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Microbiota – a key to healing the gastrointestinal tract?

Rola mikrobioty w zachowaniu zdrowego jelita

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ABSTRACT
At present, it is already known that many gastrointestinal tract diseases are caused by disorders of the intestinal ecosystem. The contribution of microbiota disorders to the development and sustaining of inflammatory bowel diseases, irritable bowel syndrome, necrotizing enterocolitis, constipation or bloating has been demonstrated in numerous scientific studies. Another matter is bacterial imbalance in food allergy and atopic diseases. The effectiveness of probiotics and prebiotics in these diseases has been repeatedly confirmed, which made researchers search for new diseases in which probiotics could be an efficient therapeutic solution. Reports have also been published on microbiota disturbances in children with a spectrum of autistic disorders, depression or metabolic syndrome, and in obese patients. New micro-organisms have been discovered, which in addition to *Lactobacillus* or *Bifidobacterium* bacteria, may significantly influence the improvement of human health. Especially promising are two bacterial species, *Akkermansia muciniphila* and *Faecalibacterium prausnitzii*, which seem to interact in continuity regeneration of the intestinal epithelium. *A. muciniphila* has the ability to degrade mucin, while producing acetic acid, propionic acid and oligosaccharides. These products become the substrate for *F. prausnitzii*, one of the main producers of butyrate in the intestine. The anaerobic butyrate-producing bacterium helps to inhibit inflammation in the gastrointestinal tract, while preventing increased intestinal permeability. Growth stimulation in bacteria (e.g. towards prebiotic therapy) may therefore prove to be a valuable nutritional option and an efficient way to restore the intestinal epithelium. While *A. muciniphila* is extensively studied in the context of obesity and diabetes, *F. prausnitzii* may become an interesting marker for Crohn's disease activity.
The aim of this paper is to present new and promising aspects of intestinal microbiota restoration in the treatment of selected diseases.
Keywords: *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, probiotics, prebiotics.

STRESZCZENIE
Obecnie wiadomo już, iż przyczyną licznych chorób przewodu pokarmowego jest zaburzenie ekosystemu jelitowego. Udział zaburzeń mikrobioty w rozwoju i podtrzymaniu nieswoistych chorób zapalnych jelit, zespołu nadwrażliwego jelita, martwiczego zapalenia jelit, zaparć czy wzdęć został wykazany w licznych badaniach naukowych. Bezsprzeczne są także zaburzenia równowagi bakteryjnej w alergiach pokarmowych i chorobach atopowych. Skuteczność probiotyków i prebiotyków w wymienionych jednostkach chorobowych została wielokrotnie potwierdzona, co skłoniło badaczy do poszukiwania nowych jednostek chorobowych, w których probiotyki mogłyby być skuteczną opcja terapeutyczną. Pojawiły się doniesienia o zaburzeniach mikrobioty u dzieci z zaburzeniami ze spektrum autyzmu, w depresji czy też u pacjentów otyłych i z zespołem metabolicznym. Odkryto także nowe drobnoustroje, które – obok bakterii z rodzaju *Lactobacillus* czy *Bifidobacterium* – mogą istotnie wpływać na poprawę ludzkiego zdrowia. Szczególnie obiecujące są bakterie z gatunku *Akkermansia muciniphila* oraz *Faecalibacterium prausnitzii*, które wydają się współdziałać w odtwarzaniu ciągłości nabłonka jelitowego. *A. muciniphila* wykazuje zdolność do rozkładu mucyny, produkując jednoczasowo kwas octowy, kwas propionowy oraz oligosacharydy. Produkty te stają się substratem dla *F. prausnitzii,* jednego z głównych producentów maślanu w jelicie. Wytwarzający maślan beztlenowiec przyczynia się do hamowania stanu zapalnego w przewodzie pokarmowym, zapobiegając jednocześnie zwiększonej przepuszczalności jelita. Stymulacja wzrostu obu bakterii (np. na drodze prebiotykoterapii) może więc okazać się wartościową opcją odżywiania i odbudowy nabłonka jelitowego. Podczas gdy *A. muciniphila* badana jest intensywnie w aspekcie otyłości i cukrzycy, *F. prausnitzii* może stać się interesującym markerem aktywności choroby Crohna.
Celem niniejszej pracy było przedstawienie nowych, obiecujących aspektów odbudowy mikrobioty jelitowej w leczeniu wybranych chorób.
Słowa kluczowe: *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, probiotyki, prebiotyki.

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Hundreds of papers have been written about the effectiveness of probiotic bacteria in the prevention and treatment of numerous diseases. The popularity of probiotics has increased in the last few years. Interest in the idea of healthy bacteria began, in fact, in ancient times. In 96 AD Gaius Plinius Secundus, also known as Pliny the Elder, prescribed to children and convalescents fermented milk drinks. He also recommended it

to patients with symptoms of gastrointestinal tract disorders. Sour milk was consumed for health reasons by the ancient Greeks and Egyptians [1, 2].

The ability of some bacteria to displace pathogenic microbes was also mentioned by Pasteur and Jaubert while examining bacterial antagonism. At the end of the 19th century attempts were made to treat patients with healthy bacteria. A perfect example of healthy bacteria properties is the successfully completed attempt to treat the gastrointestinal disorder caused by the bacterium *Proteus vulgaris* in infants using lactic acid bacteria present in whey by the Polish paediatrician and neurologist Professor Joseph Brudziński [3].

Commonly, however, the father of the idea of probiotic mechanisms is considered to be the Russian immunologist Ilya Ilyich Mechnikov, a supporter of strengthening the natural forces of the body in maintaining good health. He conducted a long-term observation of Bulgarian and Russian peasants who enjoyed excellent health and longevity. According to his observations, these features were linked with the frequent consumption of traditional thick yoghurt, rich in lactic acid bacteria (*Lactobacillus bulgaricus*). He presented the observed dependence in 1907, which resulted in a short-term fashion for diets based on milk in Europe [4, 5].

The growing popularity of probiotics was interrupted by the discovery of antibiotics, and the phase of antibiotic treatment lasted uninterruptedly for several decades. Growing bacterial multi-resistance and antibiotic side effects – largely associated with subsequent intestinal dysbiosis – led to the re-popularity of probiotics. In the 21st century they began to be widely used in prevention and treatment.

Research on animals and cell lines have demonstrated the most probable effectiveness mechanisms of probiotics in prevention and treatment. The most frequently used probiotic microorganisms – bacteria of the genus *Lactobacillus* and *Bifidobacterium* – are an important part of health-related gastrointestinal microbiota. Adequate numbers in the intestine are a guarantee of the intestinal ecosystem's balance and, indirectly, of the homeostasis of the whole organism. These bacteria are a source of digestive enzymes. They can synthesize vitamins and metabolites with a broad spectrum of activity: antimicrobial (e.g. bacteriocins), nourishing the intestinal epithelium (amines, organic acids, oligosaccharides), or lowering the pH (organic acids). The balance of the intestinal ecosystem inhibits the adhesion and proliferation of pathogens in the gastrointestinal tract. Health-promoting intestinal bacteria positively modulate the immune system by restoring the balance of Th1 : Th2 lymphocytes, inhibiting inflammation and improving intestinal peristalsis. Administered in the form of *Lactobacillus* and *Bifidobacterium* strains, probiotic preparations are intended to "mimic" health-promoting intestinal microbes, and their use is supposed to reverse intestinal dysbiosis [6, 7].

The effectiveness of probiotics in the prevention and treatment of a number of diseases has been used for years. For example, in the prevention or shortening the duration of antibiotic-associated diarrhoea and infectious diseases, maintaining remission in ulcerative colitis, the reduction of lactose intolerance, irritable bowel syndrome or the prevention of atopic eczema. Probiotics administered orally and vaginally support the treatment of recurrent vaginal infections, and aid *Helicobacter pylori* eradication therapy [8].

Therapeutic effects, previously achieved with probiotics, encouraged researchers to identify further bacterial strains beneficial to forming the host intestinal microbiota. In terms of research involving the bacterial factor in the pathogenesis of inflammatory bowel disease (IBD), the growing interest of the scientific community is on a microorganism of recognized immunomodulating properties – *Faecalibacterium prausnitzii*. The described anaerobic bacterium, belonging to the *Clostridium leptum* group, makes up about 5–10% of microbiota. This bacterium belongs to the leading producers of butyric acid, while demonstrating the ability to degrade cellulose. The major metabolite of *F. prausnitzii* – butyric acid, is one of the key elements to maintain homeostasis of the gastrointestinal tract. Butyrate, which is exclusively a product of bacterial metabolism, is a major energy substrate for the epithelial cells of the intestine, especially the colon, causing the regular regeneration of the intestinal mucus layer. Deficiency of the described metabolite is therefore analogous with the dysfunction of the intestinal barrier, which may result in the loss of selective intestinal permeability [9].

The link between the presence and abundance of *F. prausnitzii* and the condition of the intestine is close, which is reflected in numerous diseases. A correlation between an exacerbation in inflammation and disease activity in patients with IBD (Crohn's disease in particular) with a dramatic reduction in butyrate-producing bacteria has been shown [10, 11, 12]. The current knowledge indicates that butyrates can modulate the immune system of the gastrointestinal tract and inflammation in IBD, probably by inhibiting the secretion of inflammatory cytokines [13]. Studies in cell lines show that the *F. prausnitzii* A2-165 strain stimulates the release of significant amounts of interleukin-10 and interleukin-12 from monocytes of peripheral blood by inhibiting the secretion of interleukin-8 and decreasing tumour necrosis factor alpha activity. Both the strain of *F. prausnitzii* and butyrate-containing bacterial supernatant reduced the severity of inflammation in TNBS-induced colitis in mice [12]. In another study, Martin et al. reached similar conclusions – both the supply of living *F. prausnitzii* and bacterial supernatant in animals with induced mild and severe colitis exerted a protective effect [14]. Due to its unique properties, the described bacterium is now considered as one of the main microorganisms, potentially for use in the treatment of IBD in the future. Since the current state of knowledge does not allow the use of the described bacteria as a probiotic strain, it seems that the optimal solution to stimulate its growth is the implementation of prebiotic therapy.

In the intestine homeostasis with *F. prausnitzii* interacts with another bacterium with health beneficial properties – *Akkermansia muciniphila*. The functioning of both microorganisms is mutually dependent, and their corresponding numbers seem to be a very important matter for the health of the digestive tract [15]. There is also a possibility that *A. muciniphila* is a kind

of "provider" for *Faecalibacterium*. This anaerobic bacterium existing in mucus produces, during the decomposition of the mucin, short chain fatty acids such as acetic acid, propionic acid, and oligosaccharides. These compounds represent growth substrates for *F. prausnitzii*. In healthy subjects *A. muciniphila* represents about 5% of the intestinal microbiota. With the appropriate number of *A. muciniphila* and its metabolites, the *F. prausnitzii* is in equilibrium, which enables the production of sufficient amounts of butyric acid.

Akkermansia muciniphila has recently been intensively studied, especially in the context of obesity and type 2 diabetes. It has been shown that obesity and diabetes in mice, induced both by a high fat diet and genetically determined, is associated with a reduced number of the described microorganisms [16, 17].

The number of *A. muciniphila* proved to correlate negatively with body mass, fat mass, metabolic endotoxemia and inflammatory markers. Supply of the described bacteria to obese animals, with a diet rich in fats, led to the recovery of the intestinal barrier and increased thickness of the intestine mucous layer. The last information was in fact surprising, because this bacterium degrades mucin, producing short chain fatty acids. One particularly important finding was the fact that the supply of *A. muciniphila* led to the reduction of obesity in animals, which was additionally accompanied by a reduction of inflammation severity [18, 19].

Changes were also observed within the endocannabinoid system, involved in the regulation of energy balance – the level of appetite, lipid and carbohydrate metabolism or the size of lipid accumulation. The described system is based on lipid signalling and consists of endogenous bioactive lipids, with an opioid-like structure. These lipids bind and activate the G-protein-coupled receptors in the brain, affecting a number of different mechanisms. Besides the central nervous system, the endocannabinoid system affects the functioning of the immune and vegetative systems, the gastrointestinal tract and the microcirculation. Overactivity of the described system is observed in obesity.

It must be emphasized that the normalization of the endocannabinoid system was a result of the supply of *A. muciniphila* strains, because energy intake remained unchanged. The exact mechanism of *A. muciniphila*'s influence on the processes described is not yet specifically understood. It is worth mentioning that only live bacterial cultures exert the described effect. Another important finding is the fact that this microorganism directly impacts and controls the gene expression of RegIIIg in the colon [18].

It was also reported that the described bacterium showed a protective effect, inhibiting the progression of induced IBD in mice [19]. These observations indicate a synergistic activity of *F. prausnitzii* and *A. muciniphila* in the reduction of inflammation severity in the intestine. In contradiction to this observation, however, stand the results of Ganesh et al., indicating that this bacterium increases inflammation in colitis caused by infection with *Salmonella typhimurium*. The authors attribute this mucolytic effect to *A. muciniphila* properties [20]. The actual role of this microorganism in the modulation of inflammation in the gastrointestinal tract therefore needs to be determined. A much larger number of studies support the theory, where the bacterium inhibits inflammation [18, 19]. At the moment it is postulated that the easiest way to increase the number of *Akkermansia muciniphila* is dysbiosis elimination by prebiotic supply. Prebiotic therapy, which aims at increasing the number of *Akkermansia* and regulation of the endocannabinoid system, is even more intensely studied in the context of obesity treatment. Adjustment of the endocannabinoid system has a positive effect on intestinal permeability and leads to a reduction in endotoxin levels in the serum and regulation of adipogenesis [21].

Faecalibacterium prausnitzii and *Akkermansia muciniphila* play an extremely important, comprehensive role for intestinal homeostasis and the regulation of metabolic processes, which is the reason for the growing interest of the scientific community. Both characterized bacteria seem to play a key role in the intestines by nourishing and providing selective permeability of the intestinal barrier. Increasing the permeability of the intestinal barrier leads to the development of metabolic endotoxemia and even liver failure, due to excessive exposure to toxins. This condition may result in numerous diseases caused by toxins in the systemic circulation. Rebuilding microbiota by stimulating the growth of both microorganisms seems to be a promising prophylactic and therapeutic option in modern medicine. Therefore, these microorganisms were defined as the most promising microbiota elements of recent years and potential therapeutic targets at the 7th International Symposium "The Intestinal Microbiota and Probiotics: Exploiting Their Influence on Health" in London in 2013 [22].

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