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Review

Is *Blastocystis* spp. Friendly?: A Current View of the Intestinal Microbiota

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ABSTRACT

The intestinal microbiota has become the center of attention, not only in microbiology but also in all fields of medicine. There has been an intense activity in studies that investigate the composition and function of the intestinal microbiota. The imbalance in the diversity of bacteria that constitute microbiota has been defined as "dysbiosis" and associated with various diseases. *Blastocystis* spp. is a eukaryotic protist and the most prevalent protozoan of the human gastrointestinal system. The frequency of observed colonization of *Blastocystis* spp. in asymptomatic cases has made its association with diseases controversial. It was found in some studies that there is a positive correlation between *Blastocystis* and the bacterial diversity of the intestinal microbiota. This implies that the parasite may play a role in intestinal homeostasis. Human and animal studies on this subject play an important role in understanding this relationship.

Keywords: Bacterial diversity, *Blastocystis* spp., dysbiosis, intestinal microbiota

INTRODUCTION

The gastrointestinal tract is the host of "the inner microbial world" that includes thousands of different species of microorganisms. It is part of a system in which, separate from our standard knowledge, many important events related to human health occur. Fecal microbiota consists of 93% bacteria, 5.8% virus, 0.8% archaea, and 0.5% eukaryotes. Meta-taxonomic analyses reveal that humans have 63-84 bacterial phyla, and it is estimated that nearly 15 phyla are localized in the gastrointestinal tract (1). This group consists of approximately 1014 microorganisms/g stool and weighs up to 2 kg. Of the colonic microbiota, 90% is composed of two dominant phyla called *Firmicutes* and *Bacteroides*. These individually bear high variability at the species level. Although the intestinal microbiota contains a low number of phyla, high diversity is exhibited with respect to species. Despite the significant differences between individuals in the adult fecal microbiota, a stable state is achieved in the individual after a certain period (2). Changes in the microbial composition are referred to as dysbiosis. These are associated with inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), colorectal cancer, metabolic syndrome, rheumatic diseases, allergy and atopic diseases, heart diseases, and psychiatric disorders (3).

Diet during early childhood, continuing the same eating habits for a protracted period, antibiotic use, the genetic structure of an individual, and sanitation affect the variability of human fecal microbiota (4).

Blastocystis spp. is a unicellular, anaerobic, and eukaryotic microorganism that is present in the gastrointestinal tract of humans and many animal species. It is classified as being in the stramenopile phylum and is the only member of this phylum that is present in human intestines. Carrying *Blastocystis* spp. is very common globally, and its prevalence has been reported to be 22%-56% in European countries and 37%-100% in Asian and African countries. The genetic diversity of *Blastocystis* spp. is very high and includes 17 subtypes (STs). Among these subtypes, ST1-9 and ST12 are isolated from humans, whereas ST3 is the one most frequently detected. ST4 in particular, which is the second most frequently identified subtype in Europe, is rarely seen in South America, Africa, and Asia (5).

Blastocystis has been associated with diarrhea, abdominal pain, and vomiting, while its role in diseases has not yet been completely explained. Studies that investigate symptomatology with subtypes were not able to precisely define pathogenic and non-pathogenic subtypes. The detection of long-term colonization in asymptomatic cases highlights the fact that one has to consider whether this agent is a member of the intestinal environment or not (6-8). It is necessary to investigate the relation-

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ship between *Blastocystis* and intestinal microbiota to determine this. Intestinal microbiota studies gained speed, especially after 2010, with the development of next-generation sequencing techniques that have made it possible to conduct metagenomic analyses. However, the number of studies in this field that focus on *Blastocystis* is very limited (9-15).

In this review, studies that investigate the relationship between *Blastocystis* and intestinal microbiota have been reviewed and summarized, and opinions regarding whether *Blastocystis* can be a "biomarker" for healthy intestines or not have been evaluated.

Clinical and Research Consequences

The presence of high bacterial diversity in the intestinal microbiota is considered an indicator of health. The coexistence of *Blastocystis* spp. and high bacterial diversity has drawn attention to the possibility that this protist microorganism can be a biomarker of gastrointestinal system health (10).

In a meta-analysis that evaluated studies investigating the prevalence of *Blastocystis* in cases of IBS, the relative risk was reported to be 2.34 in cases with IBS that exhibit *Blastocystis* colonization. This is in comparison with cases without IBS (9). In the same study, the aim was to evaluate the relationship between the presence of *Blastocystis* and intestinal microbiota with respect to IBS pathophysiology. In this study, which employs the quantitative polymerase chain reaction method conducted in France, the prevalence of *Blastocystis* was found to be 23.2% in patients with IBS and 16.1% in controls, and the most frequently detected subtype was ST4. A significant decrease was found in *Bifidobacterium* spp. in constipated male patients with IBS infected with *Blastocystis*. There was a decrease in the amount of *Faecalibacterium prausnitzii*, which has anti-inflammatory properties, in male control patients who did not have gastrointestinal complaints who had *Blastocystis*. It was found that *Bacteroides* spp. was higher, whereas *Bifidobacterium* spp., *Desulfovibrio* spp., *Clostridium leptum*, and *F. prausnitzii* were lower in constipated patients with IBS who did not have *Blastocystis* than in the control group. *F. prausnitzii* and *Bifidobacterium* spp. are known as being protective bacteria. This is due to their anti-inflammatory, anti-carcinogenic, and immunostimulant effects. The amount of these bacteria was found to have decreased in *Blastocystis* carriers. This implies that this parasite might be associated with inflammatory events. An inverse correlation between *Blastocystis* colonization and *Bacteroides* spp. has been found. This is in addition to the other studies that will be discussed subsequently. Investigators asserted the hypothesis that *Blastocystis* and dysbiosis of the intestinal microbiota might be associated in the pathophysiology of constipation-predominant IBS (9).

In the first study, which investigates the relationship between *Blastocystis* and intestinal microbiota with metagenomic analysis, the prevalence of *Blastocystis* was found to be 20.3% in healthy individuals and 14.9% in patients with ulcerative colitis. Patients with Crohn's disease did not have *Blastocystis*. *Blastocystis* positivity was less frequently seen than *Ruminococcus* and *Prevotella* enterotypes in cases involving *Bacteroides*-predominant enterotype. This reveals a positive correlation of *Blastocystis* colonization with bacterial species diversity. The fact that patients with Crohn's disease had decreased bacterial diversity, in addition to no *Blastocystis* colonization, makes it possible to explain this hypothesis. Investigators have shown that *Blastocystis* colonization was less frequently seen in patients with dysbiosis, as in cases of Crohn's disease and ulcerative colitis. There is no information on whether *Blastocystis* selects a specific microbiota directly or indirectly. Interestingly, the same study revealed that among healthy individuals, the rate of *Blastocystis* positivity was found to be 30.9% in lean individuals and 11.1% in obese individuals (p=0.008). In this study, conducted in Denmark, lean individuals had high bacterial species diversity in their microbiota, whereas obese individuals had lower diversity. Another remarkable result was the positive correlation between bacterial species diversity in the intestinal microbiota and the relationship between *Blastocystis* and leanness (10).

Differences in the intestinal microbiota were observed between healthy controls and patients with IBS with diarrhea in a metagenomic study conducted in Australia, which compared fecal microbiota in patients with IBS with and without *Blastocystis*. It was also found that *Blastocystis* carriage had no effect on fecal microbiota (12). It is known that patients with IBS have a higher *Firmicutes*/*Bacteroides* ratio and lower fecal bacterial diversity (16). Moreover, it is thought that *Blastocystis* leads to IBS symptoms by affecting the intestinal microbiota (12).

Audebert et al. (13) investigated the intestinal microbiota of patients with and without *Blastocystis* colonization in their study conducted in France using metagenomic analysis. The study found that *Blastocystis* colonization showed a positive correlation with bacterial diversity, and that there was a higher incidence of *Clostridia* class and Ruminococcaceae and Prevotellaceae families in cases who had *Blastocystis* colonization than in those without *Blastocystis* colonization. There were more cases of Enterobacteriaceae family in patients without *Blastocystis* colonization. In addition, there was more abundance of *Faecalibacterium* and *Roseburia* genera that contain butyrate-producing bacteria in cases with *Blastocystis* colonization. Butyrate is an important metabolite for human colon health since it is the main energy source for colonic epithelial cells, possesses anti-inflammatory properties, and is able to regulate gene expression, apoptosis, and enterocyte differentiation. Data obtained from this study indicate that parasite colonization is associated with a healthy colon environment, rather than the association of intestinal dysbiosis that is linked to *Blastocystis* with infectious and inflammatory diseases of the bowel.

The first study that addressed the relationship between *Blastocystis* and intestinal microbiota in Turkey investigated the stool samples of patients with cirrhosis using metagenomic analysis (14). It was found that the prevalence of *Blastocystis* colonization (0%) is lower in cases with hepatic encephalopathy (HEP) than in cases who did not develop HEP (38.1%) (p=0.006). It is known that intestinal dysbiosis plays a role in the development of HEP in patients with cirrhosis. Decreased intestinal motility, gastric acid, and pancreatobiliary secretions, as well as portal hypertension, affect the microbiota composition in

patients with cirrhosis (17). Yildiz et al. (14) found a tendency for the negative correlation between *Blastocystis* colonization and bacterial diversity, although it was not statistically significant. In addition, they found a negative correlation between *Bacteroidetes* phylum and *Blastocystis* colonization, as seen in the previous studies (9-11, 14, 15).

Forsell et al. (15) investigated the effect of travel on *Blastocystis* carriage and its relationship with the intestinal microbiota in Swedish travelers. They found that traveling did not have any effect on *Blastocystis* carriage. There was no significant difference between the groups with and without *Blastocystis* colonization, with respect to fecal microbiota composition. Interestingly, an increased amount of *Sporolactobacillus* and *Candidatus Carsonella* was detected with *Blastocystis* colonization. In addition, a negative correlation with *Bacteroides* enterotype and increased bacterial diversity at the genus level was detected with *Blastocystis* carriage. *Sporolactobacillus* species produce lactic acid from the sugars contained in vegetables that are consumed as part of the diet. Therefore, investigators are of the opinion that *Blastocystis* colonization can be associated with a healthy microbiota and a diet that contains vegetables.

Studies involving helminths have also shown a positive correlation between helminths and increased bacterial diversity. It was found that *Trichuris trichiura* treatment is effective in the restoration of intestinal dysbiosis and the regulation of mucosal barrier functions in macaque monkeys with chronic diarrhea (18). It was seen that helminth colonization is associated with increased bacterial species diversity in Malaysian individuals infected and not infected with helminths (19).

CONCLUSION

Many recent studies have detected an increased fecal bacterial diversity in individuals who have *Blastocystis* colonization. This situation implies that this protist may be a beneficial component for intestinal homeostasis. Lukes et al. asserted that the use of protists, such as *Blastocystis*, may be beneficial in helminth treatment due to its potential of stimulating the immune system, especially in cases with allergy and IBD (20). Once this hypothesis is confirmed with future studies, commensalism and even mutualistic relationships between *Blastocystis* and individuals will need to be reshaped, at least under certain conditions.

Moreover, there are a few studies that investigate the relationships between *Blastocystis* and intestinal microbiota, and these studies have not yet provided conclusive results regarding the cause-effect relationships. Is microbiota with dysbiosis not suitable for *Blastocystis* colonization, or does *Blastocystis* affect the structuring of microbiota composition by affecting intestinal homeostasis? Answers to these questions will be found by performing long-term prospective metagenomic studies conducted on humans containing case-control groups (13). On the other hand, animal models colonized by *Blastocystis* are urgently needed to understand the functional effect of *Blastocystis* on the bacterial microbiota.

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