Intestinal microbiota transplant – current state of knowledge

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Abstract

Faecal microbiota transplantation (FMT) has induced a lot scientific interest and hopes for the last couple of years. FMT has been approved as a treatment of recurrent *Clostridium difficile* colitis. Highly sophisticated molecular DNA identification methods have been used to assess the healthy human microbiome as well as its disturbances in several diseases. The metabolic and immunologic functions of the microbiome have become more clear and understandable. A lot of pathological changes, such as production of short-chain fatty acids or components of the inflammatory cascade, caused by changes in microbiome diversity, variability and richness have been observed among patients suffering from inflammatory bowel diseases, irritable bowel syndrome, type 2 diabetes or rheumatoid arthritis. The published clinical results are encouraging, but still there is huge demand for FMT controlled clinical trials.

Key words: faecal microbiota transplantation, microbiome, inflammatory bowel disease.

In recent years, due to the numerous publications on the research of the human intestinal microbiome, the possibility of permanent modification of the microbiome through faecal microbiota transplantation (FMT) from healthy to ill individuals has become a subject of increased attention. It has been mainly the result of numerous publications confirming good results of applying FMT in the treatment of *Clostridium difficile* infections [1]. In 2014 use of FMT in *Clostridium difficile* infections coexisting with inflammatory bowel disease was included in the treatment standards of the European Crohn's and Colitis Organization [2].

The natural human intestinal microbiome constitutes a diverse biological environment, being shaped and stabilized from the moment of birth. The microbiological environment of the intestines is very complex and develops with age. Immediately after birth, this environment is very changeable and susceptible to contact with environmental stimuli and food. Approximately at the age of 3 years, this environment stabilizes, becoming similar to that of the adult human [3]. In elderly adults the microbiological intestinal environment once again, just like after birth, becomes susceptible to the influence of external factors and pathogens [4] – primarily due to its limited biodiversity. The reduction in diversity is considered a major cause of *Clostridium difficile* infections affecting elderly individuals. The microbiome diversity and variety are influenced by a number of factors, such as diet, domicile or the way of life and feeding habits associated with urban or rural lifestyle [5]. Yatsushenko et al. [3] demonstrated that the intestinal microbiome of US inhabitants is far less diverse than that of individuals living in other, less developed countries. Multiple factors can be considered as a cause of such a situation, including the growing number of caesarean sections in the American population, depriving newborns of natural contact with the bacterial environment of the vagina [6] and the common decision not to breastfeed, leading

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to the replacement of mother's milk with sterile food based on cow's milk [7]. The Western diet, rich in highly processed, ready-made, sterile dishes with preservatives added [8], use of water treated with oxygen- and chlorine-derived disinfectants and the common antibiotic use without absolute indications for such therapy [9] all play a significant role as well.

The intestinal microbiome consists of numerous bacteria, viruses and fungi living in the intestinal contents (faecal mass), as well as in the mucus covering the intestinal mucosa. It has been considered that these two habitats constitute two separate microbiological commensal ecosystems, having different functions in the interference between them and the organism of the host. Bacteria associated with the intestinal contents play mostly a metabolic role, utilizing large quantities of substrates contained in the intestinal contents. The bacteria of the mucous layer mainly take part in the regulation of the immunological processes in the mucosa and in the metabolic and immunological communication with the host [10, 11]. Bacterial obligate anaerobes dominate the biological environment of the intestines. It is presumed that they are responsible to a large extent for restraining the colonization of the intestine by other bacteria [12]. Currently, over 1000 genera of intestinal bacteria have been identified [13].

It should be noted that the exploration of this ecosystem, which coexists with the human organism, became possible only after the introduction of molecular DNA research techniques. The most common method of DNA testing used in human microbiome research is 16S ribosomal RNA (rRNA) sequencing [14]. This unit of RNA includes nine highly differentiated regions (V1-V9), which enables the bacterial species to be distinguished [15, 16]. Classical microbiological methods used to identify bacteria or fungi strains, such as microbiological culture, are ineffective in the case of the human microbiome, as the majority of bacterial strains in the intestine cannot be cultured in laboratory conditions. It has been accepted that the total number of genes of all microorganisms of the human intestinal microbiome exceeds 150-fold the number of genes in the human genome. The number of bacterial cells existing in the human digestive tract of the healthy individual reaches 100 trillion, 10 times more than the number of cells of the human body [17, 18].

Although bacteria account for 99.9% of the digestive tract microbiome, also two other groups of microorganisms, viruses and fungi, perform important functions. Particular attention should be paid to fungi, which are usually considered as pathogens, although they physiologically colonize skin, the oral cavity as well as other parts of the digestive tract [19, 20]. The role of fungi in the human microbiome has not been fully recognized, as the first publication on this subject, based on molecular identification methods, appeared in 2006 [21]. Although the *Candida* genus is most common in the digestive tract [20], the intestinal microbiome, under physiological conditions, can contain more than 50 genera of fungi, most common among them being *Candida*, *Saccharomyces* and *Cladosporium* [22, 23]. Contrary to the bacterial component of the human intestinal microbiome, the presence of fungi in the microbiome is not permanent and stable [24], and has a rather transient character. However, the results of experimental research suggest that the presence of the fungi plays a large role in the regulation of the human immune system. An example of such influence is the presence of antibodies against *Saccharomyces cerevisiae* (ASCA) in the majority of sera of Crohn's disease patients [25].

FMT is not limited to the transplantation of the microbiome only. It also includes microparticles, i.e. fragments of chitin (β-1,4-N-acetylglucosamine polymer) – a substance produced naturally by fungi and insects. Chitin microparticles, 1–10 microns in diameter, have been demonstrated to display a strong immunomodulatory influence in experimental models of bowel inflammation [26] and *in vitro*, affecting human monocytes, which take part in the inflammatory process [27].

Clinical use of FMT can be widespread, although presently the only commonly accepted indication for the use of FMT is *Clostridium difficile* infection.

Inflammatory bowel disease caused by *Clostridium difficile*

In their systematic review, Camaromota et al. [1] analyzed the results from 20 publications, including one randomized study, along with 15 case reports. Almost all patients included in the review were qualified for the study, due to the recurrence of the *Clostridium* infection after previous, unsuccessful therapy with standard drugs: metronidazole and vancomycin. In the group of 536 patients to whom FMT was applied, 467 were cured (87%). It was established that the result of the therapy depends on the way of administration of the transplant: to the stomach (81%), to the duodenum (86%), to the right part of the colon during the colonoscopy (93%), or to the left part of the colon via deep enema.

Inflammatory bowel disease

The group of inflammatory bowel diseases consists primarily of Leśniowski-Crohn's disease and colitis ulcerosa. In patients affected by these conditions, serious defects of the composition and variability of the intestinal microbiome have been observed, mainly in the form of the decreased participation of *Firmicutes* and *Bacte-*

roides species in the microbiome [28]. It is believed that the defects of the microbiome composition lead to the decreased production of short-chain fatty acids [29] – butyrates produced by *Faecalibacterium prausnitzii* in particular [30], which indirectly and directly (through the inhibition of interleukin 8) inhibit inflammatory processes in the intestinal mucosa. The lack of presence of *Faecalibacterium prausnitzii* was confirmed in Crohn's disease patients.

Currently, clinical data are insufficient for FMT to be recognized as effective in the treatment of inflammatory bowel disease. In a systematic review published in 2015, based on 45 publications [31, 32], the effects of the therapy in 112 patients with inflammatory bowel disease (including 6 patients with Crohn's disease) were evaluated. In inflammatory bowel disease, remission was achieved – depending on the institution performing the research – in 0 to 68% of cases. Such results are not encouraging. Still, it has to be considered that effectiveness of the FMT treatment of inflammatory bowel disease is dependent on the diversity of the donor's microbiome. This may account for the ambivalence of the results of the study.

Irritable bowel syndrome

Irritable bowel syndrome is a non-inflammatory disease of the intestines, of which abdominal pain and alteration of bowel habits – in the form of both diarrhoea and constipation – are primary symptoms.

Numerous defects in the composition of the intestinal microbiome have been observed in patients affected with this syndrome, as compared to the healthy population [33]. These defects include the presence of *Lactobacillus* [34] in the faeces of irritable bowel syndrome patients. Some conclude that, due to defects of the intestinal microbiome composition, the production of butyrates decreases, similarly to the situation in the inflammatory intestinal diseases, and the production of hydrogen sulfide increases [35], the latter being capable of stimulating pain receptors in the mouse colon. Clinical data concerning the effectiveness of FMT in the treatment of irritable bowel syndrome are scarce. In the systematic review by Rossen et al. [31] results of the treatment of 13 patients were included. Of that group withdrawal of symptoms was achieved in 70% of cases. In the research based on a group of 45 patients [36], improvement in the form of normalization of bowel habits was achieved in 60% of patients, the observation time ranging from 9 to 19 months.

Rheumatoid arthritis

The modification of the microbiome is gaining in significance in rheumatoid arthritis (RA) as well. It has been established [37] that in RA patients periodontitis associated with the oral microbiome occurs more often. It has been assumed that *Porphyromonas gingivalis*, a bacterium commonly present in diseases of the periodontium, plays a vital role in early RA [38, 39]. Preliminary information on the coexistence of intestinal microbiome defects and of early RA was published in 2008 [40]. Numerous experiments have shown the significant influence of modification of the intestinal microbiome on the elements of the inflammatory cascade in arthritis [41]. It has been demonstrated [42] that in the intestinal microbiome of RA patients, there is a significantly higher percentage of *Lactobacillus* species, compared to the microbiome of healthy individuals.

Type 2 diabetes and obesity

In patients with type 2 diabetes a number of changes in the composition of the intestinal microbiome, as well as in the metabolic processes that intestinal bacteria take part in, were observed. The increased production, as well as absorption, of short-chain fatty acid produced by gut microbiota has been noted in obese in as compared to slimmer individuals [43]. Changes in the microbiome composition have been observed, consisting in the reduced proportion of *Bacteroides* species and increased proportion of *Firmicutes* in it; additionally, in obese persons, an increased proportion of *Bifidobacteria* has been reported [44]. A particular role has been attributed to specific strains of the *Lactobacillus* genus. Of this group, in slim individuals, *L. gasseri* and *L. plantarum* can be found, while the quantity of *Lactobacillus reuteri* shows an almost linear correlation with body mass index [45].

Summary

Recent achievements in the field of genetics allow the exploration of the microbial intestinal environment, which accompanies the human organism from birth. Abundant data suggest that manipulation of the human microbiome content may contribute to the improvement of the treatment results of certain conditions. However, the lack of controlled clinical trials does not allow for the introduction of FMT or its modifications in the treatment of diseases other than *Clostridium difficile* caused colitis.

The authors declare no conflict of interest.

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