to form granuloma and *inhibition* of *phagosome-lysosome fusion* [95]. Another aspect that connects MAP with autoimmune diseases is the phenomenon defined as molecular mimicry. It is a mechanism, where a bacterial antigen shares structural, functional and serological similarities with self-antigens. As a consequence, immune system recognizes self peptides as a foreign and it can lead to overreactive immune response against own tissues and promote inflammatory lesions [96, 97]. After infection with the mycobacterium, an immune response is triggered against the MAP proteins which initiated it, but also against self-proteins resembling the attacked pathogen molecule. As a result, after elimination of the bacteria, the immune system still induces an inflammatory response against own tissues because it does not distinguish autoanitigens from previously eliminated bacterial antigens. Considering the fact, that MAP possesses proteins belong to family of heat shock proteins (HSP), showing homology

to heat shock proteins found in humans, it is suggested that the mechanism of molecular mimicry may be important proccess in initiating some autoimmune diseases, including IBD [97, 98].

Another bacterium whose participation in the pathogenesis of IBD was often considered is *Escherichia coli* - in particular the adherent-invasive pathotype (AIEC) [99-101]. This strain is characterized by the ability to strongly adhere to intestinal epithelial cells as well as to invade cells. Moreover, AIEC is ability to survive and to replicate extensively in large vacuoles within macrophages without triggering host cell death, and ability to induce the release of large amounts of TNF-α and interleukin 23 (IL-23) by infected macrophages [99]. Analysis of intestinal biopsies showed that this strain was significantly more common among patients with IBD compared to healthy individuals [99, 101]. The additional significance of *E.coli* in the pathogenesis of IBD is also fact that the level of antibodies directed against *E. coli* flagellin was significantly elevated among patients with CD [102].

7. THE ROLE OF INTESTINAL MICROBIOTA IN CONNECTIVE TISSUE DISEASES

Connective tissue diseases (CTD) are a *chronic, inflammatory*, autoimmune disorder affecting the connective tissue. The most common CTD include: rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, Behçet's disease, Sjögren syndrome or mixed connective tissue disease. The pathogenesis of these diseases remains unknown, but more and more data suggest that CTD is a result of disorders between immunological, environmental, genetic and microbiological factors. While intestinal dysbiosis among patients are commonly observed, it is not clear whether changes within the microbiota are a trigger in the development of these diseases enity, or only a secondary effect of a long-term disease process [103].

Disorders in the composition of the intestinal microbiome recently have been a frequent topic of interest in research on systemic lupus (SLE). Hevia et al. showed that patients with SLE were characterized by a reduced number of *Bacteroidetes* and *Firmicutes* compared to healthy people [104]. These dependencies were confirmed by He et al. who additionally documented increased colonization of the intestine by *Rhodococcus*, *Eggerthella*, *Klebsiella*, *Prevotella*, *Eubacterium* and *Flavonifractor* among SLE patients. In turn, number of *Dialiste*r and *Pseudobutyrivibrio* was significantly reduced in relation to healthy control group [105]. Moreover, Azzouz et al. showed that patients with SLE had an overall 5-fold greater representation of *Ruminococcus gnavus* (RG) of the *Lachnospiraceae* family, compared to healthy people. What's more, the number of these bacteria positively correlated with exacerbation of the disease and the presence of antibodies against these bacteria in the blood. In particular, the highest levels of serum anti-RG antibodies were detected in patients with active nephritis [106].

Other independent studies have shown that microorganisms isolated from stool samples of SLE patients promoted lymphocyte activation and T helper 17 cells (Th17) differentiation from naive $CD4^+$ lymphocytes to a greater extent compared to the microbiota isolated from stool samples of control group. Interestingly, the enrichment of microbiota obtained from faeces samples of patients with *Blautia coccoides*, *Ruminococcus obeum* and *Bifidobacterium bifidum* significantly reduced the Th17/Th1 balance and prevented $CD4^+$ lymphocyte overactivation. Further analysis of fecal microbiota showed a negative correlation between the concentration

of proinflammatory cytokine IL-17 and the number of *Firmicutes* in healthy people whereas among patients with SLE, these bacteria correlated directly with the serum levels of IFN-γ and Th1 lymphocyte. In addition, a negative correlation was showed between the frequency of *Synergistetes* and the anti-double stranded DNA (anti-dsDNA) titers and IL-6 level. In contrast, a positive correlation was observed between the numbers of *Synergistetes* and IgM antibodies against phosphorylcholine [107]. The above results show how intestinal microbiota can induce changes through the immune system. Moreover, they suggest that probiotic therapy using strains inducing T-regulatory cell (Treg) cells may prove effective in restoring balance between Treg/Th17/ Th1, whose disorder is found among patients with SLE and plays a key role in the induction of inflammation of tissues [107].

There are only few studies on the analysis of the composition of microbiota in systemic scleroderma (SSc), however, taking into account the fact that one of the most common internal organs in the course of SSc is the digestive system whose dysfunction is manifested by constipation, abdominal pain, motor disorders and mucosal changes, it is obvious that such conditions favor the formation of intestinal dysbiosis.

Studies conducted by Volkmann et al. seem to confirm the above suppositions. Thus, the analysis of the composition of the gastrointestinal microbiota of people with SSc showed that patients were characterized by reduced numbers of commensal bacteria, such as *Faecalibacterium* and *Clostridium*, while increased colonization of opportunistic pathogens, such as *Fusobacterium* and *γ-Proteobacteria*, was observed compared to healthy controls. Interestingly, *Bifidobacterium* and *Lactobacillus*, which amount is usually reduced in patients with autoimmune, inflammatory diseases, were significantly higher in SSc patients in relation to healthy group. It is also worth noting that the severity of gastrointestinal symptoms correlated with an increase number of *Fusobacterium* and the reduction of *Bacteroides fragilis* [108]. Other studies suggest that bacterial overgrowth may promote malabsorption and an impairment of intestinal motility in patients with SSc. Parodi et al. showed that small intestinal bacterial overgrowth (SIBO) in *lactulose breath test* (*LBT*) occured more frequently in SSc patients in relation to control group [109]. These dependencies were also confirmed by Marie et al. in *hydrogen* and *methane breath test. M*oreover, the researchers showed that people with a positive glucose hydrogen and methane (H_2/CH_4) breath test, were characterized by more serious symptoms and complications in the digestive tract, compared to people with negative test [110].

So far, it has been suggested that changes in intestinal microbiota among SSc patients are a secondary result of a long-term disease process, however the appearing results about the beneficial effects of antibiotic therapy on symptom alleviation of systemic sclerosis classify dysbiosis of the microbiome along with genetic and immunological predisposition as a potential factor which may contribute to the pathogenesis of SSc, in particular, to the development of intestinal symptoms in SSc patients [109, 110].

Recently, the interest around the intestinal microbiome is more and more common in the case of Behçet's disease (BD). One of the latest reports showed that Behçet's patients were characterized by a decrease number of *Roseburia* and *Subdoligranulum*, which was correlated with significant decrease of butyrate production. Considering the fact, that butyric acids is able to induct differentiation of Treg lymphocytes, their deficiency may contribute to imbalances between particular types of lymphocytes and lead to disorders of the immune system, which is important in the induction of autoimmune diseases [111]. Other independent studies in patients with BD also confirmed intestinal dysbiosis among patients. A significant increase in the

number of *Bifidobacterium* was documented, while the *Megamonas* and *Prevotella* species occurred in significantly reduced amounts compared to healthy individuals [112].

8. CONCLUSIONS

The pathogenesis of autoimmune disease remains unknown, which makes it impossible to effectively treat and prevent it. As far as it is known that the development of these diseases is a results of genetic, immunological and environmental disorders, recently the participation of intestinal microbiota in the etiology of these diseases is increasingly considered. The possibility of using molecular biology techniques increases the prospect to take a closer look at the composition of microorganisms colonizing the digestive tract which creates new cognitive abilities of interactions between the microbiome and the host organism.

Current results provide information about disorders in the composition, quantity and functioning of intestinal microorganisms among patients with autoimmune or metabolic diseases, however, further research is needed to clarify whether the observed dysbiosis is only a secondary result of the ongoing chronic process or maybe it has share in pathogenesis.

Nevertheless, the incoming results regarding the potential impact of microorganisms on the course of the diseases described above, give hope for the implementation of preventive measures in the future in order to modify the composition of gastrointestinal microbiota using antibiotics, probiotics, bacteriophage therapy or faecal transplantation, which could be a new therapeutic target in relieving symptoms these diseases or even prevent their development.

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