Support Care Cancer (2012) 20:3205–3210
DOI 10.1007/s00520-012-1446-3

ORIGINAL ARTICLE

Probiotic factors partially prevent changes to caspases 3 and 7 activation and transepithelial electrical resistance in a model of 5-fluorouracil-induced epithelial cell damage

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Received: 7 November 2011 / Accepted: 19 March 2012 / Published online: 20 April 2012
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Abstract The potential efficacy of a probiotic-based preventative strategy against intestinal mucositis has yet to be investigated in detail. We evaluated supernatants (SN) from *Escherichia coli* Nissle 1917 (EcN) and *Lactobacillus rhamnosus* GG (LGG) for their capacity to prevent 5-fluorouracil (5-FU)-induced damage to intestinal epithelial cells. A 5-day study was performed. IEC-6 cells were treated daily from days 0 to 3, with 1 mL of PBS (untreated control), de Man Rogosa Sharpe (MRS) broth, tryptone soy roth (TSB), LGG SN, or EcN SN. With the exception of the untreated control cells, all groups were treated with 5-FU (5 μM) for 24 h at day 3. Transepithelial electrical resistance (TEER) was determined on days 3, 4, and 5, while activation of caspases 3 and 7 was determined on days 4 and 5 to assess apoptosis. Pretreatment with LGG SN increased TEER ($p<0.05$) compared to controls at day 3. 5-FU administration reduced TEER compared to untreated cells on days 4 and 5. Pretreatment with MRS, LGG SN, TSB, and EcN SN partially prevented the decrease in TEER induced by 5-FU on day 4, while EcN SN also improved TEER compared to its TSB vehicle control. These differences were also observed at day 5, along with significant improvements in TEER in cells treated with LGG and EcN SN compared to healthy controls. 5-FU increased caspase activity on days 4 and 5 compared to controls. At day 4, cells pretreated with MRS, TSB, LGG SN, or EcN SN all displayed reduced caspase activity compared to 5-FU controls, while both SN groups had significantly lower caspase activity than their respective vehicle controls. Caspase activity in cells pretreated with MRS, LGG SN, and EcN SN was also reduced at day 5, compared to 5-FU controls. We conclude that pretreatment with selected probiotic SN could prevent or inhibit enterocyte apoptosis and loss of intestinal barrier function induced by 5-FU, potentially forming the basis of a preventative treatment modality for mucositis.

Keywords Probiotics · Secreted factors · Intestinal mucositis · Chemotherapy

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Introduction

The chemotherapy drug 5-fluorouracil (5-FU) is regularly prescribed to treat a wide variety of cancers, including colon, head, and neck [1].While 5-FU is effective at killing neoplastic cells, its administration also results in severe side effects, of which intestinal and oral mucositis are the most common. Those diagnosed with intestinal mucositis may suffer from a range of symptoms, such as nausea, vomiting, dyspepsia, dysphagia, and diarrhea [2]. These symptoms arise following a cascade of events within the small intestine, including upregulation of proinflammatory cytokines and transcription factors, reduced enterocyte proliferation and migration, and increased cell apoptosis [3]. The overall loss of barrier function can lead to clinical complications, such as infection and malnourishment [4]. In many cases, these side effects of chemotherapy can have such severe effects on the patient that the treatment must be ceased while the patient recovers. Despite its prevalence and the new treatment strategies being explored, there remains no definitive therapy for intestinal mucositis, and new treatment strategies are required.

Probiotics can be defined as live bacteria, which, when administered in sufficient numbers, exert beneficial physiologic or therapeutic activities [5]. Although their efficacy in the treatment of intestinal mucositis remains unclear [6], probiotics have been shown to exert beneficial effects (such as reduced enterocyte apoptosis [7], modulation of inflammation [8], and maintenance of barrier function [9]) suggesting that a probiotic-based therapy could be an effective therapeutic strategy. Recent studies have also identified probiotic supernatants (SN) as being capable of carrying out similar functions to the live bacteria from which they are derived [10]. The associated reduced risk of infection renders them ideal for use in a disorder such as mucositis in which the immune system of the individual is compromised [11, 12].

The high degree of strain specificity associated with mechanisms of probiotic action can be problematic when selecting an appropriate strain for a specific disorder. Consistent with the involvement of apoptosis and barrier disruption in the development of mucositis in the current study, we selected two probiotic strains which have previously been shown to promote cell survival and barrier function, *Lactobacillus rhamnosus* GG (LGG) [13, 14] and *Escherichia coli* Nissle 1917 (EcN) [15].

Treatment with live EcN has been shown to upregulate the tight junction molecule, zonula occluden (ZO)-1 at both the mRNA and protein levels, in addition to a reduction in intestinal permeability in mice with dextran sulfate sodium-induced colitis [15]. EcN administration led to an overall increase in intestinal barrier function, reduced body weight loss, and leukocyte infiltration. However, this study was limited to analysis of the large intestine, and it remains unexplored if EcN would also impact on small intestinal enterocytes. LGG has also been shown to exert beneficial effects on intestinal cells, preventing transepithelial electrical resistance (TEER) reduction and maintaining ZO-2 levels in Caco-2 cells treated with proinflammatory interferon-δ [13]. These changes were associated with the inhibition of proinflammatory tumor necrosis factor (TNF)-α expression, a cytokine which is also involved in the development of intestinal mucositis [16]. LGG has also demonstrated anti-apoptotic effects in a model of ex vivo staurosporine-induced apoptosis [14], in which a single dose of LGG was sufficient to inhibit caspase 3 expression, and reduce the overall percentage of cells undergoing apoptosis.

EcN and LGG are yet to be thoroughly investigated in the setting of 5-FU-induced damage. Therefore, the primary aim of this study was to test the capacity for probiotic SN derived from LGG and EcN, to protect epithelial cells from caspase activation and reduction of epithelial barrier function induced by 5-FU.

Materials and methods

SN preparation

LGG was kindly donated by Valio Ltd. (Helsinki, Finland). EcN was purchased from Ardeypharm (Herdecke, Germany) and was grown in tryptone soy broth (TSB, Oxoid, South Australia, Australia). LGG was grown on de Man Rogosa Sharpe (MRS) agar (Oxoid) at 37 °C for 24 h and then on MRS broth (Oxoid). All bacterial strains were incubated at 37 °C for 48 h and reached a concentration of 10^9 CFU/mL. Broths were then centrifuged at $1,500\times g$ for 10 min. SN were then collected and buffered with Tris/HCl to a pH of 7.0. SN were passed through a 0.20-μm filter and stored at −20 °C until use. Prior to administration, SN were diluted with 50 % Dulbecco's modified Eagle medium (DMEM).

Transepithelial electrical resistance

IEC-6 rat intestinal epithelial cells (passage 20–23) were cultured in medium comprising DMEM, 10 % fetal bovine serum (GIBCO®, Victoria, Australia), and 1 % penicillin/streptomycin (GIBCO®). IEC-6 cells were added to the upper layer of 0.33-cm^2 Transwell clear polyester permeable membranes (Corning Costar, NY, USA) at a density of 8×10^4 cells/well. Fresh DMEM (200 μL) was then added to the lower layer. Cells were allowed to adhere to the plate for 24 h prior to the beginning of the experiment. Plates were stored at 37 °C in 95 % air, 5 % CO_2 for the duration of the study.

Six treatment groups were used for the study: PBS + saline (untreated control), PBS + 5-FU (5-FU control), MRS broth + 5-FU, TSB broth + 5-FU, LGG SN + 5-FU, and EcN SN + 5-FU. The MRS and TSB groups were

included as vehicle controls to determine the effects of the media in which the SN were grown. Each treatment and DMEM (100 μL of each) were added to the upper layer of each well daily for 4 days. On day 3, 5 μM 5-FU in PBS (500 μL) was added to the DMEM in the lower layer for 24 h to induce a reduction in TEER. The medium was then replaced daily with fresh DMEM, without SN or vehicle. TEER measurements were recorded daily on days 3, 4, and 5. Treatments were performed in duplicate, while the entire experiment was carried out in triplicate to give $n=6$ for each treatment. The resistance across confluent monolayers was measured using a Millicell-ERS volt-ohm meter (Millipore, MA, USA) with electrodes. Values were expressed as ohms per square centimeter (Ω/cm^2), taking into account the surface area of the filter.

Caspase 3/7 activity

IEC-6 rat intestinal epithelial cells (passage 24–26) were cultured in medium comprising DMEM, 10 % fetal bovine serum (GIBCO®), and 1 % penicillin/streptomycin (GIBCO®). For apoptosis measurements, cells were seeded into a black 96-well plate (0.32 cm^2) at a density of 8×10^3 cells/well. Cells were allowed to adhere to the plate for 24 h prior to the addition of treatments. Plates were stored at 37 °C in 95 % air, 5 % CO_2 for the duration of the study.

The six treatment groups were as described previously. Each treatment and DMEM (100 μL of each) were added to each well daily for 4 days. On day 3, 5 μM 5-FU in PBS (200 μL) was added to each well for 24 h to induce apoptosis. The medium was then replaced daily with fresh DMEM without SN or vehicle. Apoptosis measurements were recorded daily on days 4 and 5. Treatments were performed in duplicate, while the entire experiment was carried out in triplicate to provide $n=6$ for each treatment.

Apoptosis was measured by caspase 3/7 assay (Apo-One® homogenous caspase 3/7 assay kit, Promega, WI, USA). The medium was removed from the cells and briefly stored in Eppendorf tubes, before 50 μL was returned to each well. This was done to ensure that cells which had undergone apoptosis and detached from the monolayer were included in the assay mixture and that the volume of medium was uniform for each well. Caspase reagent (50 μL) was then added to each well. The plate was covered with aluminum foil and left on a plate shaker at room temperature for 2 h. After 2 h, the plate was read on a fluorescent plate reader at 485/535 nm.

Statistical analysis

Statistical analysis was conducted using SPSS 15.0.1 for Windows (SPSS Inc., IL, USA). Data were compared by one-way ANOVA with a Tukey post hoc test and were expressed as mean ± SEM. For all data, $p<0.05$ was considered significant.

Results

Transepithelial electrical resistance

Cells were coincubated with 5 μM 5-FU overnight from days 3 to 4. TEER was recorded on days 3, 4, and 5. On day 3 (i.e., pre-5-FU) LGG SN pretreatment had significantly increased TEER compared to untreated controls ($p<0.05$, Fig. 1a). 5-FU administration significantly reduced TEER on day 4 compared to untreated controls ($p<0.05$); however, this reduction was not observed in any other treatments (Fig. 1b). All vehicle- and SN-treated groups displayed significantly higher TEER readings than 5-FU controls ($p<0.05$). Importantly, EcN treatment also increased TEER compared to its vehicle control, TSB ($p<0.05$).

The 5-FU-induced reduction in TEER was also observed on day 5 (Fig. 1c, $p<0.05$). As with the measurements on day 4, all vehicle and SN groups prevented this reduction in TEER. LGG and EcN SN + 5-FU groups exhibited increased TEER compared to untreated controls at day 5 (Fig. 1c). Furthermore, EcN SN-treated cells exhibited higher TEER than the TSB vehicle control ($p<0.05$, Fig. 1c).

Caspase 3/7 activity

Apoptosis was measured via caspase 3/7 activation on days 4 and 5. On day 4, 5-FU administration significantly increased caspase 3/7 activation compared to untreated controls (Fig. 2, $p<0.05$). All vehicle controls and SN groups displayed lower caspase 3/7 activation than 5-FU controls ($p<0.05$), and both LGG SN and EcN SN significantly reduced activation compared to their respective vehicle controls, MRS and TSB ($p<0.05$). Caspase activation for 5-FU controls remained elevated on day 5 compared to untreated cells ($p<0.05$, Fig. 3), with an increase again not observed in any vehicle- or SN-treated group. EcN SN-treated cells exhibited significantly reduced caspase 3/7 activation compared to 5-FU controls ($p<0.05$), although this reduction was not observed in the TSB vehicle control group.

Discussion

Pretreatment with EcN SN prevented 5-FU-induced reductions in TEER following a 24-h treatment with 5-FU. EcN has been shown to upregulate expression of ZO-1 [15] and ZO-2 [17], two tight junction molecules which have previously been associated with improvements in TEER [18]. Whether these changes are responsible for the increases

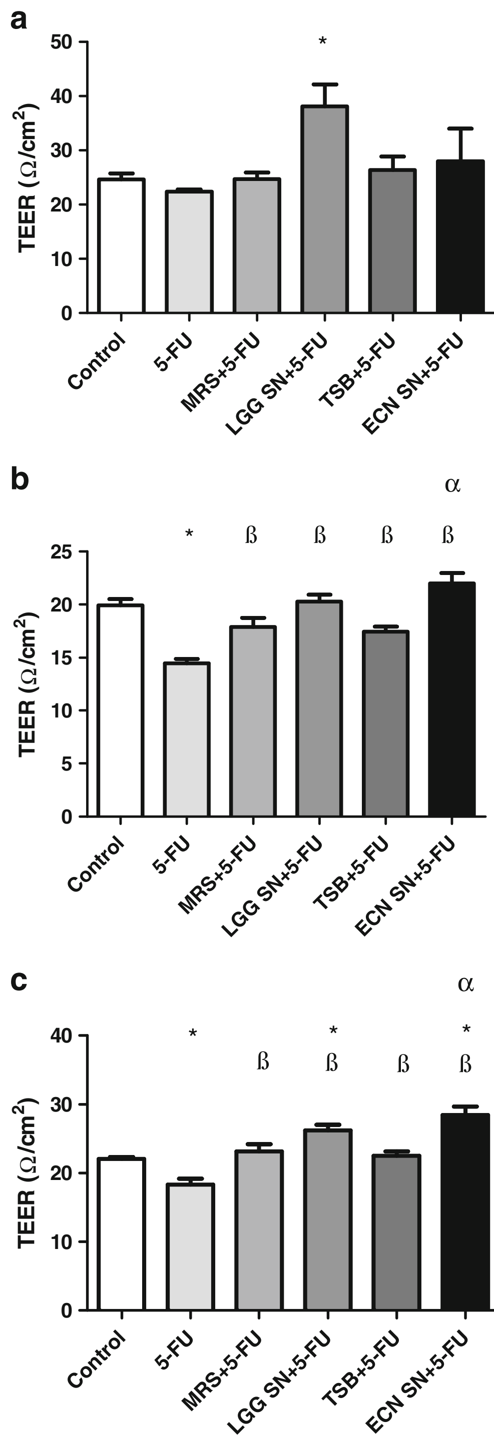

Fig. 1 Transepithelial resistance readings at **a** day 3, **b** day 4, and **c** day 5. Cells were treated from days 0 to 3 with phosphate buffered saline (control and 5-FU), de Man Rogosa Sharpe (MRS) broth, *L. rhamnosus* GG supernatant (LGG SN), tryptone soy broth (TSB), or *E. coli* Nissle 1917 supernatant (EcN SN). All treatments were added in a 1:1 dilution with Dulbecco's modified Eagle medium. Cells were treated with 5 μM 5-fluorouracil overnight on day 3. Values expressed as mean ± SEM, n=6. *p<0.05, significance compared to control treatment; $^{\beta}p$<0.05, significance compared to 5-FU treatment; and $^{\alpha}p$<0.05, significance compared to TSB + 5-FU

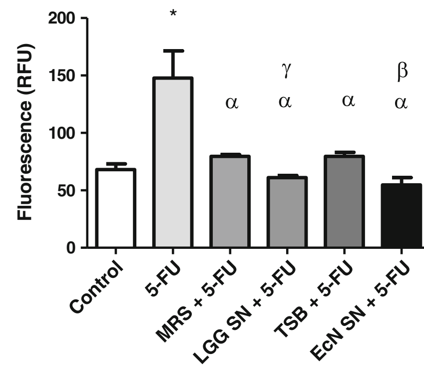

Fig. 2 Caspase 3/7 activation on day 4 (24-h post 5-fluorouracil (5-FU)). Prior to 5-FU incubation, cells were treated from days 0 to 3 with phosphate buffered saline (control and 5-FU), de Man Rogosa Sharpe (MRS) broth, *L. rhamnosus* GG supernatant (LGG SN), tryptone soy broth (TSB), or *E. coli* Nissle 1917 supernatant (EcN SN). All treatments were added in a 1:1 dilution with Dulbecco's modified Eagle medium. Values expressed as mean ± SEM, n=6. *p<0.05, significance compared to control treatment; $^{\alpha}p$<0.05, significance compared to 5-FU, $^{\gamma}p$<0.05, significance compared to MRS + 5-FU; and $^{\beta}p$<0.05 significance compared to TSB + 5-FU

observed in the current study requires further investigation. However, as the effect of 5-FU administration on the expression of ZOs remains undefined, future studies should also investigate the capacity for EcN to alter the distribution of other tight junction molecules, such as claudins. The findings of Zyrek and colleagues are particularly relevant, as an increase in ZO-1 was observed in a model of intestinal damage, whereby the authors added enteropathogenic *E. coli* E2348/69 to T84 cells to induce epithelial disruption [17]. The study revealed that coincubation of EcN with the pathogen, or the addition of EcN following damage, was able to prevent the onset of epithelial disruption and maintain barrier integrity.

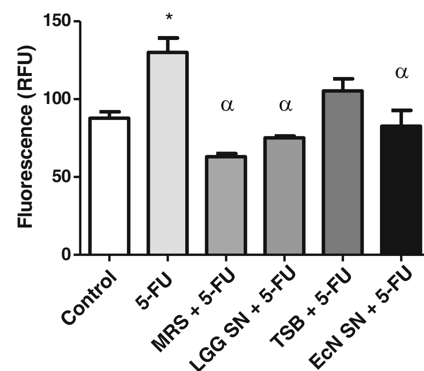

Fig. 3 Caspase 3/7 activation on day 5 (48-h post 5-fluorouracil (5-FU)). Prior to 5-FU incubation, cells were treated from days 0 to 3 with phosphate buffered saline (control and 5-FU); de Man Rogosa Sharpe (MRS) broth, *L. rhamnosus* GG supernatant (LGG SN), tryptone soy broth (TSB), or *E. coli* Nissle 1917 supernatant (EcN SN). All treatments were added in a 1:1 dilution with Dulbecco's modified Eagle medium. Values expressed as mean ± SEM, n=6. *p<0.05, significance compared to control treatment; $^{\alpha}p$<0.05, significance compared to 5-FU treatment

Given the effect of EcN SN on caspase 3/7 activity in the current study, we also propose that the reduced cell apoptosis may have contributed to an overall increase in epithelial integrity. Caspases 3 and 7 are executioner caspases which can be used as positive markers for cell apoptosis [1]. EcN SN successfully prevented the increase in caspase 3/7 activity induced by 5-FU. To our knowledge, this represents the first description of an antiapoptotic effect of EcN SN. Previously, ECN has been shown to influence the expression of genes linked to apoptosis regulation [17] and proinflammatory cytokine production [19], indicating potential mechanisms underlying the observed results.

In the current study, LGG SN decreased apoptosis and prevented barrier disruption, a finding consistent with previous studies [20, 21]. Acute improvement of epithelial integrity has been reported in T84 cells treated with LGG, where a 3-h incubation significantly increased TEER [20]. LGG administration was also able to prevent an *E. coli* 0157:H7-induced reduction in TEER while maintaining epithelial permeability and redistribution of tight junction molecules. Seth and colleagues isolated two proteins (p40 and p70) from the SN of LGG that prevented hydrogen peroxide-induced damage to barrier function in Caco-2 cells [11]. Further analysis of the LGG SN revealed a number of proteins released by LGG, including LytR and CpsA [22]. These two proteins are components of the LytR/CpsA/Psr protein family which plays a role in cell wall structural maintenance. To determine if these proteins are able to protect against 5-FU induced damage, future studies should investigate the ability of these proteins, both independently and in combination, to maintain barrier function.

The capacity for LGG to reduce enterocyte apoptosis has been described previously in other models of intestinal damage [23, 24]. Yan and colleagues studied the secreted proteins p40 and p75 from LGG and found that both proteins were able to inhibit TNF-induced apoptosis while also increasing cell proliferation [24]. Further studies are required to determine the mechanism by which these proteins were able to inhibit apoptosis. The components of the SN responsible for the beneficial results in the current study have not yet been identified.

This study further suggests an important role for the growth media from which the SN was obtained. MRS broth improved TEER following incubation with 5-FU, while both MRS and TSB prevented increases in caspase activity post 5-FU. The primary component of TSB is pancreatic digest of casein—a mix of amino acids, of which glutamine is most abundant [25]. Glutamine is an important amino acid for cell proliferation [26] and has been associated with maintenance of epithelial cell integrity [27, 28]. Indeed, early research into the use of glutamine as a treatment for chemotherapy-induced mucositis yielded promising results [29]. In a model of methotrexate-induced mucositis, glutamine supplementation following chemotherapy reduced apoptosis and promoted cell proliferation. It was hypothesized that the abnormally high availability of glutamine, the preferred fuel source of the small intestine [30], may have stimulated mucosal hyperplasia, as well as the release of enteric hormones which in turn have trophic effects on the intestinal mucosa.

Glucose is the major component of MRS broth [31]. High glucose-containing media have been shown to prevent lipopolysaccharide-induced (LPS) changes to both apoptosis and epithelial barrier function [32], with high glucose-containing media preventing LPS-induced reduction of Bcl-2 protein expression and increasing antiapoptotic Bcl-X_L. These proteins have previously been associated with the development of intestinal mucositis [1], and similar modulation in the current study may have been responsible for the protective effects of MRS. The protective effect of the growth media in the current study suggests that the changes to caspase activity and TEER observed in the SN-treated groups may not have been entirely due to the secreted factors. Future studies should attempt to separate the secreted compounds from the growth media using techniques such as super centrifugation and then compare the two individually.

We conclude that secreted factors from LGG and EcN were partially effective at preventing 5-FU-induced alterations in epithelial barrier function and apoptosis, with the growth media also contributing to protection. Future studies should focus on determining the importance of strain specificity and comparisons between the probiotic SN and their respective live bacteria. Furthermore, investigation into the effects of the probiotic-derived compounds on tight junction expression and intestinal permeability should be conducted to better understand the mechanisms underlying maintenance of intestinal barrier function.

Conflict of interest I, Luca Prisciandaro, state that there is no conflict of interest associated with this manuscript. I declare that the current work was not financially sponsored by any organization. The authorship of this manuscript is as described in the attached Authorship/Disclosure form, with myself as the first author. I have a full control of all primary data related to this manuscript and would be happy to allow the journal to review this data if necessary.

References

1. Bowen JM, Gibson RJ, Cummins AG, Keefe DM (2006) Intestinal mucositis: the role of the Bcl-2 family, p53 and caspases in chemotherapy-induced damage. Support Care Canc 14:713–731
2. Soares PM, Mota JM, Gomes AS, Oliveira RB, Assreuy AM, Brito GA, Santos AA, Ribeiro RA, Souza MH (2008) Gastrointestinal dysmotility in 5-fluorouracil-induced intestinal mucositis outlasts inflammatory process resolution. Canc Chemother Pharmacol 63:91–98

3. Daniele B, Secondulfo M, De Vivo R, Pignata S, De Magistris L, Delrio P, Palaia R, Barletta E, Tambaro R, Carratu R (2001) Effect of chemotherapy with 5-fluorouracil on intestinal permeability and absorption in patients with advanced colorectal cancer. J Clin Gastroenterol 32:228–230
4. Duncan M, Grant G (2003) Oral and intestinal mucositis—causes and possible treatments. Aliment Pharmacol Ther 18:853–874
5. Sartor RB (2004) Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics. Gastroenterology 126:1620–1633
6. Prisciandaro LD, Geier MS, Butler RN, Cummins AG, Howarth GS (2011) Probiotic factors partially improve parameters of 5-fluorouracil-induced intestinal mucositis in rats. Canc Biol Ther 11(7):671–677
7. Khailova L, Mount Patrick SK, Arganbright KM, Halpern MD, Kinouchi T, Dvorak B (2010) *Bifidobacterium bifidum* reduces apoptosis in the intestinal epithelium in necrotizing enterocolitis. Am J Physiol Gastrointest Liver Physiol 299:G1118–G1127
8. Philippe D, Favre L, Foata F, Adolfsson O, Perruisseau-Carrier G, Vidal K, Reuteler G, Dayer-Schneider J, Mueller C, Blum S (2011) *Bifidobacterium lactis* attenuates onset of inflammation in a murine model of colitis. World J Gastroenterol 17:459–469
9. Ueno N, Fujiya M, Segawa S, Nata T, Moriichi K, Tanabe H, Mizukami Y, Kobayashi N, Ito K, Kohgo Y (2011) Heat-killed body of *Lactobacillus brevis* SBC8803 ameliorates intestinal injury in a murine model of colitis by enhancing the intestinal barrier function. Inflamm Bowel Dis
10. Prisciandaro L, Geier M, Butler R, Cummins A, Howarth G (2009) Probiotics and their derivatives as treatments for inflammatory bowel disease. Inflamm Bowel Dis 15(12):1906–1914
11. Seth A, Yan F, Polk DB, Rao RK (2008) Probiotics ameliorate the hydrogen peroxide-induced epithelial barrier disruption by a PKC- and MAP kinase-dependent mechanism. Am J Physiol Gastrointest Liver Physiol 294:G1060–G1069
12. Sokol H, Pigneur B, Watterlot L et al (2008) *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci U S A 105:16731–16736
13. Donato KA, Gareau MG, Wang YJ, Sherman PM (2010) *Lactobacillus rhamnosus* GG attenuates interferon-γ and tumour necrosis factor-alpha-induced barrier dysfunction and pro-inflammatory signalling. Microbiology 156:3288–3297
14. Lin PW, Nasr TR, Berardinelli AJ, Kumar A, Neish AS (2008) The probiotic *Lactobacillus* GG may augment intestinal host defense by regulating apoptosis and promoting cytoprotective responses in the developing murine gut. Pediatr Res 64:511–516
15. Ukena SN, Singh A, Dringenberg U, Engelhardt R, Seidler U, Hansen W, Bleich A, Bruder D, Franzke A, Rogler G, Suerbaum S, Buer J, Gunzer F, Westendorf AM (2007) Probiotic *Escherichia coli* Nissle 1917 inhibits leaky gut by enhancing mucosal integrity. PLoS One 2:e1308
16. Logan RM, Stringer AM, Bowen JM, Yeoh AS, Gibson RJ, Sonis ST, Keefe DM (2007) The role of pro-inflammatory cytokines in cancer treatment-induced alimentary tract mucositis: pathobiology, animal models and cytotoxic drugs. Cancer Treat Rev 33:448–460
17. Zyrek AA, Cichon C, Helms S, Enders C, Sonnenborn U, Schmidt MA (2007) Molecular mechanisms underlying the probiotic effects of *Escherichia coli* Nissle 1917 involve ZO-2 and PKCzeta redistribution resulting in tight junction and epithelial barrier repair. Cell Microbiol 9:804–816
18. Basuroy S, Sheth P, Kuppuswamy D, Balasubramanian S, Ray RM, Rao RK (2003) Expression of kinase-inactive c-Src delays oxidative stress-induced disassembly and accelerates calcium-mediated reassembly of tight junctions in the Caco-2 cell monolayer. J Biol Chem 278:11916–11924
19. Otte JM, Mahjurian-Namari R, Brand S, Werner I, Schmidt WE, Schmitz F (2009) Probiotics regulate the expression of COX-2 in intestinal epithelial cells. Nutr Cancer 61:103–113
20. Johnson-Henry KC, Donato KA, Shen-Tu G, Gordanpour M, Sherman PM (2008) Lactobacillus rhamnosus strain GG prevents enterohemorrhagic *Escherichia coli* O157:H7-induced changes in epithelial barrier function. Infect Immun 76:1340–1348
21. Myllyluoma E, Ahonen AM, Korpela R, Vapaatalo H, Kankuri E (2008) Effects of multispecies probiotic combination on *Helicobacter pylori* infection in vitro. Clin Vaccine Immunol 15:1472–1482
22. Sanchez B, Schmitter JM, Urdaci MC (2009) Identification of novel proteins secreted by *Lactobacillus rhamnosus* GG grown in de Mann-Rogosa-Sharpe broth. Lett Appl Microbiol 48:618–622
23. Lin YP, Thibodeaux CH, Pena JA, Ferry GD, Versalovic J (2008) Probiotic *Lactobacillus reuteri* suppress proinflammatory cytokines via c-Jun. Inflamm Bowel Dis 14:1068–1083
24. Yan F, Cao H, Cover TL, Whitehead R, Washington MK, Polk DB (2007) Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. Gastroenterology 132:562–575
25. Oxoid (2010) Dehydrated culture media: modified tryptone soya broth. [Online]. Available at http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM0989&sec=2&org=72&c=UK&lang=EN (accessed on February 1st 2011)
26. Tuhacek LM, Mackey AD, Li N, DeMarco VG, Stevens G, Neu J (2004) Substitutes for glutamine in proliferation of rat intestinal epithelial cells. Nutrition 20:292–297
27. Li N, Neu J (2009) Glutamine deprivation alters intestinal tight junctions via a PI3-K/Akt mediated pathway in Caco-2 cells. J Nutr 139:710–714
28. Potsic B, Holliday N, Lewis P, Samuelson D, DeMarco V, Neu J (2002) Glutamine supplementation and deprivation: effect on artificially reared rat small intestinal morphology. Pediatr Res 52:430–436
29. Sukhotnik I, Mogilner JG, Karry R, Shamian B, Lurie M, Kokhanovsky N, Ure BM, Coran AG (2009) Effect of oral glutamine on enterocyte turnover during methotrexate-induced mucositis in rats. Digestion 79:5–13
30. Windmueller HG, Spaeth AE (1974) Uptake and metabolism of plasma glutamine by the small intestine. J Biol Chem 249:5070–5079
31. Oxoid (2010) Dehydrated culture media: MRS broth (De Man, Rogosa, Sharpe). [Online]. Available at http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM0359&c=UK&lang=EN (accessed on February 1st 2011)
32. Yu LC, Flynn AN, Turner JR, Buret AG (2005) SGLT-1-mediated glucose uptake protects intestinal epithelial cells against LPS-induced apoptosis and barrier defects: a novel cellular rescue mechanism? FASEB J 19:1822–1835