

Influence of *Saccharomyces boulardii* CNCM I-745 on the gut-associated immune system

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Background: The probiotic *Saccharomyces boulardii* CNCM I-745 (also known as *Saccharomyces cerevisiae* HANSEN CBS 5926; in the following *S. boulardii*) has proven its effectiveness in preventive and therapeutic treatment of many gastrointestinal diseases, especially diseases associated with acute diarrhea. In particular, antibiotic-associated diarrhea, *Clostridium difficile*-associated diarrhea, traveller's diarrhea, as well as acute diarrhea due to common viral and bacterial infections in children and adults.

Aim: The aim of this review is to summarize the experimental studies elucidating the molecular and immunological mechanisms by which these clinically proven effects are archived, with an emphasis on the gut-associated immune system. The main focus is laid on anti-inflammatory and immune-modulatory action of *S. boulardii* involved in bacterial or enterotoxin-mediated diarrhea and inflammation. An attempt is made to differentiate between the effects associated with cellular versus soluble factors and between prophylactic and therapeutic effects.

Methods: A literature search was performed in PubMed/PubMed Central for the effects of *S. boulardii* on the gut-associated immune system (focus acute diarrhea).

Results and conclusion: *S. boulardii* exhibits its positive effect by the direct effects on pathogens or their toxins as well as by influencing the host's infection-induced signaling cascades and its innate and adaptive immune system. The combination of these mechanisms results in a reduction of the pathogens' ability for adhesion or colonization and an attenuation of the overreacting inflammatory immune response. Thereby, the integrity of the intestinal epithelial cell layer is preserved or restored, and the diarrheic leakage of fluids into the intestinal lumen is attenuated.

Keywords: mode of action, probiotic, infectious gastrointestinal disease, diarrhea, safety

Introduction

Objective of this review

There is an expanding awareness of the role of the gut microbiome on immune function and response to pathogens. *Saccharomyces boulardii* is used worldwide for the prevention and treatment of infectious diarrhea of various etiologies. Meta-analyses have confirmed the clinical efficacy of *S. boulardii* in acute diarrhea of various causes in children¹ and in adults.²⁻⁴

Some of the effects of these infectious types of diarrhea might be evoked by a direct influence of *S. boulardii* on the modulation of the exiting gut microbes.⁵ Further mechanisms are trophic effects on enterocytes,⁶⁻⁸ reduction of bacterial virulence by toxin and pathogen binding⁹⁻¹³ as well as interference with bacterial motility and translocation^{13,14} (for review, Pothoulakis¹⁵).

This review summarizes the current knowledge on how *S. boulardii* interferes with pathogen-induced signaling pathways and how it exhibits anti-inflammatory effects.

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Also it describe how *S. boulardii* interacts with the innate or adaptive immune system to achieve its protective and therapeutic effects.

***S. boulardii* CNCM I-745, a specific probiotic**

S. boulardii is a yeast strain of the species *Saccharomyces cerevisiae* and has been used as a probiotic for >50 years. Historically, it has been thought to be a different *Saccharomyces* species before genetic analysis classified *S. boulardii* as a strain of the *S. cerevisiae* species. Therefore, the correct nomenclature for *S. boulardii* should be *Saccharomyces* (genus) *cerevisiae* (species) *var boulardii* (strain). Even though genetically very close, there are differences, which might be related to the number of genes involved in protein synthesis and stress response.^{16,17} As a consequence, *S. boulardii* exhibits a faster growth rate within the intestinal tract than *S. cerevisiae* due to its increased temperature optimum and its higher acid resistance.¹⁸ Compared to probiotics such as *Lactobacillus* and *Bifidobacterium*, *S. boulardii* has the advantage to be naturally resistant against all antibiotics by being a yeast.

CNCM I-745 is a *S. boulardii* strain produced by Biocodex Laboratories (Gentilly, France) and very well characterized by numerous preclinical and clinical data. In an expert opinion, the use of CNCM I-745 in various clinical conditions is evaluated.¹⁹

Search method

A literature search was performed in PubMed/PubMed Central for the effects of *S. boulardii* on the gut-associated immune system (focus acute diarrhea; September to October 2015). Main search terms were “*Saccharomyces boulardii*” combined with “immune”, associated with either “gastro*”, “gut”, or “intestinal”. Publication languages other than “English”, “French”, “Spanish”, or “German” were excluded.

Additional literature for specific topics (eg, gut-associated immune system or overview about diseases) and follow-up literature citations in the identified publications were added.

Interaction of *S. boulardii* and the immune system

Stimulating the immune activity in response to *S. boulardii*

Investigations on germ-free mice have shown the importance of the body's own microbiota in the development of the immune system.^{20,21} Even though germ-free mice are an artificial system, which is not comparable to a healthy

microbiome, it clearly demonstrates the importance of microbes for the development of the gut-associated immune system.

Therefore, it is not surprising that the probiotic *S. boulardii* modulates the host's gastrointestinal immune system. Apart from its anti-inflammatory abilities during infections, *S. boulardii* can assist the host immune system by inducing the release of immunoglobulins and cytokines in response to the yeast itself.

Immunoglobulin induction by *S. boulardii*

Secretory immunoglobulin A (sIgA) release is the first-line of defense against pathogens in the intestine. It prevents adhesion and forces the clearance of pathogens through several mechanisms.²² sIgA release can be enhanced by *S. boulardii* in germ-free mice^{23,24} as well as in normal BALB/c mice²⁵ or in the duodenal fluid of weanling rats.²⁶ The release of sIgA was even further increased when mice received *Clostridium difficile* toxin A during *S. boulardii* treatment.²⁵

Furthermore, in germ-free mice colonized with *S. boulardii*, there was an increase in total serum IgM as well as an increased number of Kupffer cells (liver macrophages). This leads to a more efficient clearing of enteropathogenic *Escherichia coli* (EPEC) from the blood stream compared to germ-free controls, coupled with a faster cytokine response.²⁴ This demonstrates that *S. boulardii* is able to modulate the host immune system toward a more activated state by increasing the host's resistance to enteropathogenic bacterial infections on a local level within the intestine, but also with systemic effects, for example, density of liver macrophages.

Induction of cytokines and immune cell maturation by *S. boulardii*

As early as 1986, Caetano et al were able to show in humans that *S. boulardii* can activate several cellular and humoral parameters involved in the nonspecific acute phase of defense against pathogens.²⁷ They observed an increase of erythrocytes and leucocytes together with an increase in serum complement values in response to exposure to *S. boulardii*.

The putative immunomodulatory role of *S. boulardii* in the activation of dendritic cells (DCs) prior to infection was observed by a slight transcriptional upregulation for tumor necrosis factor alpha (TNF α) and C-C chemokine receptor type 7 mRNAs after coincubation of DCs with *S. boulardii*. This upregulation before infection might make the DCs more effective in antagonizing bacteria.²⁸

Another study found that *S. boulardii* stimulated the production of several cytokines in DCs, including interleukin (IL)-1 β , IL-12, IL-6, TNF α , as well as IL-10. In addition,

S. boulardii induced high levels of the costimulatory molecules CD80 and CD86, indicative of DC maturation. Most likely, a heat-stable yeast cell wall-derived factor was responsible for the effects.²⁹

These findings suggest that *S. boulardii* leads to a general unspecific immune system activation, which can be advantageous for fighting infections.

S. boulardii-mediated immune priming

The release of immunoglobulins and cytokines in response to the yeast itself helps to explain why the preexposure to *S. boulardii* is advantageous for fighting subsequent infections.^{12,30} Anti-inflammatory cytokines increased during early stages of infection due to *S. boulardii* strengthen the host anti-inflammatory abilities.¹³

In an early stage of *Salmonella* infection (0–90 minutes), *S. boulardii* treatment was observed to lead to an increase in interferon- γ (a macrophage-stimulating cytokine) and a downregulation of IL-10 (a macrophage-inhibiting cytokine) in the small intestine, even in areas with low bacterial population. Only later during infection, *S. boulardii* led to an upregulation of IL-10, normalizing the overreacting inflammatory response.¹⁴

β -Glucan, one possible factor for immunomodulation

Several pieces of evidence point toward a small, soluble, heat-stable factor derived from the cell wall of *S. boulardii*, which can induce at least some of the immunomodulatory effects. The yeast β -glucan fraction has been identified as one candidate in this respect.³¹ β -Glucans derived from fungi and yeast consist of a (1,3)- β -linked backbone with small number of (1,6)- β -linked side chains, which are essentially known for their immune-modulating effects.³² They are found in the cell walls of nearly all fungi, including *S. boulardii*. β -Glucans are microbe-associated molecular patterns detected by pattern recognition receptors. Important pattern recognition receptors for β -glucans are the dectin-1 receptor, the complement receptor 3, and Toll-like receptor, expressed on various immune cells, for example, monocytes, macrophages, and DCs and also on intestinal epithelial cells (IECs).^{33–35} Binding to dectin-1 provokes numerous responses including production of cytokines and chemokines in DCs and macrophages³³ and forces IL-1 β secretion.³⁶ β -Glucan preparations from yeast have shown their immunomodulatory effects in human clinical trials.³⁷ There is even a connection between *S. boulardii* binding via dectin-1 receptor and the possibility to interfere with colitis.³⁸

Other, not yet identified components of *S. boulardii* may contribute to its various immunologic effects. More research is needed to elucidate all molecular mechanisms by which

S. boulardii supports the host immune system during infections or in a preventive manner.

Summary of the immunomodulatory effects

The in vivo and in vitro results presented earlier demonstrate that *S. boulardii* is able to modulate host early immune response toward a more activated state. This increases the host's resistance to microbial infections on a local level within the intestine, as well as systemically, for example, by increasing the amount of liver macrophages. In contrast, later during infections, *S. boulardii* helps to balance between pro- and anti-inflammatory immune responses by the modulation of various cytokines and chemokines and by inhibiting the maturation, migration, or proliferation of immune cells.

Anti-inflammatory action of *S. boulardii* during infections

There are many different mechanisms by which different pathogens cause diarrhea. Despite these different causalities, most cases of infectious diarrhea can be efficiently controlled by *S. boulardii*. The yeast interferes at various steps of the cascade of infection and diarrhea. One common feature is the induced inflammatory reaction of the host, which can be antagonized by *S. boulardii*.

In the early stage of infection, proinflammatory cytokines are produced by the IECs, which contribute to the defense against invading pathogens. However, high levels of proinflammatory cytokines do not only attack the invaders but cause inflammation of the host tissue leading to tissue destruction. Therefore, suppression of the proinflammatory action by anti-inflammatory mediators can achieve a beneficial balance between both the reactions. A summary of the individual mechanisms of *S. boulardii* is provided in Table 1 and is illustrated in Figures 1 and 2.

Enterohemorrhagic *E. coli*

Gram-negative *E. coli* bacteria are part of the regular intestinal microbiome. However, the genetically different bacterium enterohemorrhagic *E. coli* (EHEC) causes diarrhea and hemorrhagic colitis and can lead to other severe complications. A specific protein, intimin, located within the bacterial cell wall, facilitates bacterial adhesion to IECs, which is a prerequisite for subsequent host cell invasion and tissue colonization (for review, Nguyen and Sperandio³⁹).

Many of the EHEC pathogenic effects are caused by the cytotoxic Shiga toxin, which enters the host cell by receptor-mediated endocytosis. In addition, EHEC produces two hemolysin forms, which cause pore formation within the IECs and caspase-9-mediated apoptosis.⁴⁰

Table 1 *Saccharomyces boulardii* CNCM I-745 defense mechanisms against selected pathogens

Pathogen	Action	Mechanism	Reference
EHEC	Stops apoptosis	Interference with caspase-8 and caspase-9 pathways	42
	Preserves barrier function/tight junctions of IEC	Reduction of TNF α secretion	44
	Anti-inflammatory ability	Inhibition of MLC phosphorylation	42,44
EPEC	Preserves barrier function/tight junctions of IEC	Decrease of proinflammatory cytokine IL-8 secretion	49
	Decreases invasion of the enterocytes	Inhibition of the NF- κ B and MAPK signaling pathways	49
		Reduction of dephosphorylation of selective proteins	49
ETEC	Reduces apoptosis/preserves viability	Decrease of adhesion by interference with an upstream regulatory protein (SHC isoforms were less phosphorylated) of the ERK1/2 MAP kinase	49
	Anti-inflammatory ability	Delay of caspase-3 by EPEC activation	28
		Inhibition of proinflammatory transcriptional profile: reduction of ETEC-induced gene expression of proinflammatory cytokines (TNF α , IL-6, and GM-CSF) and chemokine (CCL2, CCL20, and CXCL8)	28
<i>Salmonella</i>	Activation/maturation of DC	Slight upregulation of mRNA for TNF α and CCR7 receptor after coincubation of DC	14,30
	Reduces adhesion to the IEC	Binding to the bacteria itself via mannose-sensitive binding	12
	Protects from invasion of IEC	Reduction of IEC cytoskeleton changes, necessary for invasion via interference of the Rac1 activation	12,13
	Protects against liver damage	Reduction of bacterial translocation	12,13
	Preserves barrier function	Reduction of adherence and inhibition of cytoskeleton changes	12,13
	Anti-inflammatory ability	Inhibition of MAPKs ERK1/2, p38, and JNK and of NF- κ B activation leading to decreased IL-8	12,13
		Decrease of inflammatory cytokines IL-6 and TNF α	30
<i>Shigella flexneri</i>	Preserves barrier function/tight junctions of IEC	Inhibition of the mRNA for the TNF α , GM-CSF, CXCL8, and CCL2 genes induced by <i>Salmonella</i>	59
	Anti-inflammatory ability	Restoration of claudin-1 levels important for tight junctions	59
<i>Clostridium difficile</i>	Inhibits binding to IEC	Reduction of cytokine IL-8 release	9-11
	Reduces toxin A toxicity	Reduction of the amount of phosphorylated (activated) ERK1/2 (pERK1/2)	25
		Reduction of phosphorylated I κ B α	25
Rotavirus	Anti-inflammatory ability	Hydrolyzation of toxin A and B by a 54-kDa serine protease leading to inhibition of toxin receptor binding	63
	Chloride secretion (thereby reduction of diarrhea)	Enhancement of the intestinal mucosal immune response by 1) the increase of total IgA concentration and 2) the increase of anti-toxin A IgA	66
	Anti-inflammatory ability	Reduction of IL-8 production via inhibition of the activation of the MAP kinases Erk1/2 and JNK/SAPK	67
<i>Candida albicans</i>	Anti-inflammatory ability	Prevention of oxidative stress via inhibition of ROS formation and reduction of chloride secretion by <i>S. boulardii</i> supernatant	67
		Restoration of reduction potential due to glutathione	68
		Reduction of proinflammatory cytokine production via IFN- γ and of IL-1 β	68
		Stimulation of the anti-inflammatory cytokines IL-4 and IL-10	68
		Reduction of TLR2 stimulation induced by <i>C. albicans</i>	68

Abbreviations: CCR7, C-C chemokine receptor type 7; DC, dendritic cell; EHEC, enterohemorrhagic *Escherichia coli*; EPEC, enteropathogenic *E. coli*; IEC, intestinal epithelial cell; IFN, interferon; IL, interleukin; JNK, Jun N-terminal kinases; MLC, myosin light chain; SHC, Src homology 2 domain containing protein; TLR2, Toll-like receptor 2.

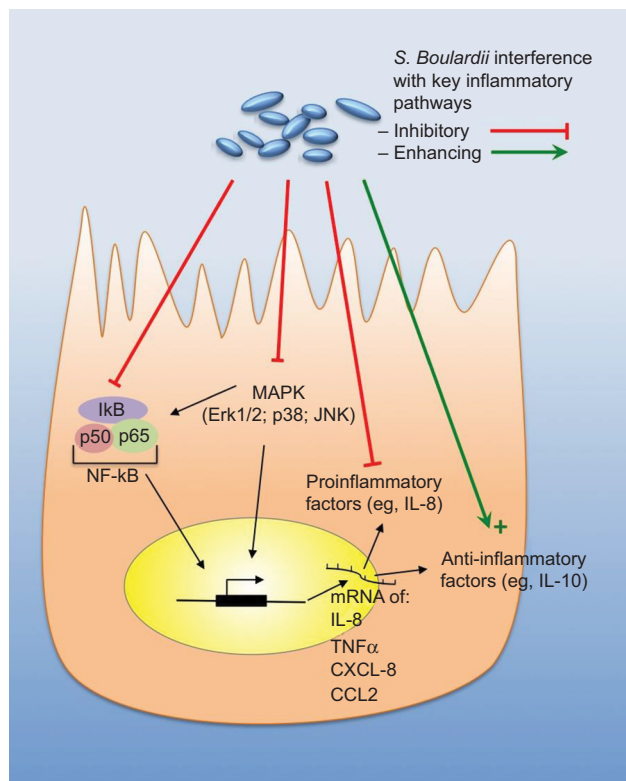


Figure 1 Anti-inflammatory abilities of *Saccharomyces boulardii*.

Notes: Summary of the interference of *S. boulardii* with key inflammatory pathways within gastrointestinal cells; red arrows indicate inhibitory actions, whereas green arrows indicate enhancing actions.

Abbreviations: IL, interleukin; JNK, Jun N-terminal kinases; mRNA, messenger RNA; TNFα, tumor necrosis factor alpha; ERK1/2, extracellular signal-regulated kinases 1/2; CCL2, CC-chemokine ligand 2; NF-κB, nuclear factor "kappa-light-chain-enhancer" of activated B-cells; MAPK, mitogen-activated protein kinase; CXCL-8, CXC-Motif-Chemokine 8.

Infection with EHEC causes inflammation and disruption of the tight junctions, leading to a breakdown of the barrier function of the intestinal epithelium. This, in turn, facilitates the invasion of IECs from their basolateral side.⁴¹ Additional apoptosis and necrosis of macrophages and lymphocytes (those host cells that are most dangerous to pathogens) worsen the EHEC pathogenicity.^{42,43}

In vitro, the apoptosis program in human colon cells (T84) triggered by EHEC can be stopped by *S. boulardii*. This is achieved by interfering with the caspase-8 and caspase-9 pathways. In EHEC-infected T84 cells, the secretion of TNFα is upregulated, which might contribute to apoptosis. This increase was significantly reduced when T84 cells were preincubated with *S. boulardii*.⁴²

Myosin light chain phosphorylation is correlated with an increase of tight-junction permeability. *S. boulardii* is able to preserve the barrier function of the epithelial cells after EHEC infections by the inhibition of myosin light chain phosphorylation.⁴⁴

Furthermore, the secretion of the proinflammatory cytokine IL-8 was decreased by *S. boulardii* via inhibition of

the NF-κB and MAPK signaling pathways. For this effect, *S. boulardii* cells must be present during the infection, as heat treatment and wash off the yeast cells no longer had this effect.⁴⁴ Mechanistically, this was achieved by blocking phosphorylation as well as degradation of IκB, which is necessary for translocation of NF-κB,⁴⁴ probably via *Saccharomyces* anti-inflammatory factor, a small molecular weight (<1 kDa), water soluble molecule, extracted from *S. boulardii*, and typically released into the culture media.⁴⁵ Additionally, *S. boulardii* was found to inhibit TNFα transcription (Figure 2 and Table 1).⁴²

EPEC

Another pathogenic *E. coli*, the EPEC causes serious and prolonged watery diarrhea, especially in children in developing countries. EPEC adheres to IECs and damages them by pore formation associated with cytotoxicity,⁴⁶ as well as by causing apoptosis.⁴⁷ In addition, tight junctions are disrupted.⁴⁸

When T84 cells were infected with EPEC in the presence of *S. boulardii*, the tight junctions were preserved, the apoptotic program was prevented or at least delayed, and the number of intracellular EPEC significantly decreased. A common mechanism by which *S. boulardii* prevents (or delays) activation of the apoptosis program is the inhibition of the pathogen-induced caspase activation.⁴⁹ EPEC induces phosphorylation of several proteins, including Src homology 2 domain containing protein (SHC). *S. boulardii* was able to reduce the degree of phosphorylation of most of these proteins, including SHC. SHC is known to be an upstream regulatory protein of the MAPK pathway, which explains the observed reduction of the EPEC-induced activation of the MAPK pathway by *S. boulardii*. Inhibition of the ERK1/2 MAPK pathway by *S. boulardii* also reduced EPEC internalization (Figure 2 and Table 1).⁴⁹

Enterotoxigenic *E. coli*

Another *E. coli* infection leading to profuse watery diarrhea is the infection with enterotoxigenic *E. coli* (ETEC). It causes 840 million gastrointestinal infections or approximately 380,000 deaths worldwide each year (for review, Gupta et al⁵⁰). In piglets, ETEC infection is the most common cause of inflammation and diarrhea, leading to reduced growth rate and increased mortality.⁵¹

In porcine intestinal cells, *S. boulardii* showed its anti-inflammatory abilities by decreasing the ETEC-induced gene expression of proinflammatory cytokines TNFα, IL-6, GM-CSF, and chemokines CCL2, CCL20, and CXCL8. In addition, *S. boulardii* was able to reduce ETEC adhesion

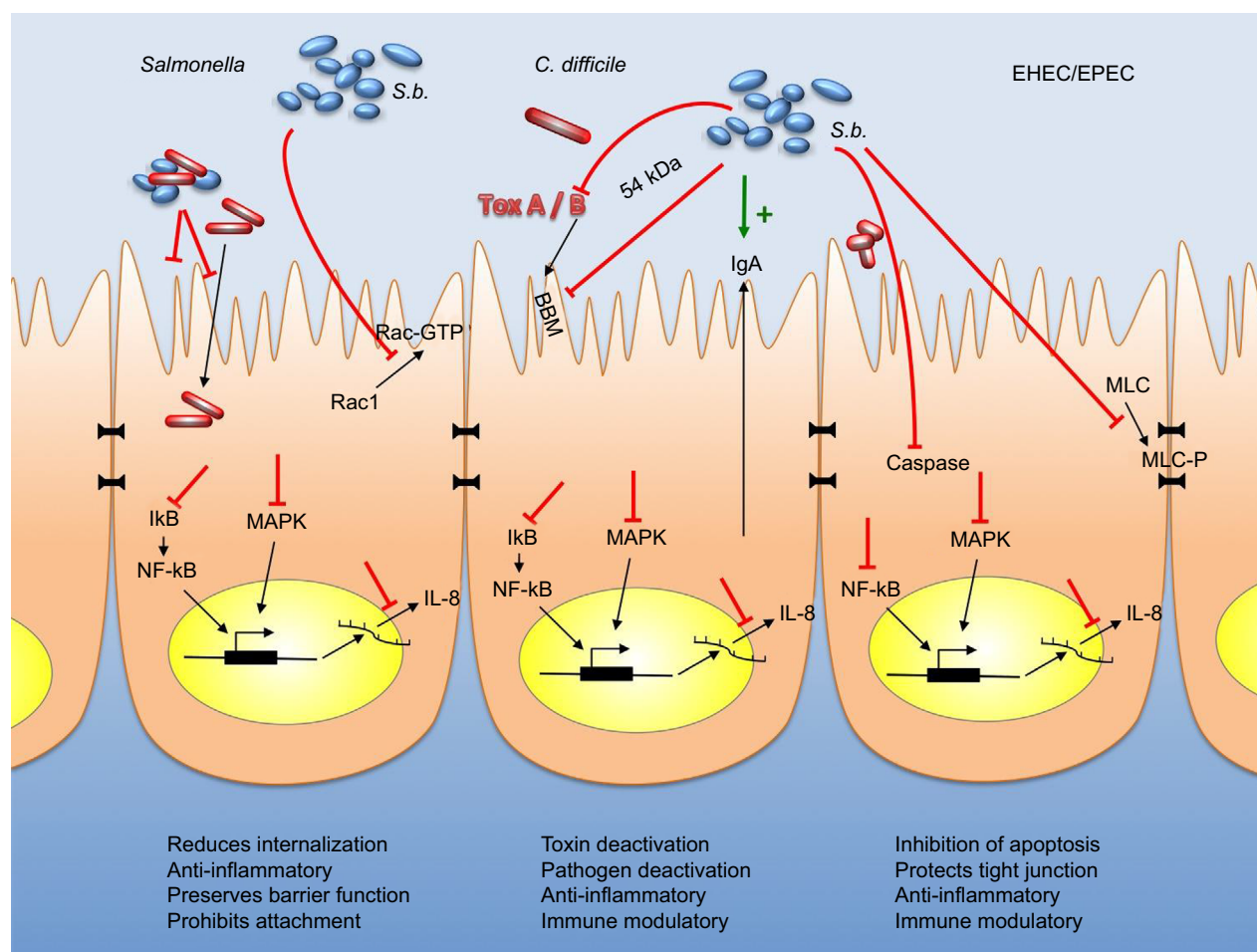


Figure 2 Effects of *Saccharomyces boulardii* in gastrointestinal cells infected by *Salmonella*, *Clostridium difficile*, or EHEC/EPEC.

Notes: Red arrows indicate inhibitory actions, whereas green arrows indicate enhancing actions.

Abbreviations: EHEC, enterohemorrhagic *Escherichia coli*; EPEC, enteropathogenic *E. coli*; IL, interleukin; MLC, myosin light chain; BBM, brush border membrane; NF-κB, nuclear factor “kappa-light-chain-enhancer” of activated B-cells; MAPK, mitogen-activated protein kinase.

to host intestinal cells²⁸ and thus reduces the possibility of bacterial internalization and enhances the elimination of the pathogen. Feeding of piglets with *S. boulardii* reduced the bacterial translocation to mesenteric lymph nodes after ETEC infection (Table 1).⁵²

Lipopolysaccharides

Bacterial endotoxin (lipopolysaccharides [LPS]) stimulation of human myeloid DCs induced the release of proinflammatory cytokines such as IL-6 and TNFα. *S. boulardii* culture supernatant, containing a <3kDa molecular weight compound,⁵³ counteracted this inflammatory response, resulting in a reduction of IL-6 and TNFα and an increase in IL-10.^{23,53}

After LPS stimulation, the same <3 kDa factor also inhibited activation and proliferation of native T-cells and the inflammation-associated migration of DC and T-cells. This was effectuated via suppression of C-C chemokine receptor type 7 expression, which is important for this migration.⁵³ Furthermore, *S. boulardii* produces a protein phosphatase

(63 kDa), which is able to reduce the inflammatory reaction and the toxicity of LPS by dephosphorylation (Table 1).⁵⁴

Salmonella

Infection with *Salmonella* causes inflammation and necrosis of the intestine, leading to gastroenteritis, including diarrhea with life-threatening consequences. *Salmonella* adheres to the host IECs and invades them through the activation of host's actin cytoskeleton by activating Rac1 GTPase.¹² The invasion results in inflammatory reactions, including the production and release of proinflammatory cytokines (eg, IL-8), activation of the MAPK pathway, as well as induction of several transcription factors such as AP-1 and NFκB.⁵⁵ These inflammatory responses cause diarrhea, lead to ulceration and destruction of the mucosa. Furthermore, *Salmonella* infections can cause systemic damage in case of translocation to liver, spleen, and lymph nodes (for review, Hurley et al⁵⁶).

S. boulardii employs multiple mechanisms to interfere with *Salmonella* infection. In vitro, *S. boulardii* was found

to reduce adhesion of *Salmonella* to IECs by (a probably mannose sensitive) binding of the yeast to the bacteria.^{12,30} In germ-free mice, *Salmonella* bound more frequently to *S. boulardii* than to epithelial cells.^{13,14} *S. boulardii* trapped the bacteria and thereby forced their elimination.

The yeast was able to interfere with host cell invasion of *Salmonella* by reducing Rac1 activation. This effect was more pronounced when HeLa cells were incubated overnight with *S. boulardii* before encountering a *Salmonella* infection.¹² These two mechanisms – reduced adherence and inhibition of cytoskeleton changers – preserve IEC barrier function and inhibit bacterial translocation to the liver, which was shown in mice treated with *S. boulardii*.^{12,13}

Furthermore, after overnight preincubation, *S. boulardii* or its supernatant could prevent the secretion of IL-8 via interference with the *Salmonella*-induced activation of MAPKs, ERK1/2, p38, and Jun N-terminal kinases and by the inhibition of phosphorylation of the I κ B- α subunit necessary for NF- κ B pathway. Additionally, the yeast could also directly (within a few hours) interfere with the IL-8 secretion process, without affecting the transcription machinery (Figure 2 and Table 1).¹²

The inhibitory effect of *S. boulardii* supernatant was completely abolished with heat treatment, indicating the presence of a heat-labile soluble factor, possibly *Saccharomyces* anti-inflammatory factor, mediating the inhibitory effect.¹²

During an ongoing *Salmonella* infection, *S. boulardii* was found to decrease the secretion of other inflammatory cytokines, namely, IL-6 and TNF α in vivo in mice. In contrast, anti-inflammatory cytokines increased in the early stages of infection due to *S. boulardii*, strengthening the host's anti-inflammatory abilities.¹³ The effects of *S. boulardii* on the immune response in *Salmonella*-infected IEC and DC cultures have also been shown by Badia et al.³⁰ At least partially, *S. boulardii* was able to inhibit the *Salmonella*-induced mRNA of TNF α expression.

Shigella flexneri

Shigella flexneri is a highly infectious human enteric pathogen, resulting in acute intestinal inflammation, abdominal cramps, severe diarrhea, and fever. The infection is associated with the disruption of tight junctions between the IECs, thereby disrupting the physical barrier and causing host cell invasion (for review, Ashida et al.⁵⁷ and Jennison and Verma⁵⁸).

In vitro, the simultaneous treatment with *S. boulardii* during *S. flexneri* infection did not reduce the number of bacteria that invaded or attached to T-84 IECs, but was at least partially

able to protect and restore the cellular barrier function, by restoring claudin-1 levels important for tight junctions.⁵⁹ In response to *S. flexneri* infection, the epithelial cells released IL-8, the key cytokine to attract polymorphonuclear leukocytes from the blood into the subepithelial region. This cytokine release was reduced when *S. boulardii* or its cell-free culture supernatant was added simultaneously, but not when added after infection, again indicating the role of a soluble factor.⁵⁹ As in other infectious diseases, *S. boulardii* acts via interference with phosphorylations of ERK1/2 (pERK1/2), and I κ B. The protective effects of *S. boulardii*, specifically the reduction of inflammation and polymorphonuclear leukocyte infiltration,⁵⁹ as well as the improved histopathology and reduced mortality,⁶⁰ were confirmed in experimental models in mice (Table 1).

C. difficile

C. difficile-induced colitis and diarrhea is one of the most common nosocomial infections. Up to 25% of all hospitalized patients treated with antibiotics will develop antibiotic-associated diarrhea – *C. difficile* can be made accountable for 10%–20% of these cases. The pathogenic effects are caused by the release of toxin A and toxin B (for review Bartlett⁶¹).

S. boulardii was found to secrete a 54-kDa serine protease, which hydrolyzes toxin A and B as well as inhibits toxin binding to its intestinal glycoprotein receptor.^{9–11} Additionally, *S. boulardii* inhibited *C. difficile* growth and toxin production in vivo.⁶² This resulted in a restoration of protein synthesis and membrane integrity.¹⁰

Like other inflammatory intestinal infections, *C. difficile* causes the release of inflammatory cytokines. In a human colonocyte cell line, *S. boulardii* supernatant was able to inhibit the toxin A-stimulated IL-8 production. Comparable to the above-described infections, *S. boulardii* supernatant inhibited the activation of MAP kinases, such as Erk1/2 and Jun N-terminal kinases/SAPK, which are involved in the IL-8 signaling pathway. Pretreatment of a mouse ileal loop with *S. boulardii* supernatant inhibited toxin A-induced pro-inflammatory reactions and reduced toxin A-induced fluid secretion, as well as tissue damage (Figure 2 and Table 1).⁶³

Rotavirus

Rotavirus infection accounts for hospitalization of up to 40% of the children <5 years of age with diarrhea.⁶⁴ Apart from the administration of selected probiotics, including *S. boulardii*,⁶⁵ no specific therapy for this viral infection is available. Rotavirus infects mature IECs. It seems that the chloride secretion, which is at least partially responsible for

the diarrhea, is induced via an oxidative stress-dependent mechanism. In vitro, rotavirus-infected Caco-2 cells produce high levels of intracellular reactive oxygen species, in parallel with a decrease of the antioxidant glutathione, leading to chloride secretion, which was altered by *S. boulardii*.⁶⁶ *S. boulardii* prevented chloride secretion by the inhibition of reactive oxygen species formation and reestablished balance of the GSH/GSSH redox system. A yeast-conditioned medium was sufficient to induce these effects (Table 1).⁶⁶

Candida albicans

S. boulardii was also found to have positive effects on yeast infection. This has been investigated on intraepithelial lymphocytes infected by *Candida albicans* in vitro. *S. boulardii* interfered with proinflammatory cytokine production (interferon- γ , IL-1 β), and it stimulated the anti-inflammatory cytokines IL-4 and IL-10.⁶⁷

In a different study, *S. boulardii* decreased mRNA levels and TNF α , which had been increased by *C. albicans* infection, while stimulating mRNA production of the anti-inflammatory cytokine IL-10.³¹ TNF α reduction due to *S. boulardii* is probably mediated via a reduced Toll-like receptor 2 mRNA expression,⁶⁸ a receptor known to be involved in yeast recognition (Table 1).⁶⁹

Nitric oxide-related effects

In a castor oil-induced diarrhea model, *S. boulardii* was able to significantly reduce diarrhea. It has been shown that the induction of diarrhea is associated with nitric oxide (NO) overproduction. *S. boulardii* inhibited inducible NO synthase activity in a concentration-dependent manner. This activity remained stable after 15 minutes at 121°C, indicating a heat-stable factor.⁷⁰ An *S. boulardii*-mediated decrease of NO levels in rat intestines was also observed in another study.⁷¹

Summary of the anti-inflammatory effects of *S. boulardii*

Independent of the pathogen, *S. boulardii* achieves its beneficial effects by inhibiting proinflammatory cytokine production or by enhancing anti-inflammatory mediators. Thereby, *S. boulardii* interferes with the host's signal transduction cascades at various positions. Reduction of the proinflammatory response is one of the protective effects of *S. boulardii* against diarrheal pathogens. Depending on the infectious agent, soluble yeast-derived factors as well as *S. boulardii* cells are responsible for the effects.

S. boulardii also reduces the pathogen number by growth inhibition of the pathogens or by direct binding and inactivating toxins by enzymatic cleavage. The reduced adhesion together

with the interference of the cytoskeleton-controlled bacterial internalization further reduces translocation and thus systemic damage. Finally, the yeast is able to preserve tight junction-mediated barrier function.

The positive effects of *S. boulardii* observed in these preclinical studies have been confirmed in many clinical trials, for example, for traveler's diarrhea,⁷² rotavirus-induced gastroenteritis,⁶⁵ or *C. difficile* infection.^{2,73,74}

Safety

The safety of *S. boulardii* has been proven in numerous clinical investigations in healthy as well as severely ill patients.^{2,65,72–74} Adverse events reported in these investigations were either none^{65,75} or low on side effects.⁷² Nevertheless, all probiotics as well as the host's own microbes bear the theoretical risk of epithelial translocation followed by systemic infection. The risk of developing fungemia due to the intake of *S. boulardii* is estimated to be 1 out of 5.6 million users.⁷⁶ The reported cases of fungemia associated with *S. boulardii* intake were in extremely ill patients, either immunocompromised or with central venous catheters.^{76,77} For all other groups, the intake of *S. boulardii* is considered to be safe.

Clinical effects on mechanistic level

Even though the mode of action has been investigated in numerous in vitro and in vivo studies, the clinical effects are not fully understood on a mechanistic level.

S. boulardii has been clinically tested in several of the acute gastrointestinal conditions described earlier. Most of the clinical trials reported a statistically significant outcome in favor of *S. boulardii*. Meta-analysis showed a protective effect of *S. boulardii* in the treatment of acute diarrhea of various etiologies in children and adults, including antibiotic-associated diarrhea.^{75,78} *S. boulardii* is also effective in primary prevention of *C. difficile* infection, an important infection due to antibiotic treatment. However, it showed only limited effects in secondary *C. difficile* infection prevention.⁷⁹

Even though there is a large body of evidence showing the overall positive effect of *S. boulardii* in these types of diarrhea, there is a small number of investigations where *S. boulardii* failed to show effectiveness.⁸⁰ The reason for failure in those studies might be insufficient power, short study duration, or being underdosed. In order to understand these outcomes, it is not only important to critically analyze the intervention itself but also to perform mode of action investigations in human. There is only a limited number of investigations evaluating the immunological effects of *S. boulardii* in humans. Only one has been found,⁸¹ which was associated with immunological effects in acute diarrhea, the

main focus of this review. In that study, the positive effect of *S. boulardii* in pediatric acute gastroenteritis was associated with enhanced immune response, indicated by increased serum IgA and decreased C-reactive protein levels. Furthermore, a significant increase in CD8 cells on day 7 was observed, indicating a late proinflammatory activity of *S. boulardii*, resulting in cytokine release and CD8 cell activation, which might help to limit the infection.⁸¹

Further knowledge about the mechanisms in human body will help to explain the effects that are not understood so far and to treat patients with the full capacity of *S. boulardii*.

Conclusion and future perspectives

S. boulardii has been used as a probiotic for >50 years. During this time, it has proven its effectiveness in many types of infectious diarrhea. It exhibits its positive effect by directly acting on pathogens and their toxins. It influences the host's infection-induced signaling cascades and its innate and adaptive immune system. In total, these mechanisms result in the reduction of the pathogens' ability for adhesion or colonization and an attenuation of the overreacting inflammatory immune response. This leads to a preserved or restored integrity of the IEC layer, and the diarrheic leakage of fluids into the intestinal lumen is attenuated.

Since *S. boulardii* interferes with bacterial infection at many stages, it becomes an interesting candidate as an antibiotic replacement in livestock farming to control pathogen-associated growth suppression. The ban of antibiotics for growth promotion requires alternatives. The first success was the introduction of *S. boulardii* to livestock feed.^{82,83}

Immunomodulatory and anti-inflammatory effects of *S. boulardii* achieve positive outcomes in a mechanistically similar manner in various infections. Therefore, it is not surprising that *S. boulardii* may prove to be effective in other gastrointestinal diseases associated with inflammation, such as *H. pylori* infection,^{84,85} Inflammatory Bowel Disease,⁸⁶ or colitis.^{31,87} Other digestion-related treatment areas might be obesity and type 2 diabetes.⁸⁸

Even treatment of cancer patients with yeast is not totally out of scope. First results have shown that human B lymphomas were inhibited by rice fermented with *S. boulardii*.⁸⁹ The yeast may also have a therapeutic or prophylactic role in intestinal neoplasia.⁹⁰ Further research is needed to demonstrate clinical efficacy of *S. boulardii* for all these new possible applications.

Acknowledgments

We are grateful to Margret I Moré for reviewing the manuscript. The work of HS was funded by Medice Arzneimittel Pütter GmbH and Co KG.

Author contributions

HS performed the PubMed search. All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work. All the authors approved the final version of the article, including the authorship list.

Disclosure

The work of HS was funded by Medice Arzneimittel Pütter GmbH and Co. KG. HS reports no other conflicts of interest in this work. SCB did not receive any funding for this article and also reports no conflict of interest in this work.

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