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ORIGINAL CONTRIBUTION

Protection against increased intestinal permeability and bacterial translocation induced by intestinal obstruction in mice treated with viable and heat-killed *Saccharomyces boulardii*

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Abstract
Background There are substantial evidences suggesting that probiotics can protect the gastrointestinal tract against inflammatory or infectious episodes. The effects of oral treatment with viable or heat-killed cells of *Saccharomyces boulardii* (*Sb*) on bacterial translocation, intestinal permeability, histological aspect of the ileum, and some immunological parameters were evaluated in a murine intestinal obstruction (IO) model.

Results Bacterial translocation and intestinal permeability in the IO group were significantly higher when compared to a Sham group ($p < 0.05$). Pretreatment with both viable and heat-killed *S. boulardii* prevented these increases, and the data obtained for IO + *Sb* and IO + heat-killed *Sb* groups were similar to those observed in the Sham group ($p > 0.05$). Histological analysis showed preservation of the ileum mucosa in mice that received both forms of the yeast when compared to the lesions observed in the IO group. The levels of serum interleukin (IL)-10 and intestinal secretory immunoglobulin A (sIgA) were higher in the animals that received both yeast treatments when compared to those from IO and Sham groups.
Conclusion Oral treatment with viable or heat-killed cells of *S. boulardii* maintained intestinal integrity and modulated the immune system in a murine IO model, preventing bacterial translocation and intestinal lesions.

Keywords *Saccharomyces boulardii* · Probiotic · Bacterial translocation · Intestinal permeability IL-10 · sIgA

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Introduction

There are substantial evidences to support the idea that a balanced gut microbiota confers important benefits for host health, such as the protection against pathogenic bacteria and inflammatory process. Under normal conditions, the composition of the microbiota is stable but can be disturbed by various factors such as diet changes, medications, and stressful states [1]. In such situations, the indigenous microbiota can be rapidly replaced by an overgrowth of pathogenic organisms, and a simultaneous increase in

mucosal barrier permeability is also frequently observed [2, 3]. Intestinal barrier dysfunction may allow the penetration of luminal antigens such as bacteria and their toxins, event known as bacterial translocation (BT) [4]. The translocation of bacteria and toxins across a leaky mucosa may be responsible for the release of systemic mediators and for the activation of immunologic cells that contribute to the development of systemic inflammation and multiple organ failure [3, 5, 6].

Three mechanisms are involved in BT: intestinal bacteria overgrowth, deficiencies in host immune defenses, and increased permeability or damage of the intestinal mucosal barrier [5, 7, 8]. Increased intestinal permeability and BT were frequently observed following shock, burn injury, obstructive jaundice, intestinal resection, hepatic transplant, or intestinal obstruction (IO) [9–13]. Studies have shown that 10% of patients hospitalized develop nosocomial infections, and millions of dollars are spent annually to treat these infections [8, 14]. It is known that some of such infections are of exogenous origin; however, a large number comes from BT of components of the indigenous microbiota.

Treatment or prevention of gut mucosal dysfunction using probiotics has been reported [11, 12, 15]. Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit to the host [16]. Although this definition emphasizes the need of probiotic viability, some authors have evidenced that heat-killed, ultraviolet-inactivated, and even components of these agents may be just as effective [17, 18]. This is an important concern for the use of live or dead microbial cells as biotherapeutic agents, since under certain clinical conditions (critical and immunocompromised patients), viable probiotic can translocate from the intestinal lumen into the internal compartment and produce systemic infection [19].

Saccharomyces boulardii is a non-pathogenic yeast that has been proven to be effective in the treatment of a variety of diarrheal diseases. This yeast has been successfully used for prevention and/or treatment of antibiotic-associated diarrhea, of acute gastroenteritis in adults and children, and of *Clostridium difficile*–associated disease [19–24]. However, there are relatively few studies about the effect of *S. boulardii* on alterations in intestinal mucosa, and the mechanisms of action are not yet fully understood. However, some evidence suggests that the yeast could act by modulation of both local and systemic immune system, as well as by a preservation of the intestinal barrier [25].

The present study evaluated the effect of viable or heat-killed cells of *S. boulardii* on BT, intestinal barrier integrity, intestinal lesions, and some aspects of the immune system in a murine IO model.

Materials and methods

Microorganisms

Viable *S. boulardii* cells were used after isolation onto Sabouraud dextrose agar (Difco, Sparks, USA) from a lyophilized commercial preparation (Floratil®, Merck S.A., Rio de Janeiro, Brazil). For the probiotic use of its heat-killed form, a suspension of *S. boulardii* cells was autoclaved at 121 °C for 15 min, and then an aliquot was spread onto Sabouraud dextrose agar for confirmation of the non-viability of the yeast. *Escherichia coli* ATCC 10536 was obtained from the American Type Culture Collection (Rockville, USA).

Mice

Swiss male mice, weighting between 25 and 35 g, were used in this study. The animals were reared in an open animal house where tap water and standard laboratory chow were given ad libitum. This study was approved by the Ethics Committee for Animal Experimentation of the Federal University of Minas Gerais (CETEA/UFMG) and complies with the guide recommended by the Institute of Laboratory Animal Resources for the care and use of laboratory animals.

Experimental design

For each experiment (intestinal permeability, BT determination, histological examination, and immunological analyses), animals were randomized into four groups: I—Sham group (gavage with 0.1 mL saline; laparotomy in which the ileum was manipulated but not ligated); II—IO group (gavage with 0.1 mL saline; laparotomy and ileal ligation); III—IO + *Sb* group (gavage with 0.1 mL of a suspension containing 10^9 colony forming units (CFU)/mL of *S. boulardii*; laparotomy and ileal ligation); and IV—IO + heat-killed *Sb* group (gavage with 0.1 mL of a suspension containing heat-killed *S. boulardii* cells; laparotomy and ileal ligation). The animals were gavaged for 10 days before the IO procedure and were monitored daily for food intake and body weight gain.

Intestinal obstruction procedure

Ten days after the above described treatment, mice of all groups were anesthetized intraperitoneally with tiazine (8 mg/Kg) and ketamine (60 mg/Kg) solutions. The abdomen was opened through a midline incision, and the terminal ileum was isolated and ligated using a single suture around the ileum with nylon. The abdominal wound was closed in two layers. Animals in the Sham group only

underwent simulated operations—laparotomy in which the ileum was manipulated but not ligated [13, 26].

Radiolabeling of *E. coli*

BT analysis was done following the procedure as described by Diniz et al. [27]. A sample of *E. coli* ATCC 10536 culture grown overnight on tryptic casein agar (Difco) was transferred to 10 mL of sterile saline solution. The bacterial concentration was adjusted to 31% of transmittance in a spectrophotometer at 580 nm, which corresponds to approximately 10^8 CFU/mL. An aliquot of bacterial suspension (2 mL) was incubated in tubes containing 1 mL of stannous chloride solution (580 mM, pH 7.0) at 37 °C for 10 min. After incubation, 37.0–55.5 MBq of technetium-99m (^{99m}Tc) obtained by elution from the sterile ^{99}Mo/^{99m}Tc generator (IPEN/Brazil) was added, and the preparation was kept at 37 °C for another 10 min. The tubes were then centrifuged at 3,000 *g* for 25 min. This procedure was repeated three times. After the last centrifugation, the radioactivity of the supernatant and precipitate was measured in a dose calibrator (CRC®-25R Dose Calibrator, Capintec, Ramsey, USA), and the percent ^{99m}Tc incorporated into the bacterial cells was determined using the following equation:

$$\%\,\text{Labeled bacteria} = \left(\frac{\text{cpm of precipitate}}{\text{cpm of precipitate} + \text{cpm of supernatant}}\right) \times 100$$

where cpm is counts per minute.

Determination of bacterial translocation

Four groups of eight animals each were treated as described above. After 10 days of treatment, 0.1 mL of a suspension containing 1.8 MBq of the ^{99m}Tc-*E. coli* was administered by gavage to all animals. After 90 min, the mice underwent surgical procedure as described above. Eighteen hours after surgery, the animals were once again anesthetized using the same technique. The blood, mesenteric lymph nodes (MLN), liver, spleen, and lungs were removed, weighed, and placed into appropriate tubes radioactivity determination [13, 26]. The samples were counted in a counter with a NaI (Tl) crystal (ANSR-Abott, Chicago, USA). Values were expressed as cpm/g or cpm/mL.

Determination of intestinal permeability

Intestinal permeability was determined by measuring radioactivity diffusion in the blood after oral administration of diethylenetriamine pentaacetic acid (DTPA) labeled with ^{99m}Tc. After 10 days of the above described treatments, all mice received 0.1 mL of DTPA solution labeled with 18.5 MBq of ^{99m}Tc by gavage. After 90 min, all mice underwent the IO procedure as previously described. Four, eight, and 18 h after the surgery, animals (five for each time and group) were anesthetized once again, and 500 μL of blood was collected and placed in appropriate tubes for radioactivity determination [28]. Data were expressed as % dose, using the following equation:

$$\%\,\text{Dose} = \left(\frac{\text{cpm of blood}}{\text{cpm of administrated dose}}\right) \times 100$$

Histopathological analysis

At least, three rings of the ileum area adjacent to the surgical intervention were obtained, fixed in buffered 4% formaldehyde, and processed for routine paraffin embedding. These samples were harvested in the 18th hour. From each sample, at least 3 histological sections (4–5 μm) were stained with hematoxylin and eosin (H&E), coded, and analyzed by optical microscopy by a single pathologist who was unaware of the experimental conditions of each group. An intestinal damaging score based on criteria of Chiu's method [29] was determined for each sample. Criteria of Chiu grading system consist of five subdivisions according to the changes in villus and gland of intestinal mucosa as follows: grade 0—normal mucosa; grade 1—development of sub-epithelial Gruenhagen's space at the tip of villus; grade 2—extension of the space with moderate epithelial lifting; grade 3—massive epithelial lifting with a few denuded villi; grade 4—denuded villi with exposed capillaries; and grade 5—disintegration of the lamina propria, ulceration, and hemorrhage. Each section was scored per animal and per group. The score is expressed as the average of the evaluation of four animals per group.

Serum IL-10 and INF-γ determination

Blood was harvested on the 18th hour and centrifuged at 1,000 *g* for 10 min, and serum was collected and stored at −70 °C until assay. The concentration of IL-10 and INF-γ was measured by ELISA using commercially available antibodies, according to the procedures supplied by the manufacturer (Biosource International INC., Camarillo, USA).

Intestinal secretory immunoglobulin A (sIgA)

After killing, the small intestine of mice from all groups was removed, and the contents were withdrawn, weighed, and suspended in PBS using 500 mg of intestinal contents per 2.0 mL PBS supplemented with an anti-protease

cocktail. After centrifugation at 2,000 *g* for 30 min at 4 °C, the supernatant was collected and kept frozen at −70 °C until use. Immunoglobulin levels in intestinal fluid were evaluated by ELISA using goat anti-mouse IgA (Sigma Chemical Co., St. Louis, USA) and horseradish peroxidase-conjugated goat anti-mouse IgA (Sigma). Color was developed with *o*-phenylene-diamine (OPD, Sigma), and absorbance at 492 nm was determined with an ELISA plate reader. The concentrations of the immunoglobulin were determined using a purified mouse IgA standard (Southern Biotechnology Associates Inc., Birmingham, USA) [30].

Statistical analysis

All experiments were performed at least in duplicate. Intestinal permeability results were compared using Student′s *t* test. The BT data were treated with the Kruskal–Wallis analysis of variance and a post hoc analysis using the Dunn's test. The differences were considered statistically significant for $p < 0.05$. All analyses were performed using the program BioEstat Version 3.0 (Mamiraua Civil Society/MCT-CNPq).

Results

Food ingestion and body weight gain were similar among the four groups throughout the 10 days of experimental treatment.

IO promoted an increase in BT as demonstrated by a significant higher uptake of ^{99m}Tc-*E.coli* in the blood, MLN, liver, spleen, and lungs of the animals in the IO group when compared with the Sham group ($p < 0.05$) (Table 1). The administration of viable *S. boulardii* reduced BT to the blood and to all the organs investigated when compared with IO group ($p < 0.05$). Similar results were obtained when animals were treated with the heat-killed cells.

Figure 1 shows that experimental IO increased intestinal permeability when compared to Sham group throughout all of the investigated times ($p < 0.05$). Pretreatment with *S. boulardii* reduced intestinal permeability, even when heat-killed cells were used, and the values were similar to those observed in the Sham group.

The histological aspects and damaging score are shown in Figs. 2 and 3, respectively. The samples obtained from the Sham group did not present histological alterations (intestinal damaging score lower than one (0.25 ± 0.5) in panoramic and detailed view (Fig. 2a, b). On the other hand, the small intestinal portions of the animals that underwent IO showed focal erosion of surface epithelium associated with intense edema and vascular congestion of the intestinal wall, mainly in the lamina propria (Fig. 2c, d). Disturbance in intestinal mucosa architecture was also characterized by an increased cellularity and enlarged villi presenting height reduction. Additionally, presence of sub-epithelial Gruenhagen's space at the top of villus and epithelial lifting with denuded villi were observed. An intestinal damaging score of four was determined for IO group (4 ± 0). On the contrary, animals that received treatment with viable *S. boulardii* (IO + *Sb*) showed discrete edema and preserved structural architecture of the intestinal mucosa (Fig. 2e, f). Almost similar aspects were observed when animals were treated with heat-killed *S. boulardii* (IO + heat-killed *Sb*). In this case, increased cellularity of lamina propria and discrete shortening of the villi height were observed, but there was no presence of

Table 1 Effects of experimental IO and oral treatment with viable and heat-killed *S. boulardii* (*Sb*) on BT, determined as biodistribution of ^{99m}Tc-*E. coli* in body organs and blood (in counts per minute/g or ml)

Organ/blood	Sham	IO	IO + *Sb*	IO + Killed *Sb*
MLN	180.23[a]	845.83[b]	160.41[a]	190.21[a]
Spleen	123.65[a]	676.25[b]	193.33[a]	213.00[a]
Liver	473.26[a]	1,794.14[b]	649.69[a]	691.38[a]
Lung	152.08[a]	629.58[b]	252.09[a]	251.00[a]
Blood	140.80[a]	465.81[b]	184.33[a]	171.25[a]

[a, b] Different letters on the same line indicate statistically significant differences ($p < 0.05$) by Kruskal–Wallis analysis of variance and post hoc analysis using the Dunn's test. Data are expressed as median. $N = 8$

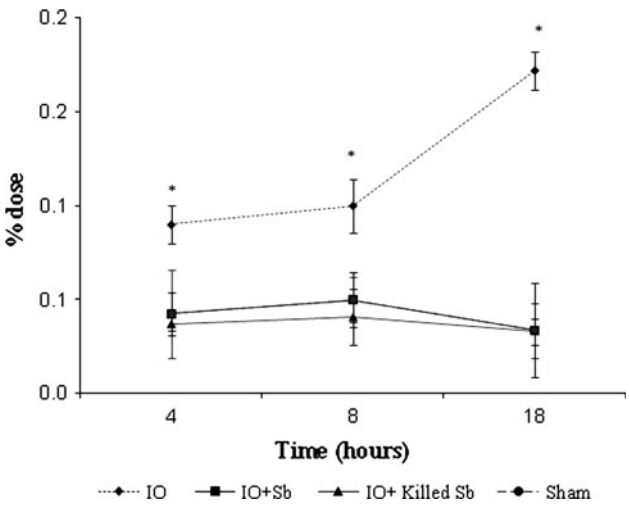

Fig. 1 Effects of experimental IO and oral treatment with viable and heat-killed *S. boulardii* on intestinal permeability. % dose = (counts per minute of blood/counts per minute of administrated dose) × 100. *Errors bars* show the SD. *Indicates statistically significant differences ($p < 0.05$) by Student′s *t* test between the IO group and the other groups throughout all investigated times. $N = 5$ for each time

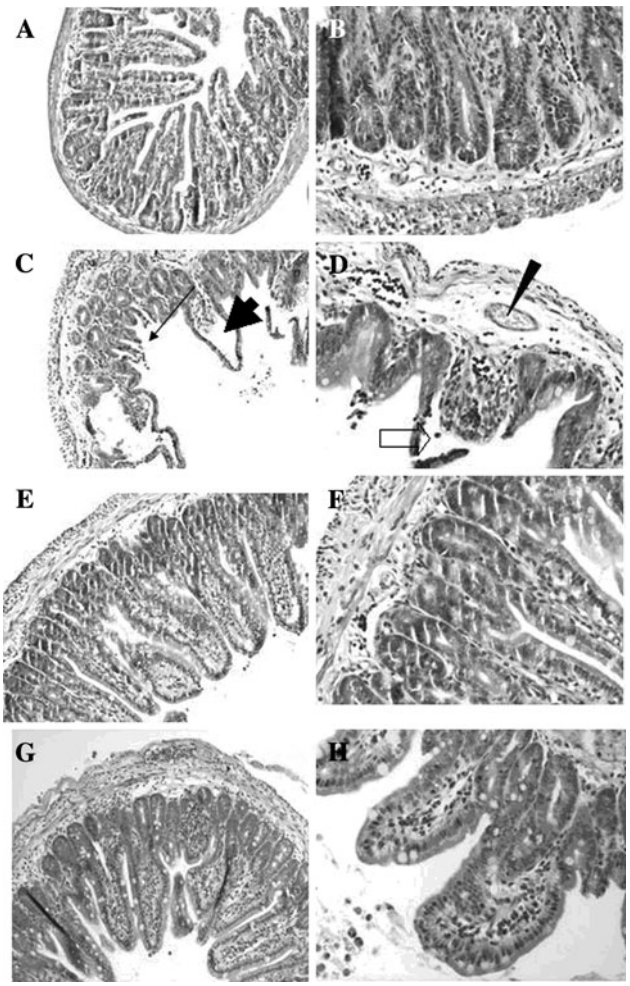

Fig. 2 Effects of experimental IO and oral treatment with viable and heat-killed *S. boulardii* on histological aspects of small intestine tracts. (**a**, **b**) Normal aspect of ileal epithelium and intestinal wall, Sham group, corresponding to score <1. (**c**, **d**) IO group, notice the epithelial and vascular significant changes: development of sub-epithelial Gruenhagen's space at the tip of villus (*large arrow* in **c**); epithelial lifting, denuded villi with exposed capillaries (*open arrow* in **d**); edema (*long arrow* in **c**); congested vessel (*arrowhead* in **d**), corresponding to score 4. In (**e**, **f**) IO + Sb and (**g**, **h**) IO + killed Sb, significant preservation of the structural aspect of ileum mucosa, corresponding both to score <1. H&E, Original magnification: **a**, **c**, **e**, **g**: 10× objective; **b**, **d**, **f**, **h**: 20× objective. $N = 4$

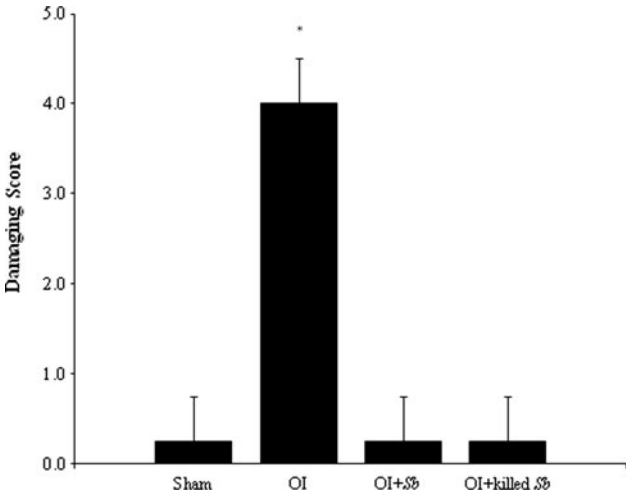

Fig. 3 Intestinal damaging score based on criteria of Chiu's method. The grading system consists of five grades according to the changes in villus and gland of intestinal mucosa as follows: grade 0—normal mucosa; grade 1—development of sub-epithelial Gruenhagen's space at the tip of villus; grade 2—extension of the space with moderate epithelial lifting; grade 3—massive epithelial lifting with a few denuded villi; grade 4—denuded villi with exposed capillaries; and grade 5—disintegration of the lamina propria, ulceration, and hemorrhage. The score is expressed as the average of the evaluation of three animals per group. *Indicates statistically significant differences ($p < 0.05$) by Student's t test between the IO group and the other groups. $N = 4$

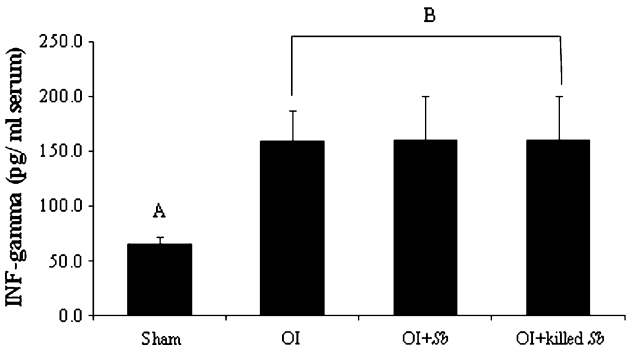

Fig. 4 Effects of experimental IO and oral treatment with viable and heat-killed *S. boulardii* on plasma levels of IL-10. *Errors bars* show the SD. Different letters indicate statistically significant differences ($p < 0.05$). Data are expressed as media. $N = 5$

sub-epithelial Gruenhagen's space at the tip of the villi or epithelial lifting with denuded villi in any of the evaluated samples from this group (Fig. 2g, h). For both treatments with the yeast, intestinal damaging scores (0.25 ± 0.5 for both) were significantly lower when compared to IO group and similar to Sham group.

The levels of IL-10 in the blood of animals that received both viable and heat-killed yeasts were significantly higher (160.0 and 159.9 pg/mL, respectively) than that observed in IO group (50.75 pg/mL), which in turn was higher than in Sham group (29.58 pg/mL) ($p < 0.05$) (Fig. 4). Figure 5 shows that levels of INF-γ were similar in all the groups submitted to IO ($p > 0.05$) and higher than in the Sham group ($p < 0.05$).

Figure 6 shows that intestinal sIgA levels were similar in IO group when compared to Sham group (742 μg/g intestinal fluid and 736 μg/g intestinal fluid, respectively) ($p > 0.05$). Treatment with both viable and heat-killed yeast significantly increased the levels of sIgA (1,390 and 1,309 μg/g intestinal fluid, respectively) when compared to IO group ($p < 0.05$).

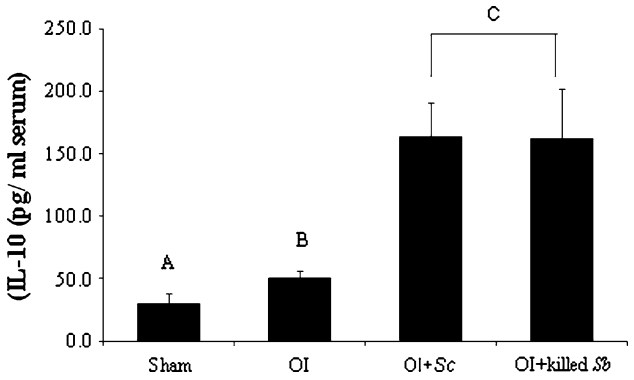

Fig. 5 Effects of experimental IO and oral treatment with viable and heat-killed *S. boulardii* on plasma levels of INF-γ. *Errors bars* show the SD. Different letters indicate statistically significant differences ($p < 0.05$). Data are expressed as media. $N = 5$

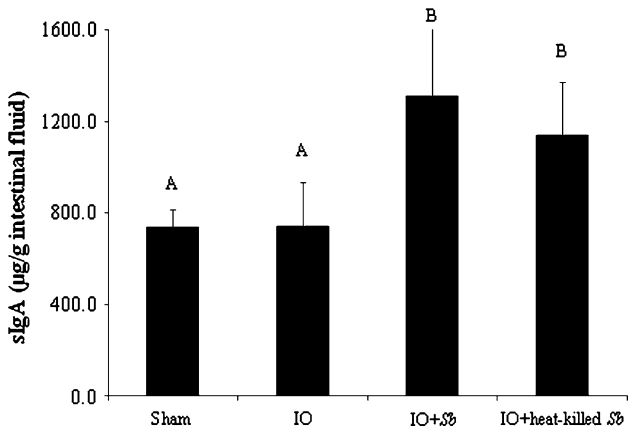

Fig. 6 Effects of experimental IO and oral treatment with viable and heat-killed *S. boulardii* on intestinal levels of sIgA. *Errors bars* show the SD. Different letters indicate statistically significant differences ($p < 0.05$). Data are expressed as media. $N = 5$

Discussion

The gastrointestinal tract has functions other than digestion and absorption of food as well as excretion of feces. Intestinal barrier function describes the ability of the gut epithelium to potentially separate harmful luminal contents such as bacteria and endotoxins from the closely regulated internal milieu of the body [5, 14]. Consequently, any gut injury or barrier failure contributes to the translocation of bacteria and endotoxins from the gut to the portal and systemic circulations.

It is well known the beneficial effect of the microbiota on intestinal and immune function. The inter-relationships between microbiota and host are clearly important for health, and an imbalance between these systems appears to drive to a wide range of mucosal and systemic immune-mediated disorders [31]. Nowadays, there is a growing interest in the use of probiotics in clinical medicine for prevention and treatment of various diseases [31, 32]. There is convincing evidence that this therapy targets to the gut improves clinical outcomes of several patients [33, 34]. Thus, the maintenance of a stable ecological balance in the gastrointestinal tract would be the greatest defense mechanism in the prevention of BT [35].

In the present study, we have used an oral treatment with a suspension of viable or heat-killed cells of *S. boulardii* to verify its potential impact on maintaining gut mucosal barrier integrity, reducing BT, intestinal lesions, and stimulating the immune system after IO in a murine model.

To analyze the influence of *S. boulardii* on BT, we used radiolabeled *E. coli*. Few studies have been performed to evaluate this aspect, and all of them have used culture-dependent methods for BT determination to MLN, liver, and spleen [11, 36]. These techniques can underestimate the levels of BT, as they do not detect fragments of bacteria and non-viable bacteria, which can also trigger an over-stimulated immune response [8, 37]. The uptake of ^{99m}Tc-*E. coli* is a very fast, direct, and simple method to determine BT, which does not require aseptic conditions and detects the presence of fragments as well as whole non-viable and viable bacteria cells in various internal compartments. According to Lichtman [7] and MacFie et al. [14], a low level of BT is a physiologic phenomenon needed for the maturation and maintenance of a competent gastrointestinal immune system. Our results are in agreement with this affirmation, since a basal count of radioactivity was observed in the blood and organs of the animals in the Sham group (Table 1). The higher uptake of ^{99m}Tc-*E. coli* to blood, MLN, liver, spleen, and lungs in animals of the IO group when compared to those in the Sham group ($p < 0.05$) (Table 1) is also in agreement with previous studies which have demonstrated that IO induced an increase in BT [13, 26, 28]. The potential mechanisms responsible for such a situation are disruption of the ecological balance of the indigenous microbiota and/or damage of the gut mucosal barrier by direct injury to the enterocytes and their junctions [38, 39]. Pretreatment with viable or heat-killed probiotic was able to reduce significantly the levels of BT ($p < 0.05$) (Table 1). Herek et al. [35] and Geyik et al. [11] found similar results using *S. boulardii* to prevent BT, but in two quite different experimental models (burn injury and jaundice obstruction).

Intestinal permeability is considered increased when permeation of molecules >150 Daltons is observed. In this study, intestinal permeability was evaluated by measuring blood radioactivity after the intake of ^{99m}Tc-diethylene-triaminopentaacetate (^{99m}Tc-DTPA) This molecule, used as a disodium complex, can be considered adequate for intestinal permeability determination due to its following characteristics: molecular weight of 549 Daltons, water soluble, non-charged, not destroyed in the gut, non-toxic,

not metabolized or sequestered, quantitatively cleared by the kidneys, and easily detectable [40, 41]. Results showed that IO induced an increase in intestinal permeability as observed in Fig. 1. As demonstrated in the same figure, oral treatment with viable or heat-killed *S. boulardii* maintained the intestinal permeability at physiological levels similar to those observed in the Sham group throughout all of the follow-up periods ($p > 0.05$). Increase in intestinal permeability was also observed in in vitro infectious models, such as of T84 cell culture infected with enteropathogenic *E. coli* [42], with enterohemorrhagic *E. coli* [43] or with *Salmonella* typhimurium [44], and in all these studies treatment with *S. boulardii* prevents the increase. In a recent clinical trial, Vilela et al. [45] observed important alterations in the intestinal mucosa integrity in patients with Crohn's disease in remission and demonstrated that an oral treatment with *S. boulardii* improved intestinal permeability. The mechanisms that could explain the maintenance of epithelial integrity by the yeast are not well known. However, the ability of *S. boulardii* to modulate the transduction pathways implicated in the control of tight-junction structure during the infection of T84 cells with enterohemorrhagic *E. coli* could be an explanation [43].

As demonstrated by histological examination (Fig. 2), IO was also associated with various serious intestinal lesions. In fact, IO is a very aggressive model, but nevertheless animals treated with *S. boulardii* presented good preservation of intestinal epithelium (Fig. 2e, g), which indicates that maintenance of mucosal barrier integrity may explain the significant decrease in BT levels in animals treated with the yeast. Similar results were reported by Aldemir et al. [38] and Geyik et al. [11], who observed higher villous height in rats treated with *S. boulardii* and undergoing intestinal loop obstruction when compared to the control group.

Inflammation is a complex reaction of the immune system that involves the accumulation and activation of leukocytes and plasma proteins at sites of infection, toxin exposure, or cell injury. While inflammation exerts a protective function in controlling infections and promoting tissue repair when balanced, it can also cause tissue damage and disease when uncontrolled. It is well known that an excessive production of pro-inflammatory cytokines, such as INF-γ, increases intestinal permeability and induces a cascade of inflammatory events resulting in mucosal injury by invading immune cells (neutrophils, tissue macrophages, and dendritic cells) which can result in sepsis [46]. To control this exacerbated systemic inflammatory situation, production of anti-inflammatory cytokines, particularly IL-10, contributes to the down-regulation of pro-inflammatory cytokines. Various studies showed that probiotics (*Escherichia coli* Nissle 1917, *S. boulardii, Lactobacillus,* and *Bifidobacterium*) have beneficial effect on balance between effector and regulatory T cells (T_H1, $T_H$reg, and T_H2) during intestinal inflammation due to acute infectious diarrhea (enteroinvasive *Escherichia coli* and *Salmonella* typhimurium) or to chronic inflammatory bowel diseases (ulcerative colitis, Crohn disease, and pouchitis) [47]. In the present study, as it could be expected, an increase in blood INF-γ was observed in mice from the IO group, and higher IL-10 blood concentrations were found in mice treated with the probiotic yeast, live or heat-killed. Considering the higher IL-10 production (regulatory cytokine) in mice treated with the yeast treatment and submitted to IO, a decrease in INF-γ would be expected, but this was not observed. However, if the ratio of the two cytokines is considered instead of the absolute value of each one (and this is important due to the opposite relationships between them), the ratio INF-γ/IL-10 was higher for the IO group (3.13) when compared to the Sham (2.19), IO + *Sb* (1.71), and IO + killed *Sb* (1.61) groups.

Other potential mechanisms underlying the reduction of BT by *S. boulardii* have been suggested. The yeast can compete with translocating bacteria for specific receptors on intestinal epithelium and/or can increase the production of IgA, seeing that these two mechanisms interfere with the bacterium adhesion. Adhesion is an initial step necessary for posterior BT, and it is well known that low concentrations of intestinal sIgA are associated with increased bacterial adherence to mucosa [30]. The intestinal levels of sIgA from animals that received the two forms of the yeast were significantly higher than those observed in animals from IO and Sham groups (Fig. 6). Rodrigues et al. [30] observed similar effects of the same yeast on the production of intestinal sIgA.

All probiotics present more than one mechanism to explain their protective effects. Combination of production of antagonistic compounds, competition for nutrients or adhesion sites, immunomodulation of the host, and fixation of bacterial toxins or pathogenic bacteria are the mechanisms more frequently observed [2]. Some of these mechanisms depend on the viability of the probiotic (antagonistic or anti-inflammatory products originated from the metabolism), and others (immunomodulatory or receptor molecules from the cellular structure) may not. The results of the present study showed that the protective ability of the yeast did not depend on its viability. This suggests that some structural components, probably from the yeast cell wall, are responsible for the reduction of BT, intestinal permeability, and intestinal lesions as well as the modulation of some aspects of the immunological system. As an opposite example, a recent study showed that only treatment with viable *S. boulardii* cells (and not with heat-killed yeast) was able to interfere with *Salmonella*-induced signaling pathways that are implicated in bacterial

internalization in a model of T84 cell culture infected with the pathogenic bacteria [44].

Conclusions

IO is an extremely aggressive model, which induced intestinal damage, including increase in intestinal permeability, destruction of the epithelium and BT. In the present study, the pretreatment with viable or heat-killed *S. boulardii* was able to preserve the intestinal barrier by maintaining intestinal permeability to physiological levels and consequently reducing BT and mucosal lesions, apparently due to mechanisms involving more of the cell structure than its metabolism. Thus, we have shown that in the most adverse situation such as IO (a rather extremely severe model), probiotic supplementation was able to positively impact on permeability and BT.

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