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ORIGINAL PAPER

Immunopathological assessments of human *Blastocystis* spp. in experimentally infected immunocompetent and immunosuppresed mice

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Abstract *Blastocystis* spp., one of the most common parasites colonizing the human intestine, is an extracellular, luminal protozoan with controversial pathogenesis. The host's immune response against *Blastocystis* spp. infection has also not been defined yet. Therefore, this research aimed to assess the potential pathogenicity of this parasite and its ability to modulate the immune response in experimental infected immunocompetent and immunosuppresed mice. These results demonstrated that the infected immunosuppressed mice were more affected than infected immunocompetent mice. Histopathological examination of the small intestine in the infected immunosuppressed mice showed that *Blastocystis* spp. infiltrated all the layers. Moreover, the epithelia showed exfoliation and inflammatory cell infiltration in submucosa compared to that of the infected immunocompetent mice. As well, examination of the large intestine of the infected immunosuppressed group showed severe goblet cell hyperplasia. *Blastocystis* spp. infiltrated all the large intestine layers compared to that of the infected immunocompetent group. Furthermore, there was a significant upregulation of the expression of proinflammatory cytokines: interleukin 12 (IL-12) and tumor necrosis factor alpha (TNF-α) in the infected immunosuppressed mice compared to that of the infected immunocompetent ones ($p \leq 0.004$ and $p \leq 0.002$, respectively). However, the expression of anti-inflammatory cytokines (IL-4 and IL-10) was significantly downregulated in the infected immunosuppressed group compared to that of the infected immunocompetent group one at 10 days postinfection ($p \leq 0.002$ and $p \leq 0.001$, respectively). The results of this study revealed that *Blastocystis* spp. affected the production of pro- and anti-inflammatory cytokines in both groups of mice compared to healthy normal (naive) group. Additionally, these data showed that there was a significant upregulation ($p \leq 0.005$) of the locally synthesized antibody: secretary IgA (sIgA) in the gut of the infected immunocompetent mice when compared to that of the infected immunosuppressed ones.

Keywords *Blastocystis* spp. · Immunosuppressed and immunocompetent mice · Cytokines production

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Introduction

Blastocystis spp. are an enteric parasite that inhabits intestinal tract of humans and many animals. This parasite has a worldwide distribution (Sohail and Fischer 2005; Tan 2008; Stensvold et al. 2009). *Blastocystis* spp. showed a dramatic increase in recent years and were considered to be a member of normal intestinal flora in the past; recently, it has been accepted as a controversial pathogen (Al-kaissi and Al-Magdi 2009). *Blastocystis* spp. infect both

immunocompetent and immunocompromised individuals. However, symptoms associated with *Blastocystis* spp. were more severe in immunocompromised patients, than those in immunocompetent individuals (Garavelli et al. 1988, 1991).

Although several reports have suggested that *Blastocystis* spp. could cause gastrointestinal disorders (Yakoob et al. 2011), *Blastocystis* species' pathogenicity has not been defined yet. The various mechanisms suggested for *Blastocystis* spp.-mediated gastrointestinal symptoms include adherence of *Blastocystis* spp. to the gut epithelium, triggering a lysis mechanism and production of diarrheagenic toxin as what is clearly done by *Entamoeba histolytica* and *Giardia lamblia* (Yakoob et al. 2011). The clinical consequences of *Blastocystis* spp. infection are mainly diarrhea or abdominal pain with nonspecific gastrointestinal symptoms such as nausea, anorexia, vomiting, weight loss, lassitude, dizziness, and flatulence (Al-kaissi and Al-Magdi 2009).

Blastocystis spp. is a polymorphic parasite as it can be present in various forms such as presents in vacuolar, granular, multivacuolar, amoeboid, nonvacuolar, and cystic forms (Zierdt 1991; Miné and Rosa 2008). In general, the vacuolar and granular forms are the most predominant in the fresh fecal and in vitro culture samples (Sukthana 2001; Abdel-Hafeez et al. 2015). The amoeboid form is generally more frequently observed in the samples of in vitro culture than in fresh fecal materials. The amoeboid form has also been recently reported to be predominantly excreted in the stool samples of symptomatic patients (Tan and Suresh 2006).

The cyst form is mainly seen in the stool samples but rare in vitro cultures (Yoshikawa et al. 2003). Human infection with *Blastocystis* spp. occurs through ingestion of contaminated food or water with by cystic form which is then transformed into the vacuolar form in the human intestine (Tan 2004; Miné and Rosa 2008; Dogruman et al. 2010).

Case reports and series have suggested that the pathogenic role of *Blastocystis* spp. is thought to be through induction of intestinal inflammation. Some studies have also suggested that inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) are associated with *Blastocystis* spp. infection (Stark et al. 2007). Several clinical and epidemiological studies implicated *Blastocystis* spp. as a pathogen, while others dismissed it as a commensal (Chen et al. 2003; Leder et al. 2005; Rossignol et al. 2005; Tan and Suresh 2006). This controversial pathogenesis of *Blastocystis* spp. deserves consideration.

Therefore, this research aimed to assess the potential pathogenicity of *Blastocystis* spp. parasite and its ability to modulate the host's immune response. Moreover, the ability of this organism to elicit an immune response through expression of proinflammatory cytokines (IFN-γ, IL-12, and TNF-α) and anti-inflammatory cytokines (IL-4 and IL-10) had been examined. Also, production of sIgA had been assessed.

Material and methods

Type of the study

The study was an experimental one and was conducted in the period from June 2014 to March 2015 in the Parasitology Department, Faculty of Medicine, Minia University, Minia, Egypt.

Source of strains of *Blastocystis* spp. isolates

Strains of *Blastocystis* spp. were isolated from the stool specimens obtained from IBS patients (Rossignol et al. 2005; Yakoob et al. 2010b). Patients were selected from wards of Tropical Medicine Department, Minia University, and those attending outpatient clinics. Those patients had been clinically diagnosed as IBS patients (Poirier et al. 2012; Nagel et al. 2014). Stool samples were collected in clean sterile cups and immediately subjected to direct parasitological examination in Parasitology Department, Faculty of Medicine, Minia University. However, *Blastocystis* spp. isolates obtained by stool culture have not been subjected to molecular genotyping for identification of its subtypes (Stensvold et al. 2009). In Egypt, two recent studies have identified the subtypes of *Blastocystis* spp. isolated from symptomatic patients in some Egyptian localities to be subtypes (ST1, ST3, ST6, and ST7). They concluded that the most predominant subtype is ST3 (Hussein et al. 2008; Fouad et al. 2011). So, we can expect that the predominant subtype in our study could be ST3, but this guess requires further future work to be confirmed.

Ethical considerations

Verbal consent was obtained from the patients. All procedures were conducted according to the ethical standards approved by the Institutional Human Ethics Committee, Faculty of Medicine, Minia University, Egypt.

Stool examination

Microscopic examination

Direct wet smear methods, both saline and lugols iodine wet mount, were applied. Giemsa stain was also used according to Garcia (2001) for confirmation of *Blastocystis* spp. Positive samples were used in culture.

Stool cultivation

Approximately, 50 mg of positively detected stool sample for *Blastocystis* spp. was cultivated in 5-ml screw caped tube containing Locke egg serum medium (LE) according to Saksirisampant et al. (2010). The medium was

sterilized by autoclaving for 20 min at 121 °C and stored in 500-ml bottles at 4 °C until use. Under sterile hood conditions, 10 % heat-inactivated (56 °C for 30 min) horse serum (Invitrogen, Groningen, Netherlands), 100 IU/ml penicillin, and 100 μg/ml streptomycin (Sigma-Aldrich, St. Louis, MO, USA) were added to the cooled medium. The inoculated medium was incubated at 37 °C for 2–3 days. The cultures were screened for *Blastocystis* spp. by standard light microscopy every 12 h and subcultured for an additional 2–3 days in fresh medium. Subcultivation was performed every 72 h when it was further needed. The organism could be maintained for more than 3 months (Saksirisampant et al. 2010).

Animals

Eight-week-old male BLAB/c mice weighing 18–20 g each were obtained from experimental house, Faculty of Medicine, Minia University. The mice were housed in standard animal house conditions. The mice had free access to standard rodent chow and water. All experimental procedures were conducted according to the ethical standards approved by the Institutional Animal Ethics Committee guidelines for animal care and use, Minia University, Egypt.

Experimental design

Forty animals were randomly divided into four groups, ten mice each, the first was apparently healthy normal group (naive), the second was infected-immunocompetent group, the third was noninfected immunosuppressed group, and the fourth was infected-immunosuppressed group. Stool samples of all mice were subjected to direct parasitological examination by wet mount and lugols iodine-staining to detect the presence of *Blastocystis* or other parasites. The mice of the second group "infected-immunocompetent" were infected intragastrically with 2×10^6/ml of *Blastocystis* spp. in sterile saline on the tenth day of the experiment. The mice of the third group "immunosuppressed" were immunosuppressed by administration of dexamethazone (DEX p) (Decadron 0.5 mg, Merk Sharb and Dom UK) 33 μg/ml in their drinking water during the whole period of the experiment. The mice of the fourth "infected-immunosuppressed" were immunosuppressed by administration of dexamethazone (DEX p) (Decadron 0.5 mg, Merk Sharb and Dom UK) 33 μg/ml in their drinking water during the whole period of the experiment, and they were infected intragastrically with 2×10^6/ml of *Blastocystis* spp. in sterile saline on the tenth day after the start of immunosuppression by dexamethazone.

Body weight of the mice

The body weight of the mice of the various studied groups was recorded on the day of infection "day 0" and on the second, fourth, sixth, eighth, and tenth days postinfection.

Evaluation of *Blastocystis* spp. infection in mice

Detection of cysts shedding in feces

Feces from all mice were examined microscopically at different periods (2, 4, 6, 8, and 10 days) postinfection. Quantitative estimation of the infection intensity in the stool samples of *Blastocystis* spp.-infected mice was performed according to the method described by Shlim et al. (1995). Cysts of *Blastocystis* spp. were counted in at least three fields with estimation of the average number/high power field (HPF) (Shlim et al. 1995).

Blood collection

Blood samples were collected from the medial canthus of the eye of mice in nonheparinized 5-ml centrifuge tube on the tenth day postinfection. Blood samples were used to separate sera by centrifugation at 1500 rpm for 10 min. Serum samples were stored at −20 °C until used.

Histolopathological examination

On the tenth days postinfection, all mice from each group were sacrificed. Tissue samples from walls of small intestine, caecum, and colon of scarifying animals were collected then fixed in 10 % neutral buffered formalin. The organs were routinely processed and sectioned at 4- to 5-μm thickness. The obtained tissue sections were collected on glass slides, deparaffinized, and stained with hematoxylin and eosin stain. The sections were then examined and observed under light microscope at ×10, ×40, and ×100 magnifications (Moe et al. 1997; Bancroft and Gamble 2008).

Cytokines measurement

IFN-γ, IL-12, TNF-α, IL-4, and IL-10 concentrations in the sera of the different groups of mice were measured at tenth day postinfection. These concentrations were assayed by a two-site sandwich enzyme-like immunosorbent assay (ELISA) using ELISA kits according to the manufacturer's instructions (Wuhan Elabscience Biotechnology Co., Ltd) (Schumacher et al. 1988).

Table 1 Changes in the body weight of the mice of the various studied groups

Groups/*N**	Body weight "g" Mean ± SD					
	Day 0	Day 2	Day 4	Day 6	Day 8	Day10
Naïve	19.30 ± 0.84	19.32 ± 0.76	19.46 ± 0.68	19.65 ± 0.46	19.73 ± 0.42	19.73 ± 0.42
Noninfected-immunosuppressed	19.32 ± 0.91	19.29 ± 0.89	19.20 ± 0.80	19.20 ± 0.73	19.20 ± 0.38	19.20 ± 0.15
Infected-immunocompetent	19.20 ± 0.86	19.10 ± 0.84	18.99 ± 0.89	17.22 ± 0.89	16.91 ± 0.89	16.65 ± 0.89
Infected-immunosuppressed	19.34 ± 0.61	18.73 ± 0.75	17.65 ± 0.53	17.00 ± 0.63	16.48 ± 0.48	14.27 ± 0.54

*N** = 10 mice in all groups except in infected immunosuppressed where *N* equals 7 mice on day 4 and 5 mice on days 6, 8, and 10. A *p* value ≤0.07 (nonsignificant)

SIgA measurement

On the tenth day postinfection, tissue samples from walls of small intestine of all scarifying animals were thoroughly rinsed in ice-cold PBS (0.01 M, pH = 7.4) to remove excess blood. Tissue specimens were weighed and then homogenized in PBS (the volume depends on the weight of the tissue) with a glass homogenizer on ice. To further breakdown the cells, sonication of the suspension with an ultrasonic cell disrupter had been done. The tissue homogenates were then centrifuged at 5000×*g* for 5 min. Then, the supernatants were separated. The concentration of sIgA in these supernatants was assayed by a two-site sandwich enzyme-like immunosorbent assay (ELISA) using ELISA kits according to the manufacturer's instructions (Wuhan Elabscience Biotechnology Co., Ltd) (Schumacher et al. 1988).

Statistical analysis

Data were presented as means ± standard deviation (SD) using Statistical SPSS for Windows, issue 15.8. Statistical significance was determined using *t* tests (Mann-Whitney), chi-square tests, and one-way analysis of variance. A *p* value less than 0.05 was considered significant.

Results

Survival rate

The infected immunosuppressed mice exhibited slow locomotion, lethargy, and losing body weight. Although the change in the body weight of mice of the various studied groups was statistically nonsignificant, there was a decrease in body weight of the mice of both infected immunocompetent and immunosuppressed groups on the tenth day postinfection (Table 1). Additionally, the survival rate of the infected immunocompetent and infected immunosuppressed mice was similar until the third day postinfection. Starting from the fourth day postinfection, the survival rate of the infected immunosuppressed mice was statistically less than that of the infected immunocompetent mice (*p* value ≤0.03) as shown in Fig. 1.

Detection of *Blastocystis* spp. in stools

By light microscopy, the vacuolar form of *Blastocystis* spp. was the most commonly detected in the mice's stool samples, followed by cystic and granular forms (Fig. S1a–c). On the contrary, the amoeboid form was

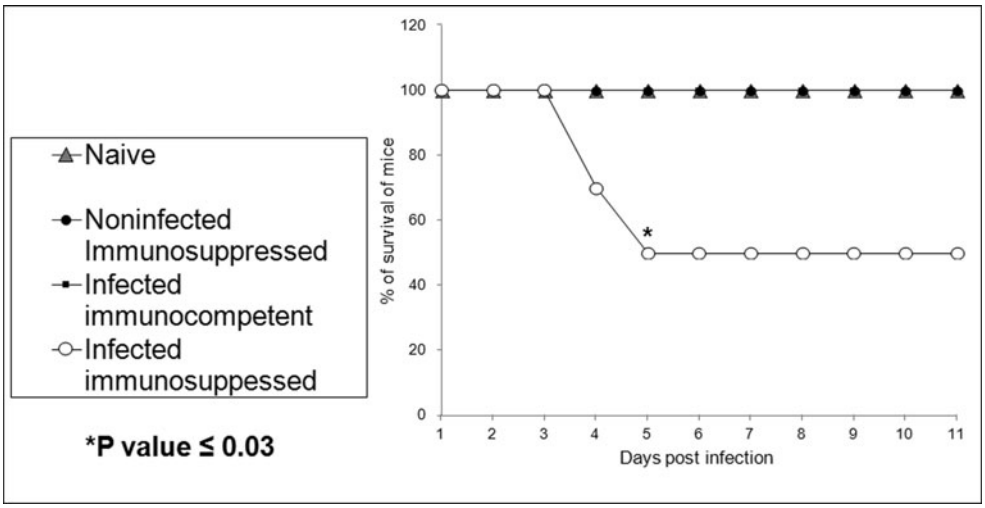

Fig. 1 Survival rate of the mice of the various studied groups. *Closed triangles* represent the data of the naive group. *Closed circles* represent the data of the noninfected immunosuppressed group. *Closed squares* represent the data of the infected immunocompetent group. *Open circles* represent the data of the infected immunosuppressed group. Data are presented as the mean ± SD

Table 2 *Blastocystis* shedding intensity in the stool of mice of the various studied groups on 2 days interval until the tenth day postinfection

Mice group/parasite load days (postinfection)	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10
Parasite load in the stool of mice of different studied groups (mean ± SD)						
Naïve	0	0	0	0	0	0
Noninfected-immunosuppressed	0	0	0	0	0	0
Infected-immunocompetent	0	2.5 ± 0.7	4.4 ± 0.5	7.2 ± 0.8	9.5 ± 0.5	11.6 ± 0.5
Infected-immunosuppressed	0	5.28 ± 0.8***	11.4 ± 0.9***	15.25 ± 0.9**	18.25 ± 0.9***	23.6 ± 1.4***

*N** = 10 mice in all groups except in infected immunosuppressed where N equals 7 mice on day 4 and 5 mice on days 6, 8, and 10

*$p \leq 0.05$; **$p \leq 0.001$; ***$p \leq 0.0001$

the least detected one. The average number of *Blastocystis* spp. forms/HPF in the stool of the infected mice is shown in Table 2. *Blastocystis* spp. shedding in all mice were initially detected on second day postinfection and continued in shedding until the end of the tenth day of the experiment. However, throughout the experiment, the intensities of *Blastocystis* spp. shedding were significantly greater in the infected immunosuppressed than those of the infected immunocompetent mice (Table 2).

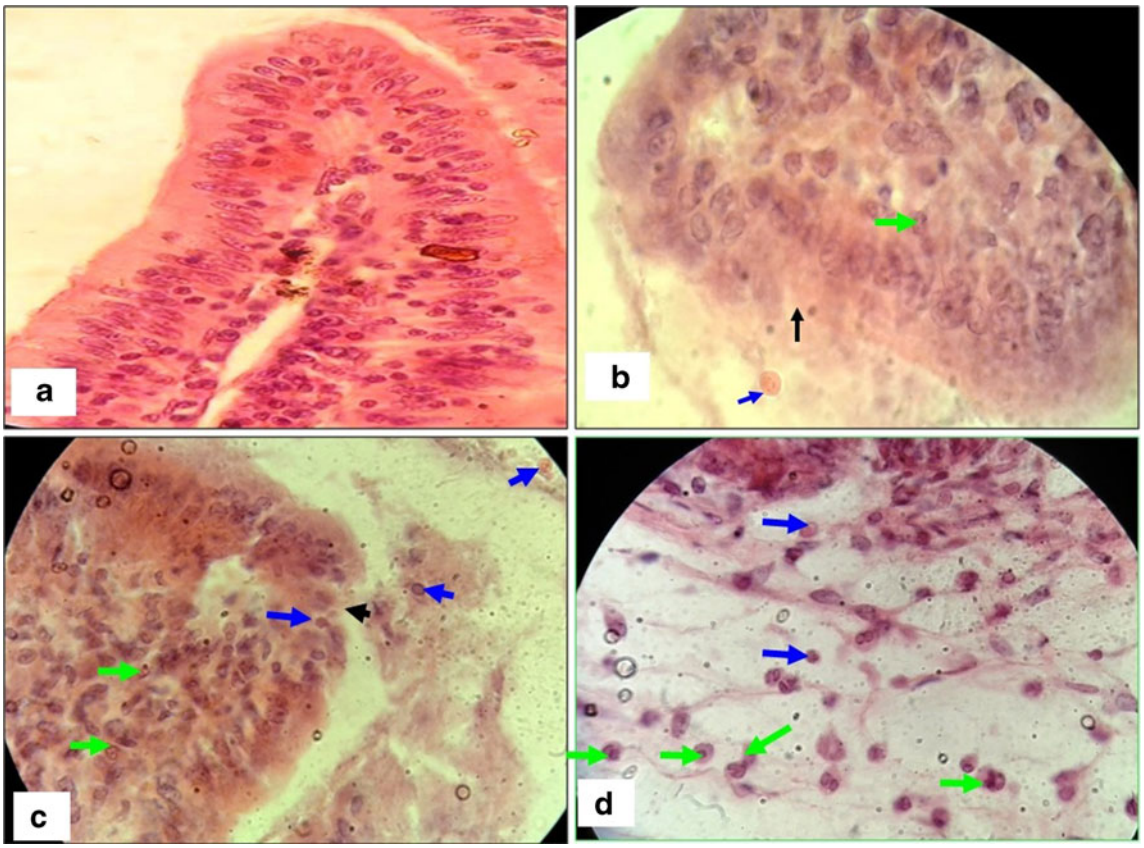

Fig. 2 Histological section of ileum stained with H&E, ×100. **a** A photomicrograph of intestinal villus in ileum of naïve mice is showing columnar epithelial liningon thin lamina propria. **b** A photomicrograph of intestinal villus in ileum of the infected immunocompetent mice is showing few luminal *Blastocystis* (*blue arrow*) with minute area of sloughed epithelial lining (*black arrow*). The lamina propria contains a very few number of eosinophils (*green arrow*). **c** A photomicrograph of intestinal villus in ileum of the infected immunosuppressed mice is showing many luminal *Blastocystis* (*blue arrow*) with large area of sloughed epithelial lining (*black arrow*). The lamina propria shows abundant eosinophils (*green arrow*). **d** A photomicrograph of intestinal villus in ileum of the infected immunosuppressed mice is showing that the *Blastocystis* was dispersed throughout the lamina propria and the submucosal layers (*blue arrow*) with a large number of eosinophils (*green arrow*)

Histolopathological examination

Histopathological examination of the whole small intestine showed that *Blastocystis* spp. (mainly granular form) were observed in the luminal content of the terminal part of the ileum. However, it was fewer in the infected immunocompetent mice than that of the infected immunosuppressed one (Fig. 2b, c). In both groups, villius exhibited shortening or attenuation (Fig. 2b, c). Though, the intestine of the infected immunosuppressed showed many areas of the mucosal layer with epithelial desquamation, minute ulcerations, and *Blastocystis* spp. infiltrating enterocytes (Fig. 2c). Furthermore, *Blastocystis* sp. was dispersed throughout all the intestinal layers (mucosal layer, the lamina propria, and the submucosal layer) and was accompanied with intense inflammatory-cell infiltration and intensive eosinophilia in the infected immunosuppressed intestine (Figs. 2c, d and S2).

Histological examination of the caecum and colon of the noninfected immunosuppressed mice showed normal columnar epitheial linning (Fig. S3). Histological examination of the caecum and colon of the infected immunosuppressed group showed edematous lamina propria and severe congestion (Fig. 3a, b). *Blastocystis* spp. dispersed throughout the muscularis mucosa layer (Fig. 3c). However, sections of infected immunocompetent ones showed minute edema and congestion (Fig. 3d). The luminal content of *Blastocystis* spp. of the infected immunosuppressed mice (Fig. 3e) was greater than that of the infected immunocompetent ones (Fig. 3f). There was severe goblet cells hyperplasia in the cecal and colonic sections of the infected immunosuppressed mice (Fig. 4a, b), while mild goblet cells hyperplasia were observed in the cecal and colonic sections of the infected immunocompetent mice (Fig. 4d). Furthermore, *Blastocystis* spp. were dispersed through all the layers of the intestine of the infected immunosuppressed mice (Fig. 4a–c).

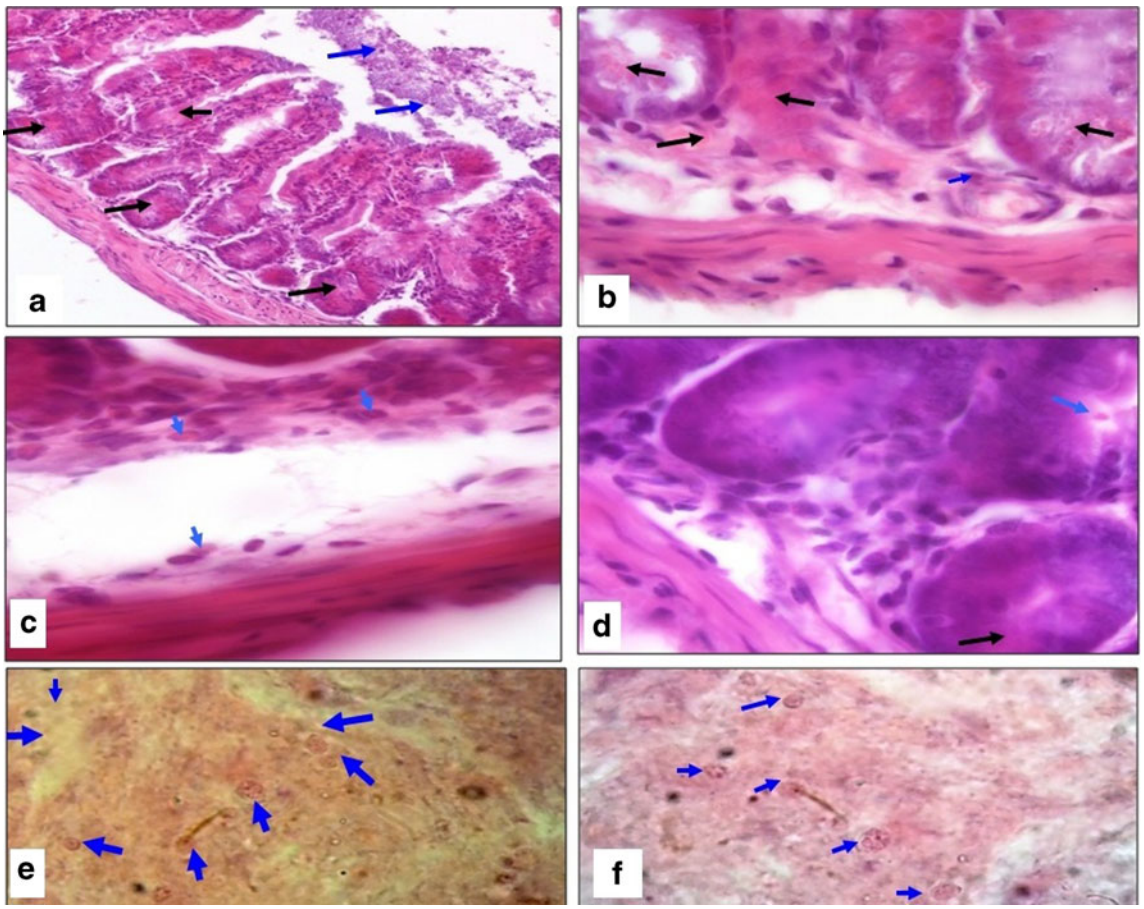

Fig. 3 Histological section of the intestinal crypts of the colon stained with H&E, ×100 (**a**). A photomicrograph of the intestinal crypts of the colon of infected immunosuppressed mice is showing severe edema and congestion of blood vessels (*black arrow*), ×10 (**b**). A photomicrograph of the intestinal crypts of the colon of infected immunosuppressed mice is showing the lamina propria containing many *Blastocystis* (*blue arrow*) (**c**). A photomicrograph of the intestinal crypts of the colon of infected immunosuppressed mice is showing that the *Blastocystis* was dispersed throughout the muscularis mucosa (**d**). A photomicrograph of the intestinal crypts of the colon of infected immunocompetent mice is showing mild edema and congestion of blood vessels (*black arrow*). The lamina propria contains *Blastocystis* (*blue arrow*) (**e**). A photomicrograph of the luminal contents of the colon of infected immunosuppressed mice is showing that the number of *Blastocystis* was greater than that of infected immunocompetent ones (**f**)

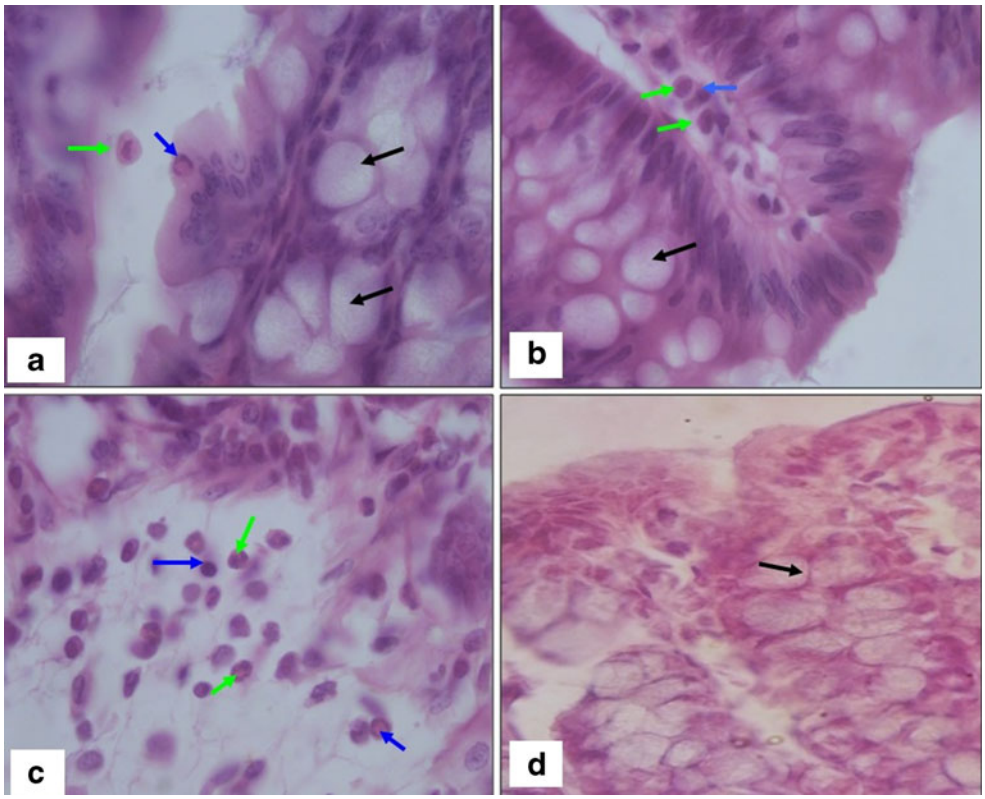

Fig. 4 Photomicrographs of the intestinal crypts of the colon showing goblet cells stained with H&E, ×100. **a** A photomicrograph of the intestinal crypts of the colon of infected immunosuppressed is showing severe hyperplasia of goblet cells (*black arrow*) and the lamina propria contains many *Blastocystis* (*blue arrow*). **b** *Blastocystis* was dispersed throughout the muscularis mucosa (*blue arrow*). There are numerous inflammatory cells (*green arrow*) (**a**–**c**). **d** A photomicrograph of the intestinal crypts of the colon of infected immunocompetent mice is showing mild hyperplasia of goblet cells (*black arrow*)

Cytokine production

Cytokine responses are shown in Fig. 5a. There was significant upregulation of the expression of proinflammatory cytokines (IL-12) and TNF-α in the infected immunosuppressed group compared to that of the infected immunocompetent group. However, the expression of anti-inflammatory cytokines (IL-4 and IL-10) was significantly downregulated in the infected immunosuppressed group compared to that of the infected immunocompetent one on the tenth days postinfection (p values were ≤0.002 and ≤0.001, respectively).

SIgA production

SIgA production is shown in Fig. 5b. There was a significant upregulation of the locally synthesized antibody of the sIgA class in the gut of the infected immunocompetent group compared to that of the infected immunosuppressed group ($p \leq 0.005$). This played a significant immunomodulatory role in the clearance of *Blastocystis* spp. infection from the gut of the infected immunocompetent group.

Discussion

Since symptoms associated with *Blastocystis* spp. infection were generally more severe in immunocompromised patients than in immunocompetent individuals (Garavelli et al. 1988, 1991), the main focus of this study was to establish an immunosuppressed mouse model for studying this parasite and comparing immunopathological changes in immunosuppressed and immunocompetent mice following *Blastocystis* spp. infection.

Furthermore, many researchers reported that clinical symptoms of blastocystosis varied among individuals as some patients are asymptomatic, while others display severe abdominal cramps, diarrhea, and fatigue. Researchers also linked *Blastocystis* spp. with IBS (Stark et al. 2007; Boorom et al. 2008; Stensvold et al. 2009). Others concluded that a clear understanding of the parasite pathogenicity still requires further studies (Yakoob et al. 2004).

In this research, the loss of body weight, lethargy, and slow movement was the common clinical symptoms in immunosuppressed mice following *Blastocystis* spp. infection. These clinical symptoms were obvious when compared them with those of the infected immunocompetent mice. This result confirmed the role of immunosuppression in increasing the susceptibility of mice for *Blastocystis* spp. infection. Additionally, the survival rate of the infected immunocompetent mice was higher than that of infected immunosuppressed ones.

Consistent with increased severity of *Blastocystis* spp. infection in immunosuppressed mice, the histopathological examination of the small intestine showed that *Blastocystis* spp. infiltrated the lamina propria, the submucosa, and the muscle

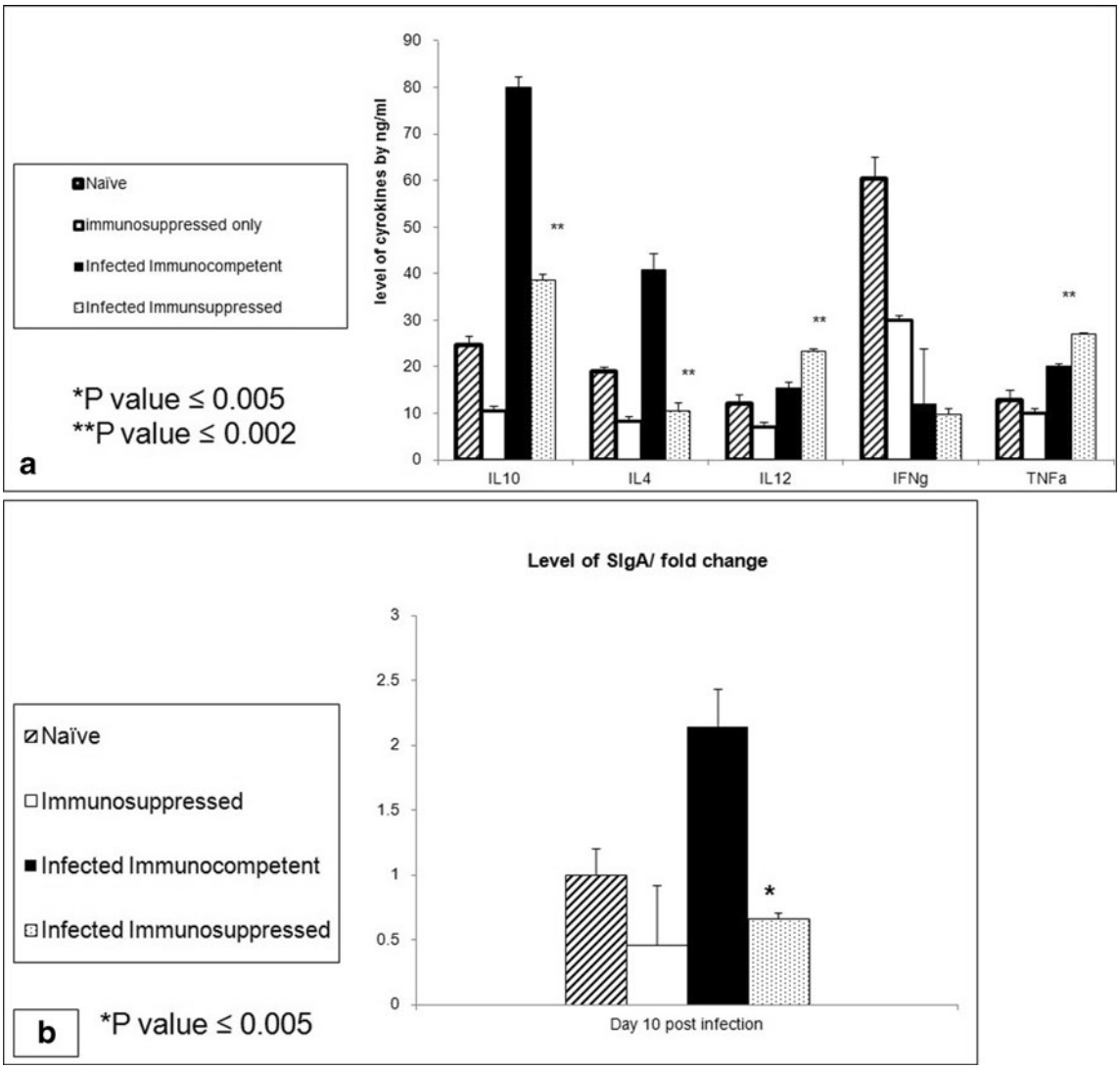

Fig. 5 **a** A histogram showing the changes in the serum levels of cytokines in the mice of different studied groups. **b** A histogram showing the changes in the intestinal tissue sIgA levels in the mice of different studied groups. Data are presented as the mean ± SD

layers. Moreover, there was severe edema, hyperemia, and congestion. The epithelia of small intestine showed exfoliation, and inflammatory cell infiltration was observed in the submucosa. These data were in agreement with Abou-El Naga and Negm (2001), Yao et al. (2005), Zhang et al. (2006), Iguchi et al. (2009), and Elwakil and Hewedi (2010).

As well, histological examination of the large intestine showed inflammatory-cell infiltration, edematous lamina propria, and mucosal sloughing. These findings were more intense in the intestine of infected immunosuppressed mice. This could be explained on the basis assuming that *Blastocystis* sp. is an invasive pathogen and capable of causing pathogenesis in BALB/c mice. The pathogenesis was more severe in immunosuppressed mice.

Moreover, the large intestine of infected immunosuppressed mice showed that *Blastocystis* spp. infiltrated the lamina propria, the submucosa, and the muscle layers forming collection of vacuolar forms. We speculate that this may be due to two reasons. The first reason is the release of cysteine protease by *Blastocystis* spp. which leads to evasion or modulation of the immune system by degradation of host immune molecules (Puthia et al. 2005). Some studies have demonstrated that cysteine proteases can increase epithelial permeability by modulating the tight junction complex (Mirza et al. 2012).

The second one is the release of hyaluronidase enzyme which leads to the degradation of extracellular matrix proteins namely hyaluronic acid facilitating the invasion by *Blastocystis* spp. into colonic epithelium (Chandramathi et al. 2010). Chandramathi et al. (2010) found elevation of hyaluronidase in urine of mice infected with *Blastocystis* spp. which was an indirect evidence of invasion of colonic epithelium by *Blastocystis* spp.

However, immunohistochemical studies have to be done to elucidate and confirm that these *Blastocystis* forms identified in the studied mice's tissues are invasive (Fayer et al. 2015).

Parasites were found mainly in the caecum and colon of the infected immunocompetent mice and in the whole intestine of the infected immunosuppressed mice. The later observation was also reported by Yao et al. (2005) who found *Blastocystis* spp. in the whole gut of experimentally infected mice. As well, severe edema, hyperemia, and congestion were observed in the tissues of caecum and colon of the infected immunosuppressed mice.

In the present work, vacuolar forms of *Blastocystis* spp. were the most common form found in the intestine; it can be postulated that the vacuolar forms developed from the inoculated cysts which are the most resistant forms able to withstand gastric digestion. This observation was also reported by El-Gebaly and Zaki (2012). This was also seen in vitro culture, where all noncyst forms arose from cysts within 24 and 48 h Abdel-Hafeez et al. (2015).

The mucous layer is an important barrier between protozoa and host epithelial cells (Ponce-Macotela et al. 2008). Thus, goblet cell hyperplasia had occurred after *Blastocystis* spp. infection.

Other researchers recorded that protein-losing enteropathy may accompany *Blastocystis* spp. infections and may be related to increased intestinal permeability (Dogruman and Hokelek 2007). An association with an alteration of goblet cell response and mucin production is observed in different intestinal infections induced by bacteria, viruses, and parasites (Boshuizen et al. 2005; Khan 2008; Hansson 2012).

Many studies demonstrated that the mechanisms of the protective role of goblet cells and mucins against parasites include the demonstration of trapping of worms in the mucus and inhibition of parasite motility and feeding capacity. In addition to enhancing the mucus barrier, goblet cells may play a role in immune activation by presenting luminal antigens to lamina propria dendritic cells (Miller 1987; Khan 2008; McDole et al. 2012).

There was a significant upregulation of the expression of proinflammatory cytokines (IL-12 and TNF-α) in the infected immunosuppressed group compared to that of the infected immunocompetent group (p values ≤0.004 and ≤0.002, respectively). These results suggest that *Blastocystis* spp. infection in mice induced to some extent an extensive local host response to the exposed antigens. These results were matching with the results obtained by Sinigaglia et al. (1999) and Iguchi et al. (2009). The infected immunosuppressed mice produced more proinflammatory cytokines than the infected immunocompetent one, and the differences were statistically significant. These data suggested that the colonization of *Blastocystis* spp. parasites provoked the activation (or influx) of T cells, monocytes/macrophages, and/or natural killer cells in local tissues (Iguchi et al. 2009).

Type 1 cytokines have been shown to be important for protective cell-mediated immune responses against a variety of intracellular pathogens (Sinigaglia et al. 1999), but it remains to be elucidated whether IFN-γ and IL-12 play a pivotal role in mucosal defense against *Blastocystis* spp. infection.

However, the expression of anti-inflammatory cytokines (IL-4 and IL-10) was significantly downregulated in the mice of the infected immunosuppressed group compared to that of the mice of the infected immunocompetent (p values ≤0.002 and ≤0.001, respectively). The infected immunocompetent mice activated production of both Th1 and Th2 cytokines. The synthesized IL-10 might be produced by the host's immune system in response to infection. IL-10 is thought to act as one of the regulatory cytokines that reduce inflammatory response Antibodies against *Blastocystis* spp. are one of the potential host defense mechanisms against parasitic infection. They have been suggested to play an important role against parasitic infection (Santos and Rivera, 2009). In mice infected with *Blastocystis* spp., sIgA was the predominant antibody isotype in intestinal secretions (Santos and Rivera 2009). In *Blastocystis* spp. infection, an increase in sIgA response was also observed. These data were matching with the results recorded by Mahmoud and Saleh (2003).

Conclusion

Blastocystis spp. infection in immunosuppressed mice was characterized by invasion of that parasite in all intestinal layers with mucosal sloughing, inflammatory cell infiltration, and severe goblet cell hyperplasia. Additionally, there was an elevation of proinflammatory cytokine (IL-12 and TNF-α), which may trigger the inflammatory process. There was a significant upregulation of the locally synthesized antibodies sIgA in the gut of the mice of the infected immunocompetent group compared to that of the mice of the infected immunosuppressed one. This played a significant immunomodulatory role in the clearance of *Blastocystis* spp. infection from the gut of the infected immunocompetent mice.

Conflict of interest None.

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