ryma p

Dataset · November 2013

1 author:

Touilboukoffa Chafia
University of Science and Technology Houari…

Immunopharmacology and Immunotoxicology

http://informahealthcare.com/ipi
ISSN: 0892-3973 (print), 1532-2513 (electronic)
Immunopharmacol Immunotoxicol, Early Online: 1–7

DOI: 10.3109/08923973.2013.790413

informa healthcare

RESEARCH ARTICLE

Beneficial role of the probiotic mixture Ultrabiotique on maintaining the integrity of intestinal mucosal barrier in DSS-induced experimental colitis

Ryma Toumi[1], Katia Abdelouhab[1], Hayet Rafa[1], Imene Soufli[1], Djamila Raissi-Kerboua[2], Zineb Djeraba[1], and Chafia Touil-Boukoffa[1]

[1]*USTHB (University of Sciences and Technology), Laboratory of Cellular and Molecular Biology (LBCM), Cytokines and NO Synthases Team, Faculty of Biological Science, Algiers, Algeria and* [2]*Clininical Pathology Laboratory, Algiers, Algeria*

Abstract

The etiology of inflammatory bowel diseases which include ulcerative colitis (UC) and Crohn disease has not yet been clarified. Several hypotheses suggest a change in composition of gut microflora along with an impaired mucosal barrier that lead to excessive mucosal immunologic responses. Increased production of nitric oxide (NO) contributes greatly to the tissue injury caused by chronic inflammation. Evidence indicates that the mucus layer covering the epithelium is altered during UC and experimental colitis. Our aim in this study was to investigate the potential therapeutic effect of probiotic during DSS-induced colitis by modulating the immune system and colonic mucus production. For that purpose, the probiotic formulation Ultrabiotique® (*Lactobacillus acidophilus*, *Bifidobacterium lactis*, *Lactobacillus plantarum* and *Bifidobacterium breve*) was administered daily for 7 d to mice with colitis. Probiotic supplementation improved clinical symptoms and histological alterations observed during DSS induced colitis. Ultrabiotique® treatment down regulated the NO production by peritoneal macrophages of DSS-treated mice and enhanced mucus production in both DSS-treated and healthy mice. In conclusion, the modification of microflora by the Ultrabiotique® played a beneficial role in maintaining the integrity of the intestinal mucosal barrier and promoted tissue repair.

Keywords

Gut microflora, mucus, nitric oxide, peritoneal macrophages, ulcerative colitis

History

Received 9 January 2013
Revised 24 March 2013
Accepted 25 March 2013
Published online 2 May 2013

Introduction

Inflammatory bowel diseases (IBD) including Crohn's disease and ulcerative colitis (UC) are a group of diseases characterized by chronic and relapsing gastrointestinal inflammation. The precise etiology of IBD remains uncertain. However, it is widely admitted that a combination of genetic susceptibility, immune disorders and environmental factors are involved in the pathogenic mechanisms behind these conditions[1,2]. Increasing evidence from animal models of IBD has highlighted the importance of the cross talk between gut flora, the mucosal epithelial barrier and the intestinal immune system. Disruption of any of these components has been shown to result in intestinal inflammation[3]. Abnormal activation of the mucosal immune responses is thought to be the major trigger of an ongoing intestinal inflammation. The chronic inflammation in IBD is characterized by a concomitant production of high levels of pro-inflammatory cytokines (TNF-α, IL-1β, IL-8, IFN-γ, IL-12) and free radicals such as nitric oxide (NO)[4,5]. The later seems to be involved in the development of tissue injury into the intestine in IBD[6–9].

The involvement of gut flora in the etiology of IBD is supported, firstly, by effective responses to treatment with antibiotics in patients and, secondly, by the highlighting of microbial agents and/or their components in intestinal inflammatory lesions[10,11]. The imbalance of gut flora known as dysbiosis leads to the alteration of the intestinal epithelial mucosa and to the abnormal and uncontrolled activation of the immune system[12]. Furthermore, in UC and DSS induced colitis, the mucus layer overlying the intestinal epithelial surface seems to be altered. The secretion of mucins by goblet cells plays a pivotal role in protecting mucosal epithelial layer against bacterial invasion, thereby preventing inflammation and tissue damage in the gastro-intestinal tract[13]. In acute DSS-induced colitis, bacteria and/or bacterial products play a major role in the enhancement of an intestinal inflammation similar to UC[14]. However, if some bacterial groups such as Enterobacteriaceae and Clostridium are potentially pathogenic and may be involved in the development of IBD. In contrast, others such as Bifidobacterium and Lactobacillus could prevent it[10]. Currently the search for new preventive and therapeutic approaches for IBD brought a real

Address for correspondence: Pr. Chafia Touil-Boukoffa, USTHB, Laboratory of Cell and Molecular Biology (LBCM), Faculty of Biological Sciences, Team Cytokines et NO Synthases, Immunity and Pathogeny BP 32 El-Alia 16111 Bab-ezzouar, Algiers, Algeria. Tel: +213 21 247-950/964. Poste 121. Fax: +213 21 24 79 04. E-mail: touilboukoffa@yahoo.fr

interest for probiotics defined as living micro-organisms that promote beneficial health effects when ingested[15]. Clinical trials and experimental studies suggest that selected probiotic species, alone or in combination, can prevent recurrent intestinal inflammation and possibly treat active IBD. The exact mechanisms of action of probiotics have not thus far been clarified. Nonetheless, it appears that their high prevalence in the gut reinforces the epithelial barrier function and maintains the balance between the pro and anti-inflammatory immune responses. The anti-inflammatory effect of oral probiotics lactobacilli and bifidobacteria has been shown in some inflammatory conditions such as pouchitis, UC and several animal models of IBD[16–18]. In our previous study, we have demonstrated that supplementation of the probiotic formula Ultrabiotique® which consists of four-strain cocktail of lactobacilli and bifidobacteria (*Lactobacillus acidophilus*, *Bifidobacterium lactis*, *Lactobacillus plantarum* and *Bifidobacterium breve*) to mice before treatment with DSS reduced the severity of colitis[19]. The increasing number of lactobacilli and bifidobacteria in the colon prevented intestinal mucosa from inflammatory lesions and reduced the local NO production. Considering these observations, in this current study we investigate the potential therapeutic effects of Ultrabiotique® when supplemented during the induction of colitis. The aim of this line of research was to have more light on the mechanisms of protection by probiotic. We, therefore, examined its immunomodulatory effects on NO production in peritoneal macrophages (pMΦ) cultures. Data from this study indicate that stimulation of colonic mucus would maintain the integrity of the epithelial barrier by decreasing bacterial translocation and promoting tissue repair of damaged mucosa.

Materials and methods

Probiotic formulation and other chemicals

Ultrabiotique® produced by Laboratoire Nutrisanté (Montaigu-France) consists of four live bacterial strains: *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Bifidobacterium lactis* and *Bifidobacterium breve*). Each capsule of Ultrabiotique® contains 4×10^9 colony-forming units (CFU) of viable lyophilized bacteria. The lyophilized probiotic was dissolved in skimmed sterile milk and diluted to a concentration of 10^9 CFU/ml of milk. DSS (molecular weight 36 000–50 000) was obtained from MP Biomedicals (Illkirch, France). Griess reagents for the determination of NO metabolites were [Naphtyl Ethylene Diamine (NED) ($C_{22}H_{20}N_2$, 2HCl) and sulfanilamide (*p*-aminobenzenesulfonamide, $C_6H_8N_2O_2S$)] obtained from Sigma Chemical (St. Louis, MO).

Animals

Male Swiss albino mice weighing 25–30 g were purchased from Pasteur Institute (Algiers, Algeria). They were exposed to 12 h light and dark cycle. Food and water were supplied *ad libitum.*

DSS-induced colitis

Dextran sodium sulfate (molecular weight 36 000–50 000) was dissolved in tap water to obtain a 2.5% DSS solution. Swiss albino mice were allowed free access to 2.5% DSS solution as drinking water for 7 d to develop a mouse model of colitis as described by Okayasu et al.[20]. The solution was freshly prepared every 2 d.

Probiotic treatment

To evaluate whether the probiotic formula Ultrabiotique® is effective in the treatment of colitis mice were randomly divided into four groups ($n = 4$ mice per group) as follows:

- (DSS) group: model group receiving 2.5% DSS in drinking water *ad libitum* for 7 d.
- (DSS-Prb) group: probiotic treatment group during colitis receiving concomitantly 2.5% DSS for modeling + 200 µl of skimmed milk containing 10^9 CFU/ml probiotic.
- (Prb group): probiotic group receiving 200 µl of skimmed milk containing 10^9 CFU/ml probiotics for 7 d. The mice were fed with 200 µL of daily-prepared suspension of the probiotic formula by oral gavage.
- (Ctrl) group: control group receiving tap water.

All the mice were monitored daily. On the 7th day, they were sacrificed.

Histopathology

After 7 d of DSS administration, mice were sacrificed. The entire colon was excised from the colo-cecal junction and its length was measured. The colon was carefully rinsed with 5 ml of cold PBS (pH 7.4) to remove the fecal remnants. One cm of the distal colon was removed, cut open longitudinally and fixed for 24 h in PBS buffered 10% formalin. The tissue was then embedded in paraffin and cut into 5 µm thick sections.

The sections were stained with Hematoxylin and Eosin (H&E) to study histological changes and with Alcian blue dye binding method to evaluate mucus secretion by goblet cells.

Cell culture

Resident macrophages were collected in sterile PBS (pH = 7.4) from peritoneal cavity of each mice. Cells were washed twice with sterile PBS (pH = 7.4), suspended in DMEM supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin containing 20 mM L-Glutamin and cultured in 24-well tissue culture plates at a density of 4×10^5 cells/well for 2 h. After a-2 h incubation at 37 in 5% CO_2 adherent macrophages were washed three times with sterile PBS (pH = 7.4) to remove non adherent cells and then cultured under the same conditions for 24 h in complete DMEM. After 24 h, the supernatants were kept at (−20 °C) for subsequent nitrite level measurement.

Nitrite level measurement

Nitrite production, an indicator of NO synthesis, was measured in all supernatants culture of pMΦ on the basis of modified Griess reaction as described by Touil-Boukoffa et al.[21]. In brief, 100 µl of culture supernatant were mixed with 100 µl of Griess reagent (5% sulfanilamide, 0.5% napthylethylenediamine dihydrochloride and 20% HCl) and 800 µL of distilled water. The resulting solution was incubated for 20 min at room temperature. Absorbance was assayed at 543 nm and compared with a standard curve obtained using sodium nitrite ($NaNO_2$).

Statistical analysis

All data were expressed as means $\pm$ standard deviation (SD). Statistical analyses were performed by one-way ANOVA with the Fisher's LSD test modified by the Bonferroni test. Probability values of $p \leq 0.05$ were considered to be significant.

Results

Probiotic treatment alleviates clinical symptoms of DSS-induced colitis

The administration of 2.5% DSS to Swiss albino mice resulted in severe colitis marked by diarrhea and gross rectal bleeding. At the end of the experimental period DSS treatment group showed many clinical signs of severe illness and moribund state. However, these symptoms were significantly attenuated in (DSS-Prb) group. No morbidity symptoms or mortality occurred in this group. Shortening of the colon is used as a marker of inflammation in DSS-induced colitis model. In (DSS) group length of colon was significantly shortened (5.75 ± 0.2, $***p < 0.0001$) compared to (Ctrl) group (9.25 ± 0.28) whereas in the (DSS-Prb) group the administration of Ultrapbiotique® prevented the inflammatory colonic shortening (8 ± 0.56, $***p < 0.0001$) (Figure 1). It remained however significantly elevated when compared to control group ($*p < 0.01$). No significant difference was observed in the (Prb) (9.12 ± 0.25) group and (Ctrl) group (Figure 1A).

Probiotic reduces nitrite levels in peritoneal macrophages cultures

During DSS-induced colitis, recruitment in the intestine of inflammatory cells and the accumulation of inflammatory mediators including NO contribute to the occurrence of intestinal lesions. Because of difficulties in isolating large amounts of macrophages from the colon, the measure of NO production by peritoneal macrophages was used as a model to evaluate the local inflammatory state during DSS colitis and after treatment of the animals with the probiotic. The results obtained showed a nitrite levels significantly higher in culture supernatants of DSS group (45.33 ± 2.08, $***p < 0.0001$) compared to the (Ctrl) group (22.53 ± 1.8). A significant decrease of nitric level was observed in culture supernatants of (DSS-Prb) group (27.52 ± 0.84, $***p < 0.0001$) in comparison to (DSS) group. On the other hand, the comparison of nitrite levels found in (Ctrl) group and (Prb) group showed a significant unexpected increase in NO levels in pMΦ culture supernatant of (Prb) group (31.16 ± 2.89, $**p < 0.001$) (Figure 2).

Probiotic treatment improves histological alterations associated with colitis

In our study, administration of 2.5% DSS in the drinking water of Swiss albino mice for 7 d was sufficient to induce acute colitis. Histological examination of distal colon of mice treated with DSS showed an altered architecture, mucosal atrophy and degeneration of intestinal crypts (Figure 3C) when compared to (Ctrl) group (Figure 4A) and (Prb) group (Figure 3B). Moreover, a depletion of goblet cells and crypt abscess formation were observed in some areas of the mucosa (Figure 4C). The lamina propria was the seat of a major polymorphic inflammatory infiltrate extending to the submucosa. In addition to these elements, the appearance of submucosal oedema in the submucosa and thickening of the muscularis were noticed (Figure 4C). In (DSS-Prb) group, Ultrabiotique® treatment decreased colonic tissue damages, promoted intestinal mucosa re-epitheliazation and crypts regeneration (Figure 4D). Unlike (DSS) group, we noted the

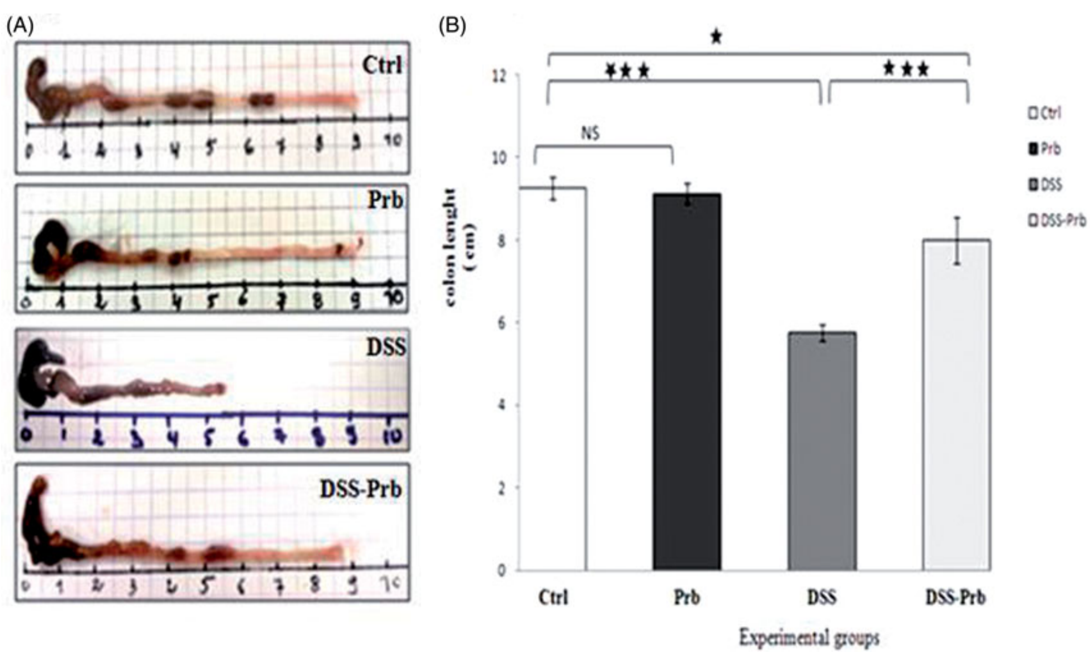

Figure 1. Oral administration of Ultrabiotique® improved the shortening of the colon. Representative photomicrographs (A) and diagram (B) of colon length. The length of large intestine was significantly longer in (DSS-Prb) group ($n = 4$) compared to (DSS) group ($n = 4$). All results are expressed as mean $\pm$ standard deviation. p Values from LSD *post-hoc* ANOVA test are indicated (NS: not significant, $*p \leq 0.01$, $***p \leq 0.0001$).

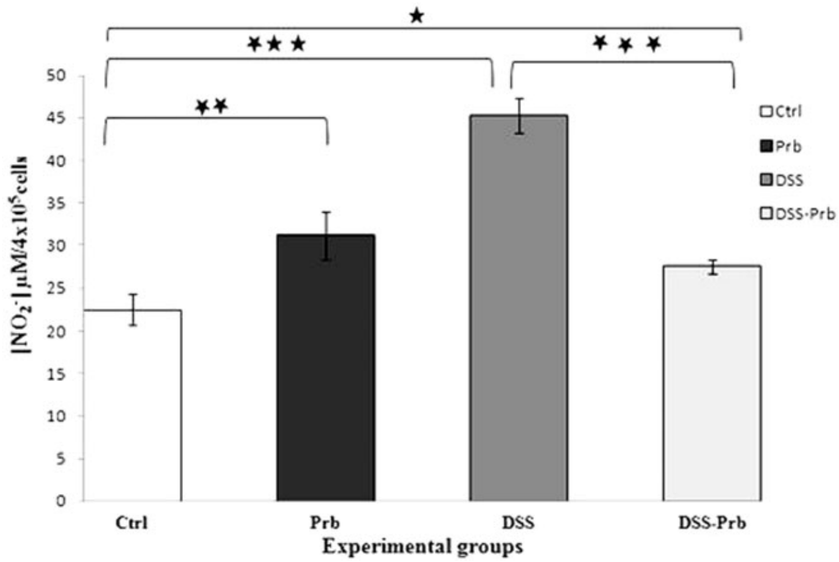

Figure 2. Oral administration of Ultrabiotique® reduces nitrite levels in peritoneal macrophages cultures of colitic mice. DSS treatment increased significantly nitrite level in culture supernatant of pMΦ compared to (Ctrl) group ($n = 4$). Oral administration of the probiotic reduced significantly nitrite level in culture supernatants of pMΦ of (DSS-Prb) treated mice ($n = 4$) compared to (DSS) group ($n = 4$) but enhanced nitritre level in healthy mice when compared to (Ctrl) group. All results are expressed as mean ± standard deviation. *p* Values from LSD *post-hoc* ANOVA test are indicated (NS: not significant, $*p \leq 0.01$, $**p \leq 0.001$, $***p \leq 0.0001$).

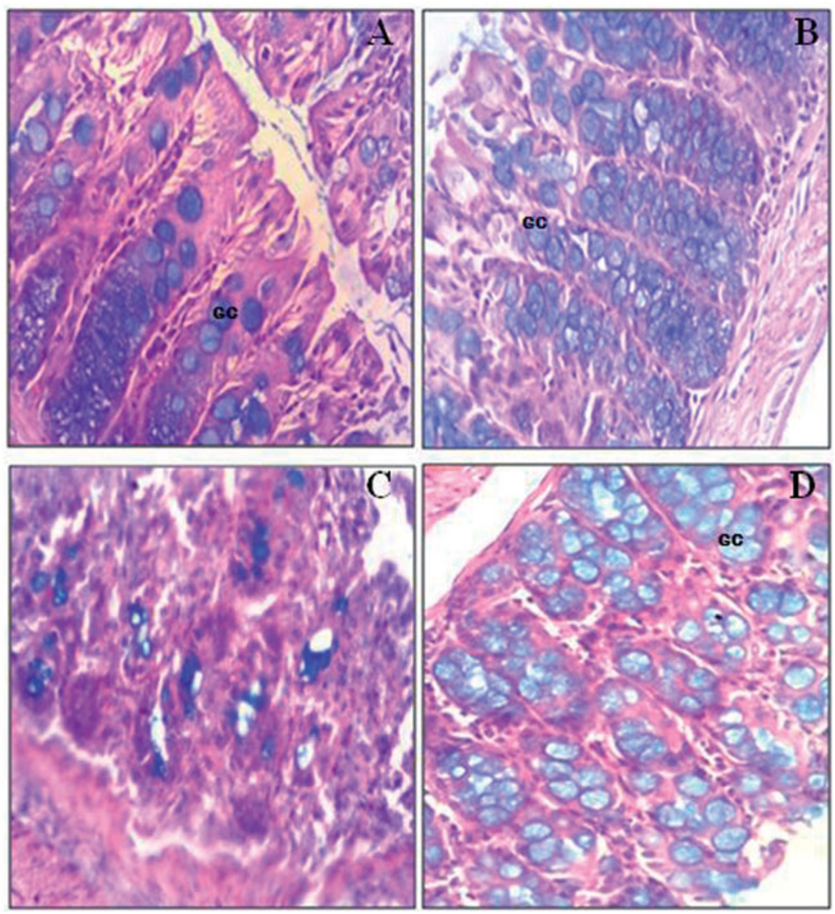

Figure 3. Representative photomicrographs of bleu Alcian stained colonic tissue. (×400). (A) (Ctrl) group ($n = 4$): normal crypts and mucus secretion. (B) (Prb) group ($n = 4$): normal crypts, presence of an important number of goblet cells that secret mucus. (C) (DSS) group ($n = 4$): gland atrophy and dramatic depletion of goblet cell accompanied by the absence of mucus. (D) (Prb-DSS) ($n = 4$): normal crypts and secretion of mucus by the goblet cells (GC).

absence of the polymorphic inflammatory infiltrate in the lamina propria and submucosa. The attenuation of colonic inflammation in (Prb-DSS) group is probably due to the anti-inflammatory properties of lactobacilli and bifdobacteria in the colon.

Probiotic treatment promotes mucus secretion by goblet cells

The histological examination (H&E staining) of distal colon from (DSS) group showed a depletion of goblet cells when compared to (Ctrl) group and (DSS-Prb) group. We suggested that the Ultrabiotique® treatment could be beneficial on the intestinal epithelium through stimulating mucus secretion by goblet cells. Thus, we performed specific staining with Alcian blue to assess the mucus production following administration of Ultrabiotique®. We noticed high mucus staining with Alcian blue in the group DSS-Prb (Figure 3D) in comparison to the (DSS) group (Figure 3C). Furthermore, histological analysis of mice receiving only Probiotic (Prb group) (Figure 3B) showed a stronger staining with Alcian blue

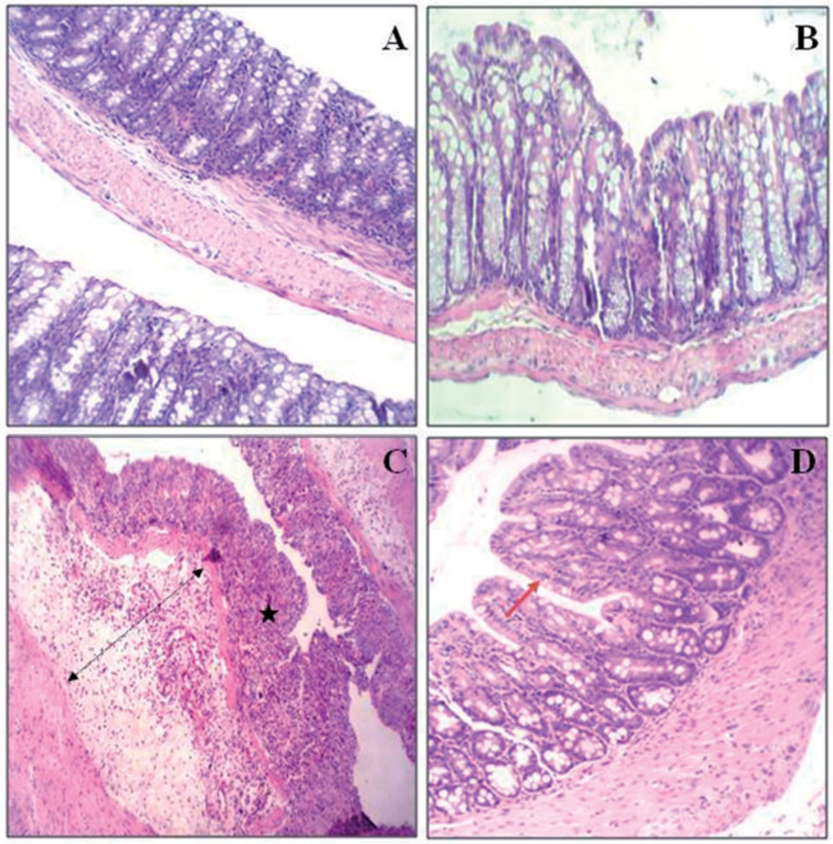

Figure 4. Representative histological photomicrographs from the distal colon of mice (H.E ×100). (A) (Ctrl) group ($n=4$), (B) (Prb) group ($n=4$): normal crypts. (C) (DSS) treatment ($n=4$): evidence of crypt destruction (Star), with infiltration of leukocytes throughout the lamina propria and extending into the submucosa. Prominent submucosal edema was also evident (double arrow). (D) (Prb-DSS) group ($n=4$): normal colonic histology, clear evidence of intestinal mucosa re-epitheliazation and crypts regeneration (simple arrow).

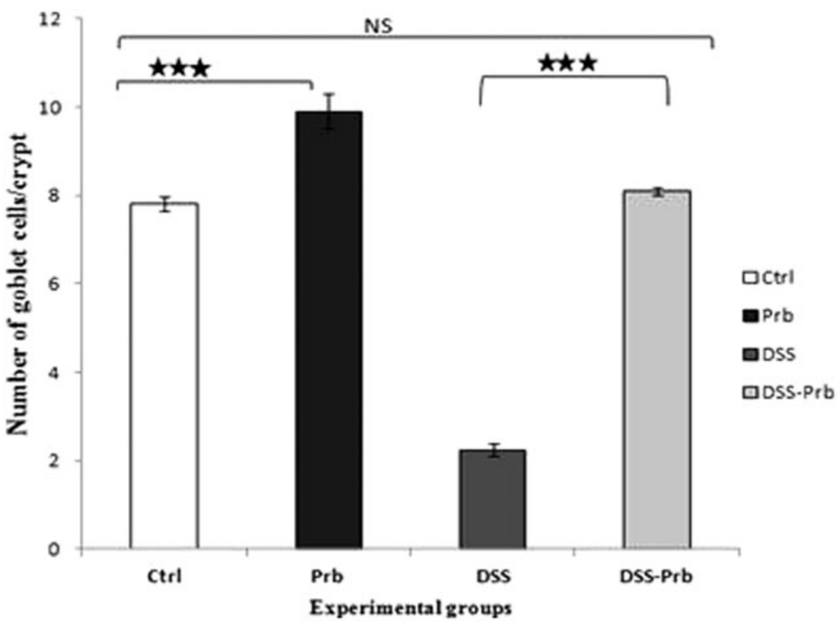

Figure 5. Changes in goblet cells number after Oral administration of Ultrabiotique®. All results are expressed as mean ± standard deviation. p Values from LSD *post-hoc* ANOVA test are indicated (NS: not significant, $***p \leq 0.0001$).

compared to (Ctrl) group (Figure 3A), (DSS) group and (DSS-Prb) group suggesting that Ultrabiotique® treatment has induced a high secretion of mucus in the colon. An elevated number of goblet cells was found in the (Prb group) in comparison with the other experimental groups (Figure 5).

Discussion

In IBD, the dysregulation of the immune system and disruption of the intestinal microbiota are the key elements of the etiopathogenesis of these conditions. This fact provided the rationale for the use of probiotics to manipulate the intestinal microenvironment and stimulate immunomodulatory mediators. Recent studies have provided evidences for the preventive and therapeutic effects of probiotics in various models of experimental colitis[22–24]. However, the molecular mechanisms whereby probiotic administration prevents or attenuates colitis have not been identified[25].

The addition of 2.5% DSS in the drinking water of mice in the (DSS) group led to deterioration of general condition of mice and the onset of bloody diarrhea which worsened towards the end of the experimental period. The significant shortening of the colon length reflected the severity of colonic inflammation that occurred in this group and indicated the presence of significant alterations in the architectural organization of colon (Figure 1). Our observations are similar to those made by Okayasu et al.[20] and other studies[26,27]. However, the concomitant administration of Ultrabiotique® with DSS improved experimental colitis symptoms and prevented the shortening the colon length. This observation indicates that Ultrabiotique® may be able to protect mice against acute colitis.

Evaluation of nitrite production *in vitro* has revealed the presence of very high levels of these metabolites in culture supernatants of pMΦ of (DSS) group in comparison to (Ctrl) group (Figure 2). These results support the involvement of NO produced by inducible NOS (iNOS) in the pathogenesis of experimental colitis. Indeed, NO produced in large quantities may, by its reactivity to form harmful radical compounds which induce mucosal damages and disruption of the intestinal epithelial barrier. Our results are consistent with those of other works on murine models of experimental colitis[19,28,29].

NO derived from iNOS is a potent pro-inflammatory mediator mainly produced by activated macrophages[30]. The expression of iNOS is primarily induced after activation of the transcription factor NF-κB in response to many stimuli, including proinflammatory cytokines (TNF-α, IFN-γ, IL-6, IL-1β), endotoxin (LPS) and many bacterial or viral components[31]. In our study, histological analysis of the colon of (DSS) group showed the presence of a major polymorphic inflammatory infiltrate in the mucosa and submucosa. Secretion of pro-inflammatory cytokines (TNF-α, IL-1β, IFN-γ, IL-12, IL-6) by inflammatory cells as well as intestinal epithelial cells has probably induced iNOS activation. NO produced in large quantities joins the superoxide anion (O_2^-) to generate peroxynitrite ion ($OONO^-$). The latter is known for its deleterious effects on tissues via the nitration of proteins and lipid peroxidation[32].

We observed with interest a decline in the rate of nitrite level in culture supernatants of pMΦ of (DSS-Prb) group in comparison to the (DSS) group (Figure 2). It seems possible that these results are due to the anti-inflammatory effect of Ultrabiotique® during colitis. Petrof et al.[33] have shown that the probiotic VSL# 3 causes inhibition of NF-κB in intestinal epithelial cells through proteasome inhibition. It is therefore possible that in our study, Ultrabiotique® acts in the colon by a similar mechanism resulting in decreased NO levels in culture supernatants of pMΦ. Moreover, this finding was consistent with the normal histological appearance of the distal colon in (DSS-Prb) group (Figure 4D). On the other hand, the evaluation of nitrite levels in the culture supernatant of pMΦ of (Prb) group showed an increase in NO levels compared with the (Ctrl) group (Figure 2). This rate is, however, lower than the rate in the DSS group. A possible explanation for this finding might be that Ultrabiotique® enhanced immune responses of healthy mice. This finding corroborates those found by of Perdigon et al.[34], who showed that oral administration of lactic acid bacteria to mice increases the phagocytic activity of peritoneal macrophages. Hence, it could conceivably be hypothesized that the effects of Ultrabiotique® differ and depend on the immune status of the host. Many studies are conducted in this way in axenic mice. They have reported the gut flora (*Lactobacilli* and *Bifidobacteria*) involvement in the maturation and development of intestinal immune system[35].

Histological examination showed a loss of Lieberkühn glands and depletion of goblet cells in the mucosa of mice treated with DSS (Figure 4A). Our results are in agreement with previous studies showing a depletion of goblet cells in experimental colitis induced with DSS[36,37]. Goblet cells synthesize the mucin MUC2 which is the most important component of mucus. They also synthesize the trefoil, TFF3 (trefoil factor 3) involved in healing process of the epithelium. UC is often characterized by a decrease in the number and size of goblet cells and therefore, a reduction in mucus production[38]. In addition, mice deficient in the gene MUC2 developed colitis spontaneously and were more sensitive to DSS compared with normal mice[39]. In our study, Alcian blue staining revealed a high mucus production by goblet cells after treatment of mice with Ultrabiotique® during colitis (Figure 3D). This production was much greater in the case of the administration of the probiotic in healthy mice (Figure 3B). These results are consistent with previous work showing the influence of probiotics on the production of mucus[40,41]. Stimulation of mucus production by probiotics is probably a strategy enhanced to increase their adherence to the epithelium. The colonization of the intestine is necessary for probiotics to fulfill their many beneficial effects.

Conclusion

In summary, we suggest that Ultrabiotique® treatment strengthens the barrier function of the intestinal epithelium by inducing mucus secretion. This will not only reduce bacterial translocation and activation of the mucosal immune system but could also have a probable role in colonic repair. On the other hand, it is also possible to envisage a direct role of probiotics on proliferation and differentiation of goblet cells and promote tissue repair providing further supports for probiotics frequent using in the treatment of IBD.

Acknowledgements

The author thanks Mr Mahfoud Labed for his technical assistance.

Declaration of interest

The authors report no declarations of interest.

References

1. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature 2007;448:427–443.
2. Calop J, Limat S, Fernandez C. Pharmacie clinique et thérapeutique. Ed.3, Elsevier Masson 2008;(3):248–268.
3. Van Deventer SJ. Tumour necrosis factor and Crohn's disease. Gut 1997;40:443–448.
4. Abraham C, Ruslan M. Interactions between the host innate immune system and microbes in Inflammatory Bowel Disease. Gastroenterology 2011;140:1729–1737.
5. Bing X, Crusius J, Meuwissen S, Peña A. Inflammatory bowel disease: definition, epidemiology, etiologic aspects, and immunogenetic studies. WJG 1998;4:446–458.

6. Rachmilewitz D, Stamler JS, Bachwich D, et al. Enhanced colonic nitric oxide generation and nitric oxide synthase activity in ulcerative colitis and Crohn's disease. Gut 1995;36:718–723.
7. Kolios G, Valatas V, Ward SG. Nitric oxide in inflammatory bowel disease: a universal messenger in an unsolved puzzle. Immunology 2004;113:427–437.
8. Rafa H, Amri M, Saoula H, et al. Involvement of interferon-γ in bowel disease pathogenesis by nitric oxide pathway: a study in Algerian patients. J Interferon Cytokine Res 2010;30:691–697.
9. Hokari R, Kato S, Matsuzaki K, et al. Reduced sensitivity of inducible nitric oxide synthase-deficient mice to chronic colitis. Free Radic Biol Med 2001;31:153–163.
10. Sartor RB. Microbial influences in inflammatory bowel diseases. Gastroenterology 2008;134:577–594.
11. Sekirov I, Russell SL, Antunes CM, Finlay BB. Gut microbiota in health and disease. Physiol Rev 2010;90:859–904.
12. Cerf-Bensussan N, Gaboriau-Routhiau V. The immune system and the gutmicrobiota: friends or foes? Nat Rev Immunol 2010;10: 735–744.
13. Renes IB, Verburg M, Van Nispen DJ, et al. Epithelial proliferation, cell death, and gene expression in experimental colitis: alterations in carbonic anhydrase I, mucin MUC2, and trefoil factor 3 expression. Int J Colorectal Dis 2002;17:317–326.
14. Hans W, Schölmerich J, Gross V, Falk W. The role of the resident intestinal flora in acute and chronic dextran sulfate sodium-induced colitis in mice. Eur J Gastroenterol Hepatol 2000;12:267–273.
15. Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO). Guidelines for the evaluation of probiotics in food; 2002.
16. Vanderpool C, Yan F, Polk DB. Mechanisms of probiotic action: implications for therapeutic applications in inflammatory bowel diseases. Inflamm Bowel Dis 2008;14:1585–1596.
17. Mimura T, Rizzello F, Helwig U, et al. Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. Gut 2004;53:108–114.
18. Osman N, Adawi D, Ahrne S, et al. Modulation of the effect of dextran sulfate sodium-induced acute colitis by the administration of different probiotic strains of *Lactobacillus* and *Bifidobacterium*. Dig Dis Sci 2004;49:320–327.
19. Abdelouhab K, Rafa H, Toumi R, et al. Mucosal intestinal alteration in experimental colitis correlates with nitric oxide production by peritoneal macrophages: effect of probiotics and prebiotics. Immunopharmacol Immunotoxicol 2012;34:590–597.
20. Okayasu I, Hatakeyama S, Yamada M, et al. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. Gastroenterol 1990;98:694–702.
21. Touil-Boukoffa C, Bauvois B, Sancéau J, et al. Production of nitric oxide (NO) in human hydatidosis: relationship between nitrite production and interferon-γ levels. Biochimie 1998;80:739–744.
22. Schultz M, Veltkamp C, Dieleman LA, et al. *Lactobacillus plantarum* 299V in the treatment and prevention of spontaneous colitis in interleukin-10-deficient mice. Inflamm Bowel Dis 2002;8: 71–80.
23. McCarthy J, O'Mahony L, O'Callaghan L, et al. Double blind, placebo controlled trial of two probiotic strains in interleukin 10 knockout mice and mechanistic link with cytokine balance. Gut 2003;52:975–980.
24. Wu X, Vallance BA, Boyer L, et al. *Saccharomyces boulardii* ameliorates *Citrobacter rodentium*-induced colitis through actions on bacterial virulence factors. Am J Physiol Gastrointest Liver Physiol 2008;294:G295–G306.
25. Kleerebezem M, Vaughan EE. Probiotic and Gut Lactobacilli and Bifidobacteria: molecular approaches to study diversity and activity. Annu Rev Microbiol 2009;63:269–290.
26. Dieleman LA, Ridwan BU, Tennyson GS, et al. Dextran sulfate sodium-induced colitis occurs in severe combined immunodeficient mice. Gastroentero 1994;107:1643–1652.
27. Kumar KG, Dhamotharan R, Kulkarni N, et al. Embelin ameliorates dextran sodium sulfate-induced colitis in mice. Int Immunopharmacol 2011;11:724–773.
28. Southey A, Tanaka S, Murakami T, et al. Pathophysiological role of nitric oxide in rat experimental colitis. Int J Immunopharmacol 1997;19:669–676.
29. McCafferty DM, Miampamba M, Sihota E, et al. Role of inducible nitric oxide synthase in trinitrobenzene sulphonic acid induced colitis in mice. Gut 1999;45:864–873.
30. Hibbs JBJ, Taintor RR, Vavrin Z, Rachlin EM. Nitric oxide: a cytotoxic activated macrophage effector molecule. Biochem Biophys Res Commun 1998;157:87–94.
31. Xie QW, Kashiwabara Y, Nathan C. Role of transcription factor NF"KB/Rel in induction of nitric oxide synthase. J Biol Chem 1994;269:4705–4708.
32. Korhonen R, Lahti A, Kankaanranta H, Moilanen E. Nitric oxide production and signaling in inflammation. Current Drug Targets – Inflammation & Allergy 2005;4:471–479.
33. Petrof E, Kojima K, Ropeleski MJ, et al. Probiotics inhibit nuclear factor-κB and induce heat shock proteins in colonic epithelial cells through proteasome inhibition. Gastroenterology 2004;127: 1474–1487.
34. Perdigon G, Galdeano MC, Valdez JC, Medici M. Interaction of lactic acid bacteria with the gut immune system. Eur J Clin Nutr 2002;56:S21–S26.
35. Macpherson AJ, Harri NL. Interactions between commensal intestinal bacteria and the immune system. Nature Rev Immunol 2004;4:478–485.
36. Renes IB, Boshuizen JA, Van Nispen DJ, et al. Alterations in Muc2 biosynthesis and secretion during dextran sulfate sodium-induced colitis. Am J Physiol Gastrointest Liver Physiol 2002;282:382–389.
37. Faure M, Moennoz D, Montigon F, et al. Mucin production and composition is altered in dextran sulfate sodium-induced colitis in rats. Dig Dis Sci 2003;48:1366–1373.
38. Tytgat K, Van der Wal J, Einerhand A, et al. Quantitative analysis of MUC2 synthesis in ulcerative colitis. Biochem Biophys Res Commun 1996;224:397–405.
39. Van Der Sluis M, De Koning BA, De Bruijn AC, et al. Muc2-deficient mice spontaneously develop colitis, indicating that Muc2 is critical for colonic protection. Gastroenterology 2006;131: 117–129.
40. Mack DR, Ahrne S, Hyde L, et al. Extracellular MUC3 mucin secretion follows adherence of *Lactobacillus* strains to intestinal epithelial cells in vitro. Gut 2003;52:827–833.
41. Caballero-Franco C, Keller K, De Simone C, Chadee K. The VSL#3 probiotic formula induces mucin gene expression and secretion in colonic epithelial cells. Am J Physiol Gastrointest Liver Physiol 2007;292:G315–G322.