Escherichia coli strain Nissle 1917 ameliorates experimental colitis by modulating intestinal permeability, the inflammatory response and clinical signs in a faecal transplantation model

Éricka L. Souza,1 Samir D. Elian,1 Laís M. Paula,1 Cristina C. Garcia,2 Angélica T. Vieira,1 Mauro M. Teixeira,3 Rosa M. Arantes,4 Jacques R. Nicoli1 and Flaviano S. Martins1

Correspondence Flaviano S. Martins flaviano@icb.ufmg.br

1Department of Microbiology, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil
2Fiocruz, Instituto Oswaldo Cruz, Laboratório de vírus Respiratórios e Sarampo, Rio de Janeiro, RJ, Brazil
3Department of Biochemistry and Immunology, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil
4Department of General Pathology, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

Inflammatory bowel diseases (IBDs) are a group of inflammatory conditions of the gut that include ulcerative colitis and Crohn’s disease. Probiotics are live micro-organisms that may be used as adjuvant therapy for patients with IBD. The aim of this study was to evaluate the effect of prophylactic ingestion of Escherichia coli strain Nissle 1917 (EcN) in a murine model of colitis. For induction of colitis, mice were given a 3.5 % dextran sodium sulfate (DSS) solution for 7 days in drinking water. EcN administration to mice subjected to DSS-induced colitis resulted in significant reduction in clinical and histopathological signs of disease and preservation of intestinal permeability. We observed reduced inflammation, as assessed by reduced levels of neutrophils, eosinophils, chemokines and cytokines. We observed an increase in the number of regulatory T-cells in Peyer’s patches. Germ-free mice received faecal content from control or EcN-treated mice and were then subjected to DSS-induced colitis. We observed protection from colitis in animals that were colonized with faecal content from EcN-treated mice. These results suggest that preventative oral administration of EcN or faecal microbiota transplantation with EcN-containing microbiota ameliorates DSS-induced colitis by modifying inflammatory responsiveness to DSS.

Received 19 June 2015
Accepted 10 January 2016

INTRODUCTION

Inflammatory bowel diseases (IBDs) are a group of chronic inflammatory conditions that affect the gastrointestinal tract. The two major forms are Crohn’s disease (CD) and ulcerative colitis (UC). CD can affect the entire gastrointestinal tract, and usually presents as discontinuous transmural lesions in the ileum and colon. UC is restricted to the distal portion of the colon and is limited to the mucosa (Baumgart & Sandborn, 2007; Blumberg, 2009; Kaser et al., 2010; Huttenhower et al., 2014). The pathogenesis of IB is still not fully understood, but an inappropriate inflammatory response against intestinal microbiota in genetically susceptible individuals seems to be a key feature of the disease (Loftus & Sandborn, 2002; Cerf-Bensussan & Gaboriau-Routhiau, 2010; Maloy & Powrie, 2011; Molodecky et al., 2012). The microbiota contribution in this process has been investigated, and it is believed that dysbiosis (microbial imbalance) is an essential event in the loss of a regulatory immune response in intestinal mucosa, triggering a chronic inflammatory process (Manichanh et al., 2012; Rigottier-Gois, 2013; Satokari, 2015).

The incidence of IBD is high in developed countries and the occurrence in developing countries is rapidly growing. This increase is associated with modifications of lifestyle and urbanization (Logan & Bowls, 2010).

The treatment of IBD involves various therapeutic approaches and surgical interventions aiming to ensure
long remission periods and to prevent possible complications for the patient. Five major classes of drugs are used, including immunosuppressive and biological agents, with anti-TNF therapy currently the most used (Triantafillidis et al., 2011). As the relationship between microbiota and IBD development has been established (Ghouri et al., 2014), probiotics have been introduced as an alternative tool in modulating the imbalance existing among the micro-organisms in the intestinal environment. According to the definition of the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO Report, 2002), probiotics are ‘live micro-organisms which when administered in adequate amounts confer a health benefit to the host’.

Previous studies have shown the clinical benefit of some probiotics in UC (Rogler, 2011). Bibiloni et al. (2005) demonstrated the efficacy of VSL#3 (a mixture of Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus bulgaricus and Streptococcus thermophilus) in mild to moderate UC on 34 adult patients. Similarly, Venturi et al. (1999) confirmed its positive effect on 20 UC patients who presented problems related to the use of standard treatment. Use of Escherichia coli strain Nissle 1917 (EcN) has already been investigated to evaluate its efficacy in UC patients. Kruis et al. (2004) found no differences between EcN treatment and mesalazine administration in maintenance of the remission period.

The precise mechanisms of action of probiotics, such as Saccharomyces boulardii, Lactobacillus spp., Bifidobacterium spp., VSL#3 and EcN, are not fully understood, but there are several suggested mechanisms for maintaining remission of UC (Triantafillidis et al., 2011; Vieira et al., 2013; Schultz et al., 2004). The major mechanisms include immunomodulation, growth suppression of pathogens and increased barrier function of the intestinal mucosa (Fedorak & Madsen, 2004).

Despite the existence of clinical studies demonstrating an improvement in UC relapses after use of probiotics, data are still limited in terms of an understanding of the complete mechanism of action involved in this process and possible side effects, especially when considering immunocompromised patients. Thus, further studies will be important in order to fill in the gaps for this new alternative/adjuvant therapy in IBD.

The aim of the current study was therefore to evaluate the effects and potential mechanism of action of oral administration of EcN in a model of colitis in mice induced by dextran sodium sulfate (DSS).

**METHODS**

**Animals.** For experiments involving clinical, histopathological, immunological and physiological determinations, conventional (CV) 6–8-week-old female BALB/c mice were obtained from CEBIO (Centre for Animal Care) from the Federal University of Minas Gerais, Brazil. Germ-free (GF) 6–8-week-old female mice (Taconic) were used for a faecal microbiota transplantation (FMT) assay. GF mice were housed in flexible plastic isolators (Standard Safety Equipment Co.) and handled according to established procedures. Experiments with conventionalized (CVZ) mice were carried out in micro-isolators (Uno Roestavsttaal). For all animals, water and a commercial autoclavable diet (Nuvital) were sterilized by steam and administered *ad libitum*, and animals were maintained in a ventilated animal caging system (Alesco) with controlled lighting (12 h light, 12 h dark), humidity (60–80 %) and temperature (22 ± 1 °C) (Martins et al., 2013). All experimental procedures were carried out according to the standards set out by the Brazilian College for Animal Experimentation (COBEA, 2006). The study was approved by the Ethics Committee in Animal Experimentation of the Federal University of Minas Gerais (CEUA/UFMG, protocol no. 046/2011).

**Experimental design.** To evaluate the effect of EcN treatment, the following four groups of CV mice (12 animals per group) were used: (i) CTL, control not treated with EcN and without DSS-induced colitis; (ii) EcN, treated with EcN and without DSS-induced colitis; (iii) DSS, not treated with EcN and with DSS-induced colitis; and (iv) EcN + DSS, treated with EcN and with DSS-induced colitis. Animal body weight, faeces consistency and presence of blood in faeces were assessed daily during experimental colitis for determination of the daily disease activity index (DAI). Mice were killed 7 days after colitis induction and the colon was removed for histology, measurement of myeloperoxidase (MPO, as indicator of extent neutrophil infiltration), eosinophil peroxidase (EPO, as indicator of extent eosinophil infiltration) and cytokine levels (KC/CXCL-1, eotaxin/CCL11 and IL-1β) in the intestinal tissue. The colon was also analysed for weight/length ratio. To assess gut barrier function, an *in vivo* intestinal permeability assay using FITC-labelled dextran was performed in another set of groups subjected to the experimental design described above.

Another experiment was conducted using two groups (10 animals per group) of GF mice conventionalized with a diluted faecal content from CV mice treated either with EcN (CVZ-EcN) or not (CVZ). Female 6–8-week-old CV mice were divided into two groups treated intragastrically with PBS or with EcN for 10 days. After this period, a pool of faecal samples from each group was resuspended in PBS (pH 7.2) at a dilution of 10−2 and homogenized. Next, 0.1 ml of this suspension was administered intragastrically to GF mice to obtain the CVZ and CVZ-EcN groups. After 21 days of conventionalization, these two groups were divided into the following subgroups (five animals per subgroup): (i) CVZ, without DSS-colitis induction; (ii) CVZ-DSS, with DSS-colitis induction; (iii) CVZ-EcN, without DSS-colitis induction; and (iv) CVZ-EcN-DSS, with DSS-colitis induction, in which DAI was determined for 7 days.

The above experimental design was performed three times to confirm the reproducibility of the results.

**Bacterial strain, growth conditions and treatment.** The probiotic EcN (Mutafior; Ardeypharm) was purchased as a pharmaceutical product available in Canada. EcN was isolated from the product and growth in brain–heart infusion broth (Acumedia, Neogen) under aerobic conditions for a period of 24 h at 37 °C. For probiotic treatment, mice received daily an intragastric gavage with 0.1 ml of a suspension containing 9.0 log10 c.f.u. EcN ml−1, 10 days before colitis induction, and treatment was maintained during experimental colitis until sacrifice.

Before sacrifice, mice were anaesthetized (60 : 80 mg ketamine : xylazine kg−1 intraperitoneally, Syntec) for blood collection and then sacrificed by cervical dislocation to collect organs for further analyses.
Induction of DSS colitis. Colitis was induced by the addition of 3.5 % (w/v) DSS (36 000–50 000 kDa; MP Biomedicals) in drinking water ad libitum for 7 days. The DSS solution was changed every couple of days.

Daily DAI. DAI assessment was carried out according to the classical scoring system as described previously (Elian et al., 2015). DAI was the cumulative scores (0–10) from the following three different parameters: faeces consistency (0, normal; 1, soft but still formed; 2, very soft; 3, diarrhoea), presence of blood in faeces (0, negative haemoccult; 1, positive haemoccult; 2, blood traces visible in faeces; 3, rectal bleeding) and weight loss (0, negative weight loss; 1, loss of 1–5 % of body weight; 2, 5–10 % body weight loss; 3, 10–20 % body weight loss; 4, > 20 % body weight loss). Faecal blood was tested using haemoccult cards (INLAB).

Histological and morphometric analysis. Samples of colon were fixed in Bouin solution and rolled up ('Swiss roll' technique). They were then fixed in 4 % formaldehyde and processed for inclusion in paraffin for microtomy. Paraffin-embedded tissue sections were cut 4 µm thick and stained with haematoxylin and eosin for general histology. Additional sections were stained with periodic acid–Schiff (PAS) and alcian blue stain in order to evaluate the production of mucins in the intestinal epithelium. For morphometric examination, the images were obtained on a Cool SNAP-Procf Colour (Media Cybernetics) microcamera built into an Olympus BX51 microscope and analysed by ImageJ software (Wayne Rasband/National Institutes of Health) (Schneider et al., 2012).

In vivo permeability assay. An in vivo permeability assay to assess intestinal barrier function was performed using an FITC-labelled dextran method, as described by Yan et al. (2009). Mice had food and water removed 4 h prior to the oral administration of FITC–dextran [60 mg (100 g body weight)⁻¹, molecular mass 4000; Sigma–Aldrich]. Serum was collected by cardiac puncture 4 h later and the fluorescence intensity of each sample measured (excitation, 492 nm; emission, 525 nm; Spectromax M3, Molecular Devices). FITC–dextran concentrations were determined using standard curves generated by serial dilution of FITC–dextran. Permeability was expressed as in µg FITC–dextran (ml serum)⁻¹.

MPO activity determination. The extent of neutrophil accumulation in the colon tissue was evaluated by assaying MPO activity, as described previously (Vieira et al., 2005). Briefly, the tissue was removed and snap frozen in liquid nitrogen. Upon thawing, the tissue was homogenized with 1.9 ml buffer [0.1 M NaCl, 0.02 M NaH₂PO₄·H₂O, 0.015 M Na₂EDTA (pH 4.7)] and centrifuged at 12 000 g for 10 min. The supernatant was discarded and the precipitate was subjected to hypotonic lysis. After further centrifugation, the precipitate was suspended in 1.9 ml 0.5 % HTAB in PBS. The samples were frozen three times in liquid nitrogen and centrifuged at 4 °C at 12 000 g for 10 min. The supernatant was used in the enzymic assay for MPO activity by measuring the change in absorbance at 450 nm using tetramethylbenzidine (Sigma–Aldrich). Results were expressed as arbitrary units.

EPO activity determination. An EPO assay was performed as described previously (Strath et al., 1985). Briefly, 100 mg colon tissue was weighed, homogenized in 1.9 ml PBS and centrifuged at 12 000 g for 10 min. The supernatant was discarded and the erythrocytes lysed. The samples were then centrifuged, the supernatant discarded and the pellet suspended in 1.9 ml 0.5 % HTAB in PBS. The samples were frozen three times in liquid nitrogen and centrifuged at 4 °C at 12 000 g for 10 min. The supernatant was used in the enzymic assay. Briefly, 10 mg o-phenylenediamine (OPD) (Sigma–Aldrich) was dissolved in 5.5 ml distilled water and then 1.5 ml OPD solution was added to 8.5 ml Tris/HCl buffer (pH 8.0), followed by the addition of 7.5 µl H₂O₂. Using a 96-well plate, 100 µl substrate solution was added to 50 µl each sample. After 30 min, the reaction was stopped with 50 µl 1 M H₂SO₄ and the absorbance read at 492 nm. Results were expressed as arbitrary units.

Cytokine determination. Tissue preparation was modified from the method of Martins et al. (2011). Briefly, colon (100 mg) was homogenized in 1 ml PBS (pH 7.2) containing protease inhibitor cocktail (0.1 mM PMSF, 0.1 mM benzethonium chloride, 10 mM EDTA, and 20 kallikrein inhibitor units of aprotinin A) and 0.05 % Tween 20. The samples were then centrifuged for 10 min at 12 000 g and the supernatant was used immediately for assays. Cytokines CXCL1/1/KC, CCL11/eotaxin and IL-1β were measured by ELISA using commercially available antibodies according to the procedures recommended by the manufacturer (R&D Systems).

Analysis by flow cytometry. Peyer’s patches (PPs) from the small intestine were collected and processed for cell extraction and further staining with specific antibodies for flow cytometry analysis. Briefly, intestines were removed and opened longitudinally to expose PPs, which were excised and mashed using microscopy slides. Tissue debris were removed by centrifugation and the cells were stained for CD3 [phycoerythrin (PE)–Cy5; BD Pharmingen], CD4 (PE–Cy7; BD Pharmingen) CD8 (FITC; BD Pharmingen) and CD25 (allophycocyanin; BD Pharmingen) on the surface. After fixation and permeabilization using a kit (BD Biosciences), cells were stained for FoxP3 (PE; ebioscience), acquired in a cytometer BD FACScanto II and analysed using FlowJo (TreeStar) software. CD3 + cells were further analysed for CD4 and CD8 expression, and CD4 + cells were assessed for CD25 expression (low and high), and then each of these subpopulations (CD3 + CD4 + CD25low and CD3 + CD4 + CD25high) were assessed for FoxP3 expression. Results were expressed as the percentage of total acquired cells.

Statistical analysis. Data were expressed as means±SEM and analyses performed using the statistical software GraphPad Prism 5.00 (GraphPad Software). Differences between means were evaluated using an ANOVA test, followed by a Newman–Keuls test. Results with P<0.05 were considered significant.

RESULTS

Treatment with EcN ameliorates DSS-induced colitis in mice

The model of acute colitis reproduced in mice by administration of DSS resulted in typical clinical symptoms of the disease such as weight loss, change in faeces consistency and rectal bleeding. The sum of each symptom score was calculated to evaluate the severity of the disease with the DAI. The administration of EcN promoted a statistically significant reduction in these symptoms, with a lower evolution of DAI in the EcN + DSS group when compared to the DSS group, especially on days 6 and 7 after disease induction (Fig. 1), despite the large SD observed on these days. This fact was related to the presence of one high score within the evaluated group of mice, but without this being considered a significant outlier compared to the rest of the group.

Colitis induction in mice promoted acute inflammation of the colon, characterized by degenerative changes of the muscular layer, cellular atypia and disorganization of...
columnar epithelial cells resulting in extensive segmental lesions in the epithelium, absence of goblet cells, presence of oedema, intense cellular recruitment with significant inflammatory infiltrate and formation of nodular infiltrate with a germinat centre (Fig. 2b). Probiotic administration did not cause any significant histological changes (Fig. 2c), maintaining an architectural pattern similar to the control group (Fig. 2a). The administration of EcN before colitis induction led to a considerable improvement in histological profile, with preservation of the organization of the intestinal epithelium and with only small areas of injury and moderate oedema (Fig. 2d). Histopathology examination also showed a significant increase in the extent of PPs after treatment with EcN and induction of colitis, which was not observed in any other group analysed. The latter observation was confirmed when morphometric analysis of PPs was conducted (Fig. 2e).

Next, we evaluated intestinal permeability by using oral administration of FITC–dextran. In this system, loss of intestinal barrier integrity allows passage of the labelled dextran through the epithelium into the bloodstream, where it can be measured. A considerable reduction in intestinal permeability was observed in mice treated with EcN (Fig. 2f), suggesting that the probiotic preserved the intestinal epithelial barrier.

**EcN treatment modulates the host immune response after DSS-induced colitis in mice**

In conventional mice, the assessment of regulatory T-cell (T_{reg}) population showed a significant increase in the CD3^{+}CD4^{+}CD25^{low}FoxP3^{+} T_{reg} population in PPs in the EcN + DSS-treated group compared to the CTL, DSS and EcN groups (Fig. 3a).

We observed higher values for both MPO activity (Fig. 3b) and CXCL1/KC levels (Fig. 3c) in the colon of DSS group when compared to the CTL group. The preventative administration of EcN significantly reduced the MPO activity and CXCL1/KC levels, demonstrating a protective effect against the acute inflammatory process.

Eosinophils are thought to play a role in the pathogenesis of IBD (Al-Haddad & Riddell, 2005). High levels of EPO activity quantified in the colon of DSS-treated animals suggested an increased influx of eosinophils in this organ (Fig. 3d), which was corroborated by increased amounts of CCL11 (eotaxin-1) (Fig. 3e), a chemoattractant for eosinophils (Adar et al., 2014). EcN administration was able to decrease EPO levels, but without changing the CCL11 concentration in target tissue.

The acute inflammatory process induced by DSS caused increased levels of IL-1β in the colon, and treatment with EcN reversed the levels of this cytokine to a level similar to that observed in the control group (Fig. 3f).

**FMT from EcN-treated conventional mice influences the course of DSS-induced colitis in GF mice**

In order to evaluate whether the microbiota containing EcN could mimic the effects of the probiotic treatment, we transferred faeces from CV mice after 10 days of probiotic treatment into GF mice, waited for 21 days and then induced colitis. GF mice that received faeces from CV untreated mice (CVZ-DSS) showed a clinical response that was similar to that of their faecal donor (Fig. 4). However, in the GF group conventionalized with the faecal microbiota of animals treated with EcN (CVZ-EcN-DSS), an improvement in clinical signs was observed, with a significant reduction in DAI when compared with the CTL group. The preventative effect against the acute inflammatory process.

Despite the difference, it was possible to observe a considerable sd in the same period in which the statistically significant difference between the groups was observed. This finding suggested that the beneficial effects of EcN treatment on the course of colitis are transferable via FMT, but are more modest compared with the effects of direct EcN administration.

**DISCUSSION**

The main findings of our work can be summarized as follows: treatment of mice for 10 days with the probiotic EcN resulted in (i) a reduction in clinical and histopathological signs of colitis; (ii) preservation of intestinal permeability; (iii) control of the inflammatory process by reducing the levels of inflammatory cells and cytokine production; and (iv) increase in T_{reg} cells. In addition, (v) the FMT from EcN pre-treated animals protected GF mice from DSS-induced colitis.

UC is a chronic inflammatory condition that affects the lining of the colon, with periods of remission and relapse.
The pathogenesis of the disease is not entirely known, although it is believed that it involves a deregulated immune response genetically triggered against intestinal microbiota antigens. These antigens may be available because of an altered epithelial barrier and may be influenced by environmental factors, such as diet (Valatas et al., 2015).

IBD treatment aims to improve the quality of life of patients by maintaining remission periods and treating relapses. Pharmaceutical treatment includes the use of immunosuppressive and corticosteroids drugs as the main choice (Triantafillidis et al., 2011). However, these therapies may have limitations and important side effects. Probiotics have emerged as an alternative in the IBD therapeutic approach. Studies have shown their effectiveness in mild to moderate cases of UC (Venturi et al., 1999; Cui et al., 2004; Kruis et al., 2004; Bibiloni et al., 2005; Zocco et al., 2006). However, these findings should be treated with caution, as further studies are necessary in order to prove the beneficial effects of probiotics in clinical management, as well as possible complications attributed to their use such as bacterial translocation and possible adverse effects in immunosuppressed patients.

Fig. 2. (a–d) Histopathological views of colon mucosa of conventional mice not treated with EcN and without DSS-induced colitis (a; CTL); not treated with EcN and with DSS-induced colitis (b; DSS); treated with EcN and without DSS-induced colitis (c; EcN); and treated with EcN and with DSS-induced colitis (d; EcN + DSS). *: Inflammatory infiltrate; #: oedema area; thick arrow, epithelial damage; thin arrow, nodular lymphoid activation. Haematoxylin and eosin stain; magnification: ×40; insets, ×100. (e, f) The percentage area of the colon perimeter with PPs (e) and in vivo intestinal permeability (µg ml⁻¹) (f) of CTL, EcN, DSS and EcN + DSS mice. Asterisks above columns represent a significant difference between each column and CTL group. The horizontal bars indicate the difference between the related columns. **P<0.01; ***P<0.001.
Fig. 3. Immunological determination in colon tissue of conventional mice not treated with EcN and without DSS-induced colitis (CTL); not treated with EcN and with DSS-induced colitis (DSS); treated with EcN and without DSS-induced colitis (EcN); and treated with EcN and with DSS-induced colitis (EcN + DSS). (a) CD3+ CD4+ CD25low FoxP3+ population in PPs. (b) Qualitative assessment of the presence of neutrophils (MPO activity). (c) Quantification of chemokine recruiter for neutrophils (KC/CXCL-1). (d) Qualitative assessment of the presence of eosinophils (EPO detection). (e) Quantification of cytokine recruiter for eosinophils (eotaxin/CCL11). (f) Proinflammatory cytokine IL-1β in the colon. Asterisks above columns represent significant differences between columns and the CTL group; horizontal bars indicate differences between related columns. [n=6, except for (a) where n=5]. *P<0.05; **P<0.01; ***P<0.001; NS, not significant.
IBDs are characterized by a dysbiosis process related to loss of a physiological immune response, triggering a chronic inflammatory process (Satokari, 2015). It has been observed that animals genetically modified to develop spontaneous colitis remain healthy when kept under GF conditions, showing that the presence of microbiota is a determining factor for the development of UC (Orel & Kamhi Trop, 2014). In this context, the intestinal microbiota exhibit a reduced diversity and stability and prevalence of certain micro-organisms (Manichanh et al., 2012). Members of the two most prevalent phyla in healthy individuals, Firmicutes and Bacteroidetes, have abundance reduced in IBD, with an increase in Proteobacteria, particularly of facultative anaerobes of the family Enterobacteriaceae (Kaser et al., 2010; Satokari, 2015). Lepage et al. (2011), using a metagenomic platform to analyse mucosa biopsies from 62 participants, both healthy and UC patients, observed an increased proportion of Proteobacteria and Actinobacteria and a reduced proportion of Bacteroidetes in UC patients. In this sense, our research shows a pioneering aspect as we could relate the beneficial effects of EcN in UC via FMT. The slightly high SD in the FMT model performed can be justified by the non-uniformity of clinical response within the CVZ-EcN-DSS group, although no outlier point occurred in this group.

Another important aspect of this analysis is related to the fact that clinical improvement was more modest compared with the findings involving the direct administration of the probiotic. Possibly, this difference in response is related to indirect beneficial effects on the CVZ group, as the probiotic does not colonize the intestinal environment and its clinical effect occurs due to a possible microbiota modulation that has been lost over time due to suspension of its use. Further analyses would seem to be necessary in order to characterize possible changes that may occur in the intestinal microbiota by probiotic action that were able to promote improvements in clinical signs in this model.

The surface of the intestinal epithelium is the first barrier against intestinal luminal aggression against the mucus layer (Dongarrà et al., 2013). It is covered by multilayered mucus structures that limit the access of micro-organisms to the epithelium, maintaining homeostatic conditions (Chassaing et al., 2015). In UC, the major change that allows the development of the disease is the modification of the sulfation of mucins that constitute this layer of protective mucus. The damage caused to the epithelial barrier leads to increased permeability due to defective cell–cell junctions (Ordás et al., 2012; Perše & Cerar, 2012). A recent study performed by Chassaing et al. (2015) related the intake of emulsifying agents present in the diet to the development of UC, these compounds being able to induce a mild inflammation in WT mice, and severe colitis in mice predisposed to this intestinal disorder.

EcN treatment seems to be promising and it was able to reduce the increased permeability in our colitis model. Other studies have demonstrated the efficacy of probiotic treatment in the re-establishment of epithelial barrier integrity, which suggests that this may be a common feature of most probiotics. Tiago et al. (2015) observed that Saccharomyces cerevisiae UFMG A-905 was able to diminish inflammation and intestinal permeability in a murine model of colitis. Johnson-Henry et al. (2008) demonstrated the beneficial effect of Lactobacillus rhamnosus GG in the preventative treatment of enterohaemorrhagic E. coli infection via barrier function increase. Similarly, Klingberg et al. (2005) demonstrated the ability of L. plantarum MF1298 to reinforce the barrier function in a dose-dependent manner associated with increased expression ofZO-1.

In addition to clinical parameters of the disease, we also evaluated the profile of the immune response to chemically induced colitis and possible alterations due to treatment with the probiotic. Dendritic cells found in the lamina propria capture micro-organisms from the intestinal lumen and present them to CD4⁺ T-cells in secondary lymphoid organs such as PPs (Abraham & Medzhitov, 2011). This region of organized lymphoid tissue plays a critical role in antigen presentation and is considered an inducible site of the immune response, and also of its regulation (Kelly & Mulder, 2012; Dongarrà et al., 2013). There was a significant increase in the perimeter of PPs in the DSS-induced colitis group treated with EcN in relation to all other groups. This suggests possible activation of regulatory pathways of the immune response.

UC is a chronic, idiopathic, inflammatory disease that affects the colonic mucosa, and its pathogenesis is characterized by a disturbance of the immune response (Ordás et al., 2013). It is covered by multilayered mucus structures that limit the access of micro-organisms to the epithelium, maintaining homeostatic conditions (Chassaing et al., 2015). In UC, the major change that allows the development of the disease is the modification of the sulfation of mucins that constitute this layer of protective mucus. The damage caused to the epithelial barrier leads to increased permeability due to defective cell–cell junctions (Ordás et al., 2012; Perše & Cerar, 2012). A recent study performed by Chassaing et al. (2015) related the intake of emulsifying agents present in the diet to the development of UC, these compounds being able to induce a mild inflammation in WT mice, and severe colitis in mice predisposed to this intestinal disorder.

EcN treatment seems to be promising and it was able to reduce the increased permeability in our colitis model. Other studies have demonstrated the efficacy of probiotic treatment in the re-establishment of epithelial barrier integrity, which suggests that this may be a common feature of most probiotics. Tiago et al. (2015) observed that Saccharomyces cerevisiae UFMG A-905 was able to diminish inflammation and intestinal permeability in a murine model of colitis. Johnson-Henry et al. (2008) demonstrated the beneficial effect of Lactobacillus rhamnosus GG in the preventative treatment of enterohaemorrhagic E. coli infection via barrier function increase. Similarly, Klingberg et al. (2005) demonstrated the ability of L. plantarum MF1298 to reinforce the barrier function in a dose-dependent manner associated with increased expression ofZO-1.

In addition to clinical parameters of the disease, we also evaluated the profile of the immune response to chemically induced colitis and possible alterations due to treatment with the probiotic. Dendritic cells found in the lamina propria capture micro-organisms from the intestinal lumen and present them to CD4⁺ T-cells in secondary lymphoid organs such as PPs (Abraham & Medzhitov, 2011). This region of organized lymphoid tissue plays a critical role in antigen presentation and is considered an inducible site of the immune response, and also of its regulation (Kelly & Mulder, 2012; Dongarrà et al., 2013). There was a significant increase in the perimeter of PPs in the DSS-induced colitis group treated with EcN in relation to all other groups. This suggests possible activation of regulatory pathways of the immune response.

UC is a chronic, idiopathic, inflammatory disease that affects the colonic mucosa, and its pathogenesis is characterized by a disturbance of the immune response (Ordás et al., 2013). It is covered by multilayered mucus structures that limit the access of micro-organisms to the epithelium, maintaining homeostatic conditions (Chassaing et al., 2015). In UC, the major change that allows the development of the disease is the modification of the sulfation of mucins that constitute this layer of protective mucus. The damage caused to the epithelial barrier leads to increased permeability due to defective cell–cell junctions (Ordás et al., 2012; Perše & Cerar, 2012). A recent study performed by Chassaing et al. (2015) related the intake of emulsifying agents present in the diet to the development of UC, these compounds being able to induce a mild inflammation in WT mice, and severe colitis in mice predisposed to this intestinal disorder.

EcN treatment seems to be promising and it was able to reduce the increased permeability in our colitis model. Other studies have demonstrated the efficacy of probiotic treatment in the re-establishment of epithelial barrier integrity, which suggests that this may be a common feature of most probiotics. Tiago et al. (2015) observed that Saccharomyces cerevisiae UFMG A-905 was able to diminish inflammation and intestinal permeability in a murine model of colitis. Johnson-Henry et al. (2008) demonstrated the beneficial effect of Lactobacillus rhamnosus GG in the preventative treatment of enterohaemorrhagic E. coli infection via barrier function increase. Similarly, Klingberg et al. (2005) demonstrated the ability of L. plantarum MF1298 to reinforce the barrier function in a dose-dependent manner associated with increased expression ofZO-1.
et al., 2012). Beyond the unbalance between T-helper 1 (Th1) and Th2 cell responses, known as part of the IBD aetiology, it is believed that failures in the mediation of regulatory immune response are also involved in this process (Himmel et al., 2008). This idea is supported by some evidence that indicates a key role of FoxP3 in intestinal homeostasis (Himmel et al., 2008; Atarashi et al., 2013; Geem et al., 2015) and its role in reducing colitis severity (Rudensky, 2011; Grant et al., 2015; Raphael et al., 2015; Wang et al., 2015). Powrie et al. (1993) conducted a trial assay with immunodeficient mice in which the transfer of naïve CD4+ T-cells in the absence of Treg cells resulted in colitis dependent on the presence of commensal bacteria. The transfer of Treg cells promoted the stabilization and reversion of the disease.

The intestinal microbiota play a key role in IBD due to changes in their composition. However, more recently, another key function has been connected, the stimulation of Treg cells, which depends on microbiota signals for their appropriate development and function in inflammation suppression (Furusawa et al., 2013). Recent studies have demonstrated that strains within Clostridia clusters affect the differentiation, accumulation and function of Treg cells in mice (Atarashi et al., 2013). Likewise, short-chain fatty acids, such as butyrate and propionate, resulting from fibre fermentation by intestinal bacteria, have been associated with the differentiation of colonic Treg cells in mice (Furusawa et al., 2013) and improvement of inflammatory processes in colitis when evaluating the effect of propionate in Treg cells, when both were administered in mice (Smith et al., 2013). Our findings regarding the stimulation of Treg cells by EcN in colitis allowed us to associate the direct participation of the probiotic in this process via PPs. It has been suggested that the capacity of probiotics to act as CD28 superagonists may underlie the expansion of Treg cells and consequently the decrease in disease severity observed in our experiments (Rudensky, 2011). Another hypothesis is related to a possible modulation of the intestinal microbiota composition by EcN, favouring specific members involved in Treg stimulation via short-chain fatty acid production.

It is known that during IBD there is an increase in the number of innate immune cells in the intestinal mucosa (Valatas et al., 2015). Neutrophils are the first line of defence to be recruited during an infectious or inflammatory process. Furthermore, it has been shown that the level of infiltration of neutrophils in the intestine correlates with tissue damage (Kruger et al., 2015). Therefore, strategies that inhibit their recruitment are suggested to be promising to treat IBD. Some inflammatory mediators are responsible for this recruitment, mainly CXC chemokines such as IL-8 (CXCL8), MIP-2 (CXCL2) and KC (CXCL1) (Kobayashi, 2008). Treatment with EcN significantly reduced the recruitment of neutrophils and CXCL1 production in the intestine and also protected intestinal injury after colitis induction. A similar result was observed by Sha et al. (2014), who showed that EcN treatment decreased disease severity and neutrophil influx in a murine model of colitis induced by 2,4,6-trinitrobenzenesulfonic acid.

Eosinophils are pro-inflammatory leukocytes classically known to be associated with the Th2 cytokine profile (T-cells expressing IL-4, IL-5, IL-10 and IL-13) and which are resident in the gastrointestinal lamina propria of healthy subjects (Woodruff et al., 2011). They are recruited by chemokines that act on CCR3, especially eotaxin, and are able to secrete toxic inflammatory mediators, such as EPO, which is stored within vesicles (Al-Haddad & Riddell, 2005; Woodruff et al., 2011). Eosinophils are thought to play a major pro-inflammatory role in IBD and contribute to diarrhoea, tissue destruction and fibrosis formation (Vieira et al., 2009; Albert et al., 2011). It has recently been suggested that eosinophils may also contribute to tissue repair during IBD (Travers & Rothenberg, 2015). Treatment with EcN was able to reduce the levels of EPO, but not CCL11 levels in the intestine. The maintenance of high levels of CCL11 chemokine in the probiotic-treated group may be secondary to an attempt by the tissue to recruit more eosinophils to facilitate tissue repair, as seen recently by other researchers (Travers & Rothenberg, 2015).

Treatment with EcN also reduced the levels of the pro-inflammatory cytokine IL-1β, probably by reducing the number of inflammatory cells after colitis induction. Antigen-presenting cells, in response to activation by microbial antigens, produce a number of pro-inflammatory cytokines, including IL-1β. Members of the IL-1 family are thought to contribute to the initiation, but not the maintenance, of the inflammatory process in models of experimental colitis in mice (Neurath, 2014). The observed effect may be due to lower recruitment of inflammatory cells, as the literature data demonstrate that the production of cytokines by inflammatory cells is pathological (Neuman, 2007).

Given the role of microbiota in the regulation of the immune response and the beneficial effect of the use of probiotics in this context, EcN has demonstrated its effectiveness in improving clinical and histopathological signs, re-establishing an epithelial barrier and regulating immune responses, as well as promoting significant benefits in the evolution of clinical signs in an FMT model. Taken together, these results suggest a possible biotherapeutic strategy using EcN for the maintenance of remission in patients with colitis.

ACKNOWLEDGEMENTS

The authors are grateful to Clélia N. Silva for valuable technical help, and Marcelo Gomes and Valner Mussel for the animal care. This work was supported by grants from the Brazilian National Council for Scientific and Technological Development (CNPQ; grant no. 470533/2012-9), the Ministry of Science and Technology; the Foundation for Research Support of the State of Minas Gerais (FAPEMIG), Brazil (grant no. APQ-01619-14); and the Higher Council for Scientific and Technological Development (CNPQ; grant no. APQ-01619-14); and the Higher Council for Scientific and Technological Development (CNPQ; grant no. APQ-01619-14).
Level Personnel Training Coordination (CAPES), Brazilian Ministry of Education. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

REFERENCES


