frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Mini Review
PUBLISHED 31 August 2022
DOI 10.3389/fcimb.2022.967724

OPEN ACCESS

EDITED BY
Kevin S. W. Tan,
National University of Singapore,
Singapore

REVIEWED BY
Lei Deng,
National University of Singapore,
Singapore

*CORRESPONDENCE
Liliana Rojas-Velázquez
rojasvelazquezliliana@gmail.com
Cecilia Ximénez
cximenez@unam.mx

SPECIALTY SECTION
This article was submitted to Parasite and Host, a section of the journal Frontiers in Cellular and Infection Microbiology

RECEIVED 13 June 2022
ACCEPTED 04 August 2022
PUBLISHED 31 August 2022

CITATION
Rojas-Velázquez L, Morán P, Serrano-Vázquez A, Portillo-Bobadilla T, González E, Pérez-Juárez H, Hernández E, Partida-Rodríguez O, Nieves-Ramírez M, Padilla A, Zaragoza M and Ximénez C (2022) The regulatory function of *Blastocystis* spp. on the immune inflammatory response in the gut microbiome. *Front. Cell. Infect. Microbiol.* 12:967724. doi: 10.3389/fcimb.2022.967724

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

Liliana Rojas-Velázquez[1]*, Patricia Morán[1], Angélica Serrano-Vázquez[1], Tobías Portillo-Bobadilla[2], Enrique González[1], Horacio Pérez-Juárez[1], Eric Hernández[1], Oswaldo Partida-Rodríguez[1], Miriam Nieves-Ramírez[1], Angeles Padilla[1], Martha Zaragoza[1] and Cecilia Ximénez[1]*

[1]Unidad de Investigación en Medicina Experimental, Facultad de Medicina, Universidad Nacional Autónoma de México (UNAM), Mexico City, Mexico, [2]Red de Apoyo a la Investigación, Coordinación de la Investigación Científica, Universidad Nacional Autónoma de México (UNAM) e Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico

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; Stenzel 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 asymptomatic healthy individuals infected with *Blastocystis* show an anti-inflammatory response characterized by lower concentration of fecal inflammatory markers and a higher alpha diversity in the bacterial community (Partida-Rodríguez et al., 2017; Nieves-Ramírez et al., 2018).

On the other side is the issue of *Blastocystis* pathogenicity or non-pathogenicity related to genetic differences of the subtypes (Stensvold et al., 2009; Wu et al., 2014). Some subtypes had been associated with unhealthy changes in humans as the case of ST1, ST4, and ST7 and, on the contrary. ST3 has been related as a non-pathogenic subtype (Domínguez-Márquez et al., 2009; Eroglu et al., 2009; Stensvold et al., 2009). However, it is early to say which subtypes are pathogenic or not based on the little information that is currently available. In addition, it was demonstrated that some subtypes could alter the gut microbiome, for example, ST7 produces a decrease in beneficial bacterial populations, such as *Bifidobacterium* and *Lactobacillus* (Yason et al., 2019), and ST3 was associated to an eubiotic state characterized by beneficial species that are 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 infiltration 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-Ramírez 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) (Puthia et al., 2005). In addition, individuals colonized by *Blastocystis* displayed lower levels of fecal calprotectin (Nieves-Ramírez 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 stablished 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 epithelia, 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 helminths 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-Ramírez 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). In a previous

TABLE 1 Modifications of *Blastocystis* spp. on the gut microbiota.

Blastocystis subtype	Country	Origin of samples	Clinical state	Bacterial		Gut microbiota composition		Reference
				Richness	Diversity	Increase	Decrease	
ST1 to ST4	France	Humans	IBS and no GI (ASI)	–	–		*Faecalibacterium prausnitzii*, *Bifidobacterium* sp.	(Nourrisson et al., 2014)
ST1 to ST4 and ST6	Spain and Denmark	Humans	IBS and ASI	↑	–	*Prevotella* and *Ruminococcus* enterotypes		(Andersen et al., 2015)
NS-ST	Denmark	Humans	ASI	–	–	*Prevotella*	*Bacteroides* and clostridial cluster XIVa	(O'Brien Andersen et al., 2016)
Blastocystis	France	Humans	IBS, IBD, GI and ASI	–	↑	Clostridia and Mollicutes (classes), Lactobacillales, Clostridiales (orders), Ruminococcaceae, and Prevotellaceae (families)	Enterococcaceae, Streptococcaceae, Lactobacillaceae, and Enterobacteriaceae (families)	Audebert et al., 2016
ST1 to ST8	Australia	Humans	IBS, IBD and ASI	–	–	Non-significant	Non-significant	(Nagel et al., 2016)
Blastocystis	Côte d´ Ivoire	Humans	ASI and GI	–	–	*F. prausnitzii*/*Escherichia coli* ratio		(Iebba 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 Proteobateria	(Beghini et al., 2017)
ST1 to ST4 and ST8	Sweden	Humans	ASI	↑	–	*Sporolactobacillus* and *Candidatus carsonella*	*Bacteroides*	(Forsell et al., 2017)
ST2 and ST3	Mexico	Humans	GI andASI	↑	–	*Prevotella copri*, *Ruminoccoccus bromii*, *Debaryomyces hansenii*, *Mucor mucedo*, *Aspergillus flavus*, *Mucor racemosus*, and *Issatchenkia terricola*	*Hymenolepis nana*	(Nieves-Ramírez et al., 2018)
ST1 to ST4 and ST7, ST8	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	Humans	ASI	↑	↑	Firmicutes, Elusimicrobia, Lentisphaerae, and Euryarchaeota (phylum); *F. prausnitzii* and *Roseburia* sp.	Actinobacteria, Proteobacteria, unassigned bacteria, and Deinococcus–Thermus	(Kodio et al., 2019)
Blastocystis	India	Humans	VL	–	↑	Clostridiales vadin BB60	*Bacteroidaceae* and *Escherichia–Shigella*	(Lappan et al., 2019)
ST1 to ST4 and ST7	Italy	Humans	IBD, IBS, and chronic diarrhea	–	↑	*Prevotella*, *Methanobrevibacter* and *Ruminococcus*	*Bacteroides*	(Gabrielli et al., 2020)
Blastocystis	Colombia	Humans	ASI	↑	–	*Prevotella*	*Akkermansia*	(Alzate et al., 2020)
Blastocystis	Colombia	Humans	ASI	↑	↑	*Faecalibacterium*	*Prevotella*, *Bacteroides*, and *Akkermansia*	(Castañeda et al., 2020)
ST3		rat model (human donor)	Colitis	–	–	Clostridiales (Firmicutes),*Bilophila*, and *Butyricimonas*	Bacteroidales (*Bacteroidetes)*, Defluviitaleaceae	(Billy et al., 2021)
ST1 to ST3 and ST6	VC2	Humans	AS and type 1 diabetes	↑	–	Ruminococcaceae	*Bifidobacterium*	(Cinek et al., 2021)
Blastocystis	Côte d´ Ivoire	Humans	ASI	↑	↑	*Succinivibrio*	Bacteroides-driven enterotype	(Di Cristanziano 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 Dipodascaceae	Aspergillaceae, *Aspergillus*, and *Penicillium*	(Nourrisson et al., 2021)
Blastocystis	Korea	Humans	ASI	↑	–	Clostridia, Ruminococcaceae, Prevotellaceae, and Faecalibacterium		(Kim et al., 2021)

(Continued)

TABLE 1 Continued

Blastocystis subtype	Country	Origin of samples	Clinical state	Bacterial		Gut microbiota composition		Reference
				Richness	Diversity	Increase	Decrease	
ST1 to ST5 and ST7	Mexico	Humans	ASI and metabolic ill	–	–		Firmicutes/BacteroidetesRatio	(Muñoz Yañez et al., 2021)
ST2 to ST3 and ST5	Colombia	Humans	*C. difficile* infected, ASI, and diarrhea	↓	–	Saccharomycetales family Ascomycota and Basidiomycota Prevotellaceae*Faecalibacterium*, *Dorea*, and Lachnospiraceae	*Akkermansia*	(Vega et al., 2021)
Blastocystis	VC3	Humans	ASI	↑	–	Firmicutes, Bacteroidetes, *Prevotella*, *Faecalibacterium*, *Flavonifracter*, *Clostridium*, *Succinivibrio*, and *Oscillibacter*		(Stensvold et al., 2022)
ST4	Singapore	Wistar rats (*in vitro*)	ASI	–	–		*Bacteroides vulgatus*	(Deng and Tan, 2022)

VC, various countries; Italy, Tanzania, USA, Denmark, China, Mongolia, Germany, Spain, Peru, and France.
VC2, Azerbaijan, Jordan, Nigeria, Sudan, Tanzania, and Czechia.
VC3, Algerian, Egypt, Turkey, United Kingdom, and Denmark.
IBS, irritable bowel syndrome; IBD, irritable bowel disease.
GI, diarrhea, vomiting, bloating, constipation, and abdominal pain.
ASI, asymptomatic infected.
VL, visceral Leishmaniasis.

study, we observed that *Blastocystis* was associated with an increment of *Ruminococcaceae bromii* (Nieves-Ramírez et al., 2018), which is well known for its ability to degrade resistant starches in the human gut (Ze et al., 2012). Data suggested that microbial fermentation of resistant starches in the colon leads to SCFAs, such as lactic acid, acetate, propionate, and butyrate, which are the major end products of the microbial fermentation pathway (Ze et al., 2012; Maier et al., 2017; 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 immune tissue express cysteine proteases that cleave sIgA and secrete anti-lysozyme and anti-lactoferrin-factors that lead to immune evasion (Puthia et al., 2005; Gutzeit 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 valgatus* to compromise the intestinal epithelial barrier (Deng and Tan, 2022). *Bacteroides vulgatus* can produce mucin-degrading enzymes such as glycosidase, sialidases, 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 *Rag1*$^{-/-}$ 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 determined 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; Begue 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 as 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 protist 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.

Funding

The present work was supported by grant numbers IN217821 and IV200420 from PAPIIT- DGAPA, Universidad Nacional Autónoma de México (UNAM).

Acknowledgments

The authors highly appreciate the informatics assistance of Angélica Serrano-Ahumada and Marco E. Gudiño-Zayas in the preparation of the manuscript.

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.

Publisher's note

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