The regulatory function of *Blastocystis* spp. on the immune inflammatory response in the gut microbiome

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*Blastocystis* spp. is a unicellular organism that resides in digestive tract of various vertebrates, with a worldwide distribution and a variable prevalence. For many years, *Blastocystis* spp. was considered a cyst of a flagellate, a fungus, or a saprophyte yeast of the digestive tract; in 1996, it is placed in the group of stramenopiles (heterokonts). Since its new classification, many questions have arisen around this protist about its role as a pathogen or non-pathogen organism. Recent evidence indicates that *Blastocystis* spp. participates in the immune inflammatory response in the intestinal microbiome generating an anti-inflammatory response, showing a lower concentration of fecal inflammatory markers in infected human hosts. Here, we review recent findings on the regulatory function of *Blastocystis* spp. in the immune inflammatory response to comprehend the purpose of *Blastocystis* spp. in health and disease, defining if *Blastocystis* spp. is really a pathogen, a commensal or even a mutualist in the human gut microbiome.

**KEYWORDS**

*Blastocystis*, immune inflammatory response, regulatory function, gut microbiome, human gut microbiota
Introduction

*Blastocystis* spp. (Heterokonta or Stramenopiles) is an enteric protist. It has a worldwide distribution that inhabiting the digestive tract of several vertebrates, being the most prevalent protist of the human intestine (Silberman et al., 1996; Stensel and Boreham, 1996; Tan, 2008). *Blastocystis* spp. (named *Blastocystis* hereafter) exhibits great genetic diversity; by the moment, 26 subtypes have been established on the basis of the small subunit of the ribosomal RNA gene (SSU rRNA), and each subtype has a distinct distribution and different types of host around the world (Aynur et al., 2019; Maloney et al., 2019). So far, it has been reported that humans could be infected by specific subtypes as ST1 to ST10 and ST12, but an undoubted fact is that ST1, ST2, ST3, and ST4 are the most frequently subtypes identified in humans (Moosavi et al., 2012; Alfellani et al., 2013; Fontanelli Sulekova et al., 2019; Jiménez et al., 2019). The pathogenicity or non-pathogenicity of *Blastocystis* depends on several factors such as the interaction with the intestinal microbiota, the infecting subtype, and the host’s immune response. Some studies carried out in different settings suggest that *Blastocystis* is part of the normal gut microbiota of humans and other mammals, being able to colonize the intestinal tract and establish itself for prolonged periods without causing disease (Parfrey et al., 2011; Scanlan et al., 2014; Pandey et al., 2015). An interplay with the immune system is established. There is evidence, for example, that *Blastocystis* colonization may be related to an anti-inflammatory response favoring changes in the bacterial composition of the gut microbiota, increasing levels of Firmicutes and promoting a greater bacterial diversity (Nourrisson et al., 2021). Our group reported that members of the phyla Firmicutes and Bacteroidetes, such as those of the genera *Ruminococcus* and *Prevotella*, respectively (Iebba et al., 2016a).

Some initial reports indicate that *Blastocystis* infection might be related to the inflammatory state of the intestine that is typical of the irritable bowel syndrome (IBS) (Giacometti et al., 1999). As far as one knows, the associated symptoms of *Blastocystis* infection are the outcome of the innate immune response that follows the breakdown of the intestinal barrier. There is inflammation and damage of the intestinal epithelium that involves activation of membrane receptors such as TLRs and CD8 T lymphocytes, macrophages, and neutrophils activation, including Immunoglobulin M (IgM), IgG, and IgA production (Vitetta et al., 2016). However, the function of *Blastocystis* colonization associated with gastrointestinal symptoms remains unresolved.

Apparently, *Blastocystis* has developed ways to take advantage of the host immune inflammatory response to settler and to continue host colonization without causing disease. Here, we review recently described strategies by which *Blastocystis* could regulate the immune inflammatory response in the gut microbiome.

The effect of *Blastocystis* on the immune inflammatory response

The human gut microbial community comprises a highly complex ecosystem (The Human Microbiome Project Consortium, 2012). Bacteria, nematodes, and protozoan parasites are common in the gastrointestinal tract. They have co-evolved and adapted to a variety of circumstances in an interplay with the host, so it is not surprising that they become important elements as regulators and/or modulators of the host immune response (Reynolds et al., 2015). Under certain conditions, this can undermine the host ability to initiate an effective immune safeguarding mechanism, allowing the colonization and persistence of infection of parasites and other microorganisms (Round and Mazmanian, 2010; Bancroft et al., 2012; Glendinning et al., 2014), which adapt to the new ecological niches, as could be the scenario of *Blastocystis*.

In the intestine, *Blastocystis* has an interplay with the intestinal epithelium and the underlying immune system (Belkaid and Hand, 2014). IgA is the most abundant mucosal antibody that has a fundamental function conserving homeostasis with the microbiome by joining and neutralizing invading pathogens near the mucus layer (Gutzeit et al., 2014). IgA secretion in the intestinal lumen is caused by parasitic infections with helminths to limit the fertility of the parasite and provide immune protection against reinfections (Johansen et al., 1999; McCoy et al., 2008). Individuals colonized with *Blastocystis* have presented lower levels of fecal IgA compared with non-colonized individuals (Nieves-Ramirez et al., 2018).
Blastocystis is also correlated with decreased neutrophil counts in blood (Cheng et al., 2003) and is known to produce serine proteases that degrade secretory IgA (sIgA) (Pathua et al., 2005). In addition, individuals colonized by Blastocystis displayed lower levels of fecal calprotectin (Nieves-Ramirez et al., 2018). The calprotectin is a protein used as a marker of intestinal inflammation and is derived from the secretion of cytosolic proteins from neutrophils (Walsham and Sherwood, 2016). It seems that the interaction of Blastocystis in the intestine established an anti-inflammatory habitat.

Serum cytokines have been reported in cultures of in vitro cell lines incubated with Blastocystis that favor the production of interleukin-8 (IL-8) and granulocyte-macrophage colony-stimulating factor (Long et al., 2001). In a murine model through histopathological examinations, the pathogenicity of Blastocystis and its capacity to modulate the immune response were evaluated, finding changes in the epithelium, with an exfoliation and inflammatory cell infiltration in the submucosa, severe hyperplasia of caliciform cells, and Blastocystis infiltrated in all layers of the large intestine. In addition, mice infected with Blastocystis show a greater expression of cytokine IL-12 and tumor necrosis factor-alpha (TNF-α) and a lower expression of cytokine IL-4 and IL-10 (Abdel-Hafeez et al., 2016). Meanwhile, it was demonstrated that Blastocystis ST7 induces the expression of IL-1β and IL-6 proinflammatory cytokines through the activation of the mitogen-activated protein kinases in mouse intestinal explants (Lim et al., 2014). In addition, in an experimental model that used mice colonized with ST4, the production of short-chain fatty acids (SCFAs) and the proportion of anti-inflammatory cytokine IL-10 were increased (Deng and Tan, 2022). Meanwhile, in patients with IBS infected with Blastocystis subtypes ST1, ST2, and ST3 through the evaluation of serum by enzyme-linked immunosorbent assays, it was found that there was an increase in the concentration of the cytokines IL-6 and TNF-α (Azizian et al., 2016).

On the basis of these studies and although they are scarce until now, it seems that Blastocystis and some specific subtypes could generate an anti-inflammatory scenario, of course, future research on this issue is required to elucidate how this microorganism interacts with the host immune inflammatory response.

The modulation of Blastocystis on the intestinal microbiota and its interaction with the immune inflammatory response

The microorganisms in healthy intestinal microbiota consist of trillions of virus, bacteria, and other protozoa and fungi that are all in proximity with the intestinal mucosa, developing specific and important biological interactions and physiological functions critical for the host (Sekirov et al., 2010; Brown et al., 2013). This long-term and dynamic coevolution with the human intestinal immune system eventually permits or favors mutualistic host-microbial relationships that have significant impact to the maturation, development, and modulation of the immune response. There is a fine regulation of the expression of immune mediators that impact the recruitment and differentiation of local immune cell populations in harmony with microorganisms and that direct the establishment of the intestinal microbiome (Sjögren et al., 2009; Caballero and Pamer, 2015).

Likewise, intestinal parasites such as helmints and protozoa regularly secrete molecules that modify the ecological niche and therefore can modulate the structure and function of the gut microbiota (Sekirov et al., 2010). The intestinal environment can be interpreted from different levels of interactions between biotic and abiotic factors that have an impact on biochemical networks, microbial communities, and the function of the host immune system. An example is the human plasminogen’s increased effect on Bifidobacterium cell adhesion to enterocytes or bacteria role as modulator of cell behavior in immune and inflammatory processes (Candela et al., 2008; Keragala and Medcalf, 2021). Only recently, the relationship between human-associated gut protists and the gut bacterial community has been explored to elucidate their role in dysbiosis or pathogenesis (Burgess and Petri, 2016; Barash et al., 2017). We are in the process of understanding the key factors that modify the balance between health and disease in relation to the diversity of the microbiome and its changes in the composition, structure, and function (Brown et al., 2013). One of the evident difficulties is that the intestinal microbiota behavior can be highly variable in the human host, determined by various factors such as diet, sociodemographic status, health and disease condition, or the use of antibiotics and, to a lesser extent, by the genetic component (Cho and Blaser, 2012; Yatsunenko et al., 2012; Goodrich et al., 2016).

Currently, Blastocystis is clearly associated with changes in the composition of the microbiota in the human host (Table 1). The unavoidable question is whether these protozoa are capable of regulating the intestinal inflammatory immune response in humans modulating bacterial populations, if so, by which mechanisms they act to do it. This will remain controversial until new research emerged. So far, it has been found that Blastocystis colonization is strongly related with broad shifts in the gut-resident bacterial community and with an increase in bacterial diversity (Nieves-Ramirez et al., 2018; Deng et al., 2021). Bacterial diversity is associated to healthy microbiota and favorable immune response in the host (The Human Microbiome Project Consortium, 2012); some hypotheses state that Blastocystis could have a predatory effect on bacteria population, feeding on abundant taxa and modifying in this way the diversity, increasing or decreasing bacterial populations (Matz and Kjelleberg, 2005; Kurm et al., 2019).
| Blastocystis subtype | Country | Origin of samples | Clinical state | Bacterial richness | Gut microbiota composition |
|----------------------|---------|-------------------|----------------|-------------------|-----------------------------|
| ST1 to ST4           | France  | Humans            | IBS and no GI (ASI) | –                | Faecalibacterium prausnitzii, Bifidobacterium sp. (Nourrisson et al., 2014) |
| ST1 to ST4           | Spain and Nicaragua | Humans | IBS and ASI | † –            | Preotrella and Ruminococcus enterotypes (Anderson et al., 2015) |
| NS-ST                | Denmark | Humans            | ASI            | –              | Bacteroides and clostridial cluster XIVa (O’Brien et al., 2016) |
| Blastocystis         | France  | Humans            | IBS, IBD, GI and ASI | – †          | Clostridia and Mollicutes (classes), Lactobacillales, Clostridiales (orders), Ruminococccaceae, and Prevotellaceae (families) Enterococcus spp. (Stefan, et al., 2016) |
| ST1 to ST8           | Australia | Humans | IBS, IBD and ASI | – –            | Non-significant Non-significant (Ngul et al., 2016) |
| Blastocystis Côte d’Ivoire | Humans | ASI and GI | – –            | † –          | F. prausnitzii/Escherichia coli ratio (Labbe et al., 2016) |
| Blastocystis VC      | Humans  | Colorectal cancer, type 2 diabetes, liver cirrhosis obesity and IBD | – –            | † –          | Clostridiales, Firmicutes, and archaea organisms (Methanobrevibacter smithii) Bacteroides and Bifidobacterium (Beghini et al., 2017) |
| ST1 to ST4 and ST8   | Sweden  | Humans            | ASI            | † –            | Spreuclastosbacillus and Candidatus carsonella Bacteroides (Forsell et al., 2017) |
| ST2 and ST3          | Mexico  | Humans            | GI and ASI     | † –            | Preotella copri, Ruminococcus bromii, Debaryomyces Hansenii, Masur muscula, Aspergillus flavus, Masur racemous, and Isathenkenia terricola Hymenolapia nana (Nievers-Ramirez et al., 2018) |
| ST1 to ST4 and ST7   | Belgium | Humans            | IBD and ASI    | † †            | Bacteroides enterotype, Akkermansia (Tito et al., 2019) |
| ST7                  | Singapore | Mouse model (human donor) | – –            | – –          | Lactobacillus and Bifidobacterium (Yason et al., 2019) |
| Blastocystis Mali     | Mali     | Humans            | ASI            | † †            | Firmicutes, Elusimicrobium, Lentisphaerae, and Euryarchaeta (phylum), F. prausnitzii and Roseburia sp. Actinobacteria, Proteobacteria, unassigned bacteria, and Deinococccus-Thermus (Kodis et al., 2019) |
| Blastocystis India   | India    | Humans            | VL             | – †            | Clostridiales svain BB60 Bacteroidesaceae and Escherichia-Shigella (Lappan et al., 2019) |
| ST1 to ST4 and ST7   | Italy    | Humans            | IBD, IBS, and chronic diarrhea | † – | Preotella, Methanobrevibacter and Ruminococcus Bacteroides (Gabbrielli et al., 2020) |
| Blastocystis Colombia | Colombia | Humans            | ASI            | † –            | Preotella Akkermansia (Alice et al., 2020) |
| Blastocystis Colombia | Humans | ASI | † †            | Faecalibacterium | Prevotella, Bacteroides, and Akkermansia (Casafiada et al., 2020) |
| ST3                  | Rat model (human donor) | Colitis | – –            | † †            | Clostridiales (Firmicutes), Bifidobacteria, and Butyricimonas Bacteroidales (Bacteroidetes), Dolfiuraicaleae (Bilby et al., 2021) |
| ST1 to ST3 and ST6   | VCC2     | Humans            | AS and type 1 diabetes | † – | Ruminococcaceae Bifidobacterium (Cink et al., 2021) |
| Blastocystis Côte d’Ivoire | Humans | ASI | † †            | Succinobacteria Bacteroides-driven enterotype (G. Cristianiana et al., 2021) |
| ST1 to ST4 Cameroon  | Humans   | ASI | † –            | Clostridiales Bacteroides-driven enterotype (Even et al., 2021) |
| Blastocystis France  | Humans   | IBS and ASI       | † –            | Fumicutes, Bacteroidetes, Ruminococcaceae, Tenericutes, and Clostridiales Aspergillaceae, Aspergillus, and Penicillium (Nourrisson et al., 2021) |
| Blastocystis Korea   | Humans   | ASI | † –            | Clostridia, Ruminococcaceae, Prevotellaceae, and Faecalibacterium (Kim et al., 2021) |

(Continued)
Blastocystis subtypes opens a new pathway to understand a capacity of IgA in the bacterial microbiome (Moon et al., 2015). Levels of this immunoglobulin, which corroborates the regulatory of species with low level of IgA suffering from a decrease in the lactoferrin-factors that lead to immune evasion (Puthia et al., 2005; Gutzzeit et al., 2014). Experiments with mice in cohousing diversity of the bacterial microbiota is interacting with the intestinal epithelium, preventing the growth of pathogenic microorganisms and promoting the proliferation of beneficial bacteria (Shen et al., 2017). An adequate degradation of resistant sugars prevents chronic and inflammatory diseases [e.g., IBS, irritable bowel disease (IBD), and ulcerative colitis (UC)] (Ott, 2004; Nishida et al., 2018; Pushpanathan et al., 2019; Tan et al., 2021; Sobh et al., 2022). Butyric acid is an important resource for nourishing the colonocytes and maintains healthy the colonic epithelium. Propionic acid and acetic acid have a protective effect lowering the pH in the large intestine, preventing the growth of pathogenic microorganisms and promoting the proliferation of beneficial bacteria (Shen et al., 2017). An adequate degradation of resistant sugars prevents chronic and inflammatory diseases [e.g., IBS, irritable bowel disease (IBD), and ulcerative colitis (UC)] (Ott, 2004; Nishida et al., 2018; Pushpanathan et al., 2019), so it possible that Blastocystis can indirectly regulate proinflammatory and inflammatory cytokines by modulating the intestinal microbiota.

Other way on which Blastocystis could affect the abundance and diversity of the bacterial microbiota is interacting with the intestinal epithelium and the underlying tissue express cytokine proteases that cleave sIgA and secrete anti-lysozyme and anti-lactoferrin-factors that lead to immune evasion (Pathia et al., 2005; Gutzzeit et al., 2014). Experiments with mice in cohousing and fecal transfer have been noted differences in IgA production, where the species with high content of IgA acquired the microbiota of species with low level of IgA suffering from a decrease in the levels of this immunoglobulin, which corroborates the regulatory capacity of IgA in the bacterial microbiome (Moon et al., 2015).

Recently, the existence of studies regarding to specific Blastocystis subtypes opens a new pathway to understand a little more how this microorganism exert a modulation on the microbiota intestinal population and its repercussion in the immune inflammatory response, for example, ST3 creates an eubiotic state characterized by favorable species of the phyla Firmicutes and Bacteroidetes (Andersen et al., 2015; Iebba et al., 2016a; Nieves-Ramírez et al., 2018; Gabrielli et al., 2020). The deterioration of the integrity of the microbiota and its barrier function favors the invasion of opportunistic and exogenous pathogens, generating dysbiosis, stimulating an inflammatory reaction of the host, and leading to a disorder of the intestinal nutritional environment and, frequently, to secretory diarrhea, with serious effects on the microbiota ecosystem. Therefore, it is of interest to preserve a eubiotic condition in the intestinal microbial ecosystem to guarantee a good state of health (Iebba et al., 2016a). Hence, again, ST3 indirectly could help in the immune response of the host, promoting the increased of beneficial bacterial populations.

In the case of ST7 that seems behave as a pathobiont, it produces a decrease in Bifidobacterium and Lactobacillus populations, which are considered as beneficial bacteria (Yason et al., 2019). ST7 was shown to have significantly greater cysteine protease activity compared with ST4 (Wu et al., 2014). Blastocystis ST7 has been shown to be more resistant to anti-parasitic drugs (Mirza et al., 2011; Yason et al., 2018) and against the host innate immune response (Yason et al., 2016). Some functions of Bifidobacterium are to maintain the epithelial barrier and to exert anti-inflammatory properties that can reduce the production of pro-inflammatory cytokines like IL-6 and TNF-α (Ling et al., 2016). In contrast, ST7 disrupts epithelial barrier and increases the levels of pro-inflammatory cytokines to trigger an inflammatory response (Long et al., 2001; Lim et al., 2014). Lactobacillus has also been found to significantly increase IgA levels (Carasi et al., 2015).
Epidemiological studies have shown that reductions in *Lactobacillus* and *Bifidobacterium* contribute to increased susceptibility to gastrointestinal disorders, for example, patients with UC and Crohn’s disease CD had lower levels of *Lactobacillus* and *Bifidobacterium* populations, respectively (Jonkers, 2003; Ott et al., 2008). On this basis, *in vivo* studies have been carried out using the dextran sulfate sodium (DSS) colitis mouse model, in which an improvement in both colitis symptomatology and mucus production was observed after the administration of *Lactobacillus* and *Bifidobacterium* (Abdelouhab et al., 2012; Toumi et al., 2013). Therefore, a reduction of both intestinal bacteria would eliminate a protective element of the intestinal epithelium, which would provide an ideal environment for the pathogenesis of *Blastocystis*.

In contrast, ST4 *in vitro* plays a similar probiotic role, inhibiting the capacity of *Bacteroides vulgatus* to compromise the intestinal epithelial barrier (Deng and Tan, 2022). *Bacteroides vulgatus* can produce mucin-degrading enzymes such as glycosidase, siaiades, and neuraminidase, which can profoundly weaken the mucosal barrier function and exaggerate inflammation (Ohkusa et al., 2009; Derrien et al., 2010). *In vitro* experiments demonstrated that *B. vulgatus* can invade colonic epithelial cells (SW-480 and HT-29) and activate the expression of pro-inflammatory cytokines (Ohkusa et al., 2009). Interestingly, Deng et al. reported in a second work (Deng et al., 2022) that ST4-altered microbiota from Ragy−/+ mice reduces inflammation in experiment-induced colitis through an increase in “beneficial” microbes such as *Akkermansia*. Bacteria belonging to *Akkermansia* are associated with gut health, and the expansion of *Akkermansia* can increase mucus production to ameliorate intestinal inflammation (Everard et al., 2013). Moreover, fecal microbiota transplantation (FMT) from ST4-colonized mice increased the SCFA production and the proportion of anti-inflammatory cytokine IL-10 more profoundly than FMT from control mice. *Blastocystis* ST4 improves the intestinal inflammation in a mouse model. They also observed that *Blastocystis* ST4 colonization activates Th2 immune responses in normal healthy mice and DSS-induced colitis mice. It has been demonstrated that Th2 cells are important sources of type 2 cytokines (IL-4, IL-5, and IL-13) and are also important effector cells during the inflammatory process (Gause et al., 2020). In addition, they also found that *Blastocystis* ST4 colonization increases the number of IL-10–producing Treg in the colonic mucosa of DSS-induced mice. The cytokine IL-10 produced by Treg cells is required for containment of inflammatory responses in mucosal tissues (Rubtsov et al., 2008). Both humans and mice deficient in IL-10 or IL-10 receptor are prone to develop severe intestinal inflammation (Glocker et al., 2009; Beuge et al., 2011). Furthermore, we can agree more with them that future studies should focus on understanding the mechanistic connection between *Blastocystis* ST4 colonization, IL-10 signaling, and bacterial-derived SCFAs using relevant animal models, not just for this subtype, otherwise for all subtypes of *Blastocystis* that infect the human population.

Although there is still little information about the modulatory function of *Blastocystis* on the intestinal microbiota, these research studies give us an enormous advance in the knowledge of this microorganism and its potential behavior as a pathobiont, commensal, mutualist, or even a probiotic in the human intestine that could promotes regulation of the inflammatory immune response.

**Conclusion and personal perspectives**

An undoubted fact in relation to *Blastocystis* is its interaction with the host at an immunological level and microbiota. It has been said a lot not only about its actual role in disease conditions (e.g., IBS and IBD) causing some gastrointestinal symptoms (e.g., diarrhea, vomiting, bloating, constipation, and abdominal pain) but also about merely being an organism that infects and establishes itself without causing any harm or even being beneficial to the host. The existence of many studies on *Blastocystis* trying to clarify and define the real character of *Blastocystis* as a pathogen, non-pathogen, commensal, mutualist, or even an engineer of the host gut, has not been successful, so this controversy will continue and, hopefully, it will be clarified soon. For the moment, with the current information on *Blastocystis*, we can say that it interferes with or modifies the intestinal inflammatory immune response and the structure of the intestinal microbiota of the host. *Blastocystis* behavior in the host–parasite relationship appears to be a beneficial protost in the gut, shaping bacterial populations profiles associated to healthy intestinal microbiota rather than a pathogenic organism. Nevertheless, there are some recent pieces of evidence suggesting that *Blastocystis*, under particular circumstances, might display a pathogenic behavior.

There is a long way to go in the study of *Blastocystis*, and our personal interest about this microorganism is to establish how it acts in the modulation of the microbiota, because it may have a potential role as an intestinal probiotic. In this way, we could also determine its non-pathogenicity in vulnerable infected groups, whose immune status makes them susceptible to damage, and provide valuable information regarding the beneficial role of *Blastocystis* rather than just assuming the harmful behavior of this microorganism for humans.

**Author contributions**

LR-V and CX conceptualized the review content. LR-V and CX wrote the first draft of the manuscript. TP-B participated in writing of specific paragraphs. LR-V, CX, PM, AS-V, EG, HP-J and TP-B edited the manuscript. EH, OP-R, MN-R, AP, and MZ participated in editing.
of specific paragraphs and re-reading of the text. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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