EDITORIAL

PROBIOTICS, PREBIOTICS AND SYMBIOTICS IN INFLAMMATORY BOWEL DISEASES: STATE-OF-THE-ART AND NEW INSIGHTS

E. SINAGRA[1,2], G. TOMASELLO[3,4], F. CAPPELLO[4,5,6], A. LEONE[5], M. COTTONE[7], M. BELLAVIA[4], F. ROSSI[2], T. FACELLA[2], P. DAMIANI[8], M.N. ZEENNY[5,9], F. DAMIANI[1], A. ABRUZZO[1], G. DAMIANO[3], V.D. PALUMBO[3], M. COCCHI[6], A. JURJUS[9], G. SPINELLI[3], A.I. LO MONTE[1,3] and D. RAIMONDO[2],

[1]PhD Course in Surgical Biotechnology and Regenerative Medicine, University of Palermo, Italy; [2]Fondazione Istituto S. Raffaele- G. Giglio, Gastroenterology and Endoscopy Unit, Cefalù, Italy; [3]Department DICHIRONS, University of Palermo, Italy; [4]Euro-Mediterranean Institute of Science and Technology, Palermo, Italy; [5]Department BIONEC, University of Palermo, Italy; [6]Institute "Paolo Sotgiu" for Research in Quantitative and Quantum Psychiatry and Cardiology, LUdeS University, Lugano, Switzerland; [7]Department DIBIMIS, University of Palermo, Italy; [8]Policlinico Paolo Giaccone, Hospital, Unit of Geriatry, Palermo, Italy; [9]Department of Morphology, American University of Beirut, Beirut, Lebanon

Received July 10, 2013 - Accepted November 15, 2013

E. Sinagra and G.Tomasello share first authorship for this paper.

Inflammatory bowel disease (IBD) consists of two distinct clinical forms, ulcerative colitis (UC) and Crohn's disease (CD), with unknown aetiology, which nevertheless are considered to share almost identical pathophysiological backgrounds. Up to date, a full coherent mechanistic explanation for IBD is still lacking, but people start to realize that the pathogenesis of IBD involves four fundamental components: the environment, gut microbiota, the immune system and the genome. As a consequence, IBD development might be due to an altered immune response and a disrupted mechanism of host tolerance to the non-pathogenic resident microbiota, leading to an elevated inflammatory response. Considering the available data arising from the scientific literature, here reviewed, in CD, a benefit of probiotics remains unproven; in UC, a benefit of probiotics remains unproven, even if *E. coli Nissle 1917* seems promising in maintaining remission and it could be considered an alternative in patients intolerant or resistant to 5-ASA preparations; in pouchitis, small controlled trials suggest a benefit from VSL#3 in the primary and secondary prevention of pouchitis; in IBD-associated conditions, a benefit of probiotics remains unproven. However, well-designed randomized control clinical trials are necessary to understand the undoubted role of these agents in the management of gut physiology in health and disease.

Inflammatory bowel disease (IBD) consists of two distinct clinical forms with unknown aetiology, ulcerative colitis (UC) and Crohn's disease (CD) which nevertheless share almost identical

Key words: Probiotics, prebiotics, synbiotics, inflammatory bowel disease

Mailing address: Angelo Leone, MD. Sp. Ortho., PGCAPHE, BioNec, School of Medicine, section of Histology and Embryology. University of Palermo, Via delle Scienze 90128 Palermo, Italy Tel.: +39 091 6553581 Fax: +39 091 6553586 e-mail: angelo.leone@unipa.it

0393-974X (2013)

pathophysiological backgrounds.

CD is predominantly associated with a type 1 helper-T-cell (Th1) and type 17 helper-T-cell (Th17) immune responses, characterized by increased production of interleukin IL-12, IL-23, IL-27, interferon-γ (IFN-γ) and tumour necrosis factor TNF-α. Diversely, UC seems to be associated with a type 2 helper-T cell (Th2) immune response, mainly leading to raised levels of IL-5 and transforming growth factor-β (TGF-β). The etiology of IBD is complex and multifactorial, where environmental, genetic and immunological components appear to play a role (1-3).

After the emergence of IBD about a century ago, numerous hypotheses were suggested as the possible mechanism, which included infection, toxicants, psychogenic disturbances, nutritional deficiencies, allergy to pollens or foods, abdominal trauma, impaired vascular or lymphatic circulation, lysozymes and other enzymes (4-7), or the excessive or deficient immune response due to reduced exposure to bacteria or helminthes (8-9). Most of them were invalidated and forgotten. Up to date, a full coherent mechanistic explanation for IBD is still lacking, but people start to realize that the pathogenesis of IBD involves four fundamental components: the environment, gut microbiota, the immune system and the genome (10-12).

As a consequence, IBD development might be due to an altered immune response and a disrupted mechanism of host tolerance to the non-pathogenic resident microbiota, leading to an elevated inflammatory response. The strength of this hypothesis arises from many evidences, such as the fact that inflammation mainly occurs in the intestinal sites with the highest bacterial concentration (in UC), that antibiotic treatment often results in amelioration of disease symptoms (13), and that germ-free mice do not spontaneously initiate colitis (14).

We analyzed the international literature about the role of microbiota in IBD, using PubMed as primary source, focusing on the use of probiotics as complementary therapeutic approach in CD, UC and pouchitis. Aim of this review is to summarize the pathophysiological rationale for the use of probiotics in IBD, and to focus on clinical trial of such emerging therapeutic options.

Microbiota and etiopathogenesis of inflammatory bowel disease

The human normal flora, or microbiota, is extensive, both in its absolute quantitative mass and qualitative diversity. Conventional microbiological techniques fail to give a detailed inventory of the normal microbiota, but the development of recent high-throughput sequencing and molecular taxonomic methodologies have greatly increased our understanding of the population composition, dynamics, and ecology of the gut microbiota. The composition of intestinal flora is remarkably stable at different anatomic locations along the gut, but absolute numbers vary greatly, ranging from 10^{11} cells/g content in the ascending colon to 10^{7} to 10^{8} in the distal ileum and 10^{2} to 10^{3} in the proximal ileum and jejunum; anaerobes are several orders of magnitude more abundant than aerobes in the bacterial community, and a majority of the population (60-90%) comprises two divisions: the *Bacteroidetes and Firmicutes* (15).

The composition of the microbiota has been suggested to influence susceptibility to IBDs (16), which are mediated by both innate and adaptive arms of the host immune system. It is possible that distinct members of the commensal microbiota engage specific components of the immune system and, in doing so, they regulate intestinal immune homeostasis (15).

An important question is whether specific commensal microorganisms regulate the homeostasis of effector T cells in the lamina propria. For example, it has been reported that the gut commensal *Bacteroides fragilis* affects systemic Th1 responses through the action of the bacterial-derived polysaccharide A (PSA) (17). The lamina propria of the small intestine at steady state contains two populations of CD4 T cells, Th17 cells, and regulatory T cells (Treg); in particular, the former has been assumed to play a role in Crohn's disease and ulcerative colitis (18, 19, 20). Interestingly, Ivanov et al. (21) found that Th17 cells could be induced in the lamina propria of the small intestine in response to specific components of the commensal microbiota belonging to the *Cytophaga–Flavobacterium–Bacteroides* phylum, suggesting that the composition of the intestinal microbiota is likely to influence intestinal immunity, tolerance, and IBD susceptibility (22). More recently Ivanov et

al. (23) showed that Segmented Filamentous Bacteria (SFB) are potent inducers of Th17 cells in the lamina propria of the small intestine of mice. In particular, SFB colonization induced production of serum amyloid A (SAA) in the terminal ileum and SAA acted on dendritic cells in the lamina propria to promote Th17 cell differentiation. Also, the aforementioned CD4 Treg cells can be stimulated by commensal microbiota as evidenced by O'Mahony et al. (24), who showed that in mice the deliberate consumption of the commensal organism *Bifidobacterium infantis 35624* resulted in the induction of Treg cells, which protected the host from excessive inflammation during the course of infection caused by *Salmonella typhimurium*.

In particular, the reduction in the phlogistic response was achieved through the control of excessive pathogen mediated activation of nuclear factor kappa B, a transcription factor often involved in innate proinflammatory signalling in response to microbial exposure.

Natural killer (NK) cells play an important role in the innate immune system. It has been shown (25) that, in a germ-free mice, NKp46þ IL-22 producing cells were markedly reduced, suggesting that an environmental niche, operative in the gut, generated these unique effectors cells. Interestingly, more recently, Takayama et al. (26) conducted a clinical study that showed that NKp46þ cells were predominant in the intestinal mucosa of patients with Crohn's disease compared with controls or patients with ulcerative colitis. Upon interaction with intestinal inflammatory macrophages, these cells were also activated via IL-23 and produced IFNγ. Another interesting point concerning intestinal chronic diseases is the role of epithelial antimicrobial proteins as innate immune effectors; they probably play an important role in maintaining mutually beneficial host–microbe relationships by restricting contact between resident microbes and mucosal surfaces, and their deficiencies are associated with IBD. In particular, using a germ-free murine model, it has been shown (27) that resident gut bacteria drive intestinal epithelial expression of a C-type lectin that binds peptidoglycan and has direct antimicrobial activity; interestingly, the human counterpart of this protein (HIP/PAP) is usually overexpressed in intestinal mucosa of IBD patients (28) and it is also believed to be a biomarker of pancreatic ductal adenocarcinoma.

Commensal microbiota could also be implicated in stimulating immunoregulatory pathways through expression of specific heat shock proteins (HSPs). Molecular chaperones of the HSP family are evolutionarily conserved proteins that modulate a variety of intracellular functions including the folding of proteins, folding of multimeric proteins, the translocation of proteins across membranes, the degradation of proteins, and signal transduction. Apart from their chaperone activities, HSPs are involved in the regulation of innate immunity and mucosal immunity. Several reports have shown HSP reactive T cells to have an immunoregulatory phenotype, indicating that HSPs, particularly HSP60 and HSP70, constitute a group of autoantigens with the potential to trigger immunoregulatory pathways, which can suppress immune responses that occur in various human inflammatory diseases, such as rheumatoid arthritis (29, 30), type 1 diabetic mellitus (31, 32), atherosclerosis (33), and allergy (34). Intragastric administration or nasal immunization with HSPs was effective as preventive/therapeutic interventions of atherosclerosis in animal models, suggesting that mucosal immunization with HSPs is valuable for the regulation of chronic inflammation (35). Interestingly, it has been shown that the bacterial HSP60 GroEL, whose production is related to intestinal microflora, could generate CD4+CD25+ FoxP3+ T cells from naive T cells; owing to their ability to secrete GroEL, intestinal flora could be involved in intestinal homeostasis and UC pathogenesis (36). Luminal bacteria may also exert a protective role because some of their byproducts, such as small-chain fatty acids (SCFAs), are able to simulate intestinal expression of cytoprotective HSP25 and HSP72 (37-41).

Our group also reported his experience in this field. In a first work we investigated three heat shock protein/molecular chaperones: HSP10, HSP70, and HSP90. We found that the levels of these proteins are increased in UC patients at the time of diagnosis and decrease after therapy, supporting the notion that these proteins deserve attention in the study of the mechanisms that promote the development and maintenance of IBD, and as biomarkers of this disease (42). In another study we wanted to determine

Table I. *Controlled trials of probiotics for the induction and maintenance of remission in adults with Crohn's disease.*

Authors, year	Study design	Number of patients	Regimen	Rationale of the use of the probiotics employed	Duration (months)	Outcomes
Schultz et al., 2004	Randomized controlled trial	11	Lactobacillus GG versus placebo	- Inhibition of apoptosis of intestinal epithelial cells. - Decreased translocation of commensal bacteria via the mesenteric lymph nodes and liver.	6	No difference in obtaining remission
Malchow et al., 1997	Randomized controlled trial	28	E. coli Nissle 1917 versus placebo	- Downregulation of the expansion of newly recruited T cells into the mucosa - Intestinal inflammation regulation via TLR-2 and TLR-4 - Restoration of disrupted epithelial barrier in the colonic epithelial cell line T84	12	No difference in obtaining maintenance of remission
Guslandi et al., 2000	Randomized controlled trial	32	Saccharomyces boulargii 1g daily plus mesalamine versus mesalamine alone	- Limitation of infiltration of T-helper 1 cells into the mucosa NF-kB blocking and IL-8 downregulation	6	Probiotics plus mesalamine were superior in obtaining maintenance of remission, evaluated through Crohn's Disease Activity Index
Schultz et al., 2004	Randomized controlled trial	11	Lactobacillus GG versus placebo	- Inhibition of apoptosis of intestinal epithelial cells - Decreased translocation of commensal bacteria via the mesenteric lymph nodes and liver	6	No difference in obtaining maintenance of remission
Prantera et al., 2002	Randomized controlled trial	45	Lactobacillus GG versus placebo	- Inhibition of apoptosis of intestinal epithelial cells - Decreased translocation of commensal bacteria via the mesenteric lymph nodes and liver	12	No difference in preventing clinical post-surgical recurrence
Marteau et al., 2006	Randomized controlled trial	98	Lactobacillus johnsonii LA1 versus placebo	- Upregulation of intestinal MUC3 and MUC3 mRNA expression	6	No difference in preventing clinical post-surgical recurrence
Van Gossum et al., 2007	Randomized controlled trial	70	Lactobacillus johnsonii LA1 versus placebo	Upregulation of intestinal MUC3 and MUC3 mRNA expression	3	No difference in preventing endoscopic post-surgical recurrence

in colon mucosa of CD and ulcerative UC in relapse: a) the levels of the chaperonins HSP60 and HSP10; b) the quantity of inflammatory cells; and c) if the levels of chaperonins parallel those of inflammation cells. In this study, we found that HSP60 and HSP10 occurred in the cytoplasm of epithelial cells in CD and UC but not in negative controls.

HSP60 and HSP10 co-localised to epithelial

TableII. *Controlled trials of probiotics for the induction and maintenance of remission in adults with Ulcerative colitis.*

Authors, year	Study design	Number of patients	Regimen	Rationale of the use of the probiotics employed	Duration (months)	Outcomes
Rembacken et al., 1999	Randomized controlled trial	120	E. coli Nissle 1917 versus mesalamine	- Downregulation of the expansion of newly recruited T cells into the mucosa. - Intestinal inflammation regulation via TLR-2 and TLR-4. - Restoration of disrupted epithelial barrier in the colonic epithelial cell line T84.	3	No difference in induction rates
Tursi et al., 2004	Randomized controlled trial	90	VSL#3 plus balsalazide versus balsalazide alone or mesalamine alone	-Reduction of secretion of TNF-a and interferon-g . - Improvement of the colonic barrier function. - Inhibition of Salmonella Dublin invasion into T-84 cells. - Convertion of linoleic acid into conjugated linoleic acid. -Inhibition of TNF-a-induced IL-8 secretion, mitogen-activated protein kinase activation and NF-kB activation in HT-29 cells (CpG DNA). - Upregulation of mucin expression.	2	Better significant induction of remission in the probiotic group
Kato et al., 2004	Randomized controlled trial	20	Probiootic milk (Bifidobacterium breve, Bifidobacterium bifidum and Lactobacillus acidophilus 1) versus placebo	- Increase in IL-10 secreted by mesenteric lymph nodes (Bifidobacterium-fermented milk) - Reduction of MPO activity, tissue contents of immunoglobulin, TNF-a. - Upregulation of intestinal MUC3 and MUC3 mRNA expression.	3	Significantly better change in clinical activity index, histological score in the probiotic group, in evaluating the induction of remission
Furrie et al., 2005	Randomized controlled trial	18	Symbiotic (Bifidobacterium longum and fructooligosaccharide/ inulin mix) plus Standard treatment versus Standard treatment alone	- Upregulation of IL-10 expression in dendritic cells. - Inhibition of disorderd T-cell activation.	1	Improved sigmoidoscopy scores and cytokine profiles in the probiotic group, in evaluating the induction of remission
Kruis et al., 1997	Randomized controlled trial	120	E. coli Nissle 1917 versus mesalamine	Downregulation of the expansion of newly recruited T cells into the mucosa. - Intestinal inflammation regulation via TLR-2 and TLR-4. - Restoration of disrupted epithelial barrier in the colonic epithelial cell line T84	3	No statistically significant difference in the relapse rate in evaluating the maintenance of remission
Ishikawa et al., 2003	Randomized controlled trial	21	probiotic milk (Bifidobacterium Breve, Bifidobacterium Bifidum and Lactobacillus acidophilus) with Standard tretment versus Standard treatment alone	- Increase in IL-10 secreted by mesenteric lymph nodes (Bifidobacterium-fermented milk). - Reduction of MPO activity, tissue contents of immunoglobulin, TNF-a - Upregulation of intestinal MUC3 and MUC3 mRNA expression	12	Fewer relapses in probiotic group, ikn evaluating the maintenance of remission
Kruis et al., 2004	Randomized equivalence trial	312	E. coli Nissle 1917 versus mesalamine	- Downregulation of the expansion of newly recruited T cells into the mucosa. - Intestinal inflammation regulation via TLR-2 and TLR-4. - Restoration of disrupted epithelial barrier in the colonic epithelial cell line T84	12	Equivalence in the relapse rate, in evaluating the maintenance of remission
Zocco et al., 2006	Randomized controlled trial	186	Lactobacillus GG versus mesalamine versus both	- Inhibition of apoptosis of intestinal epithelial cells. - Decreased translocation of commensal bacteria via the mesenteric lymph nodes and liver.	12	Lactobacillus GG is showed to prolonge the time of relapse

Table III. *Controlled trials of probiotics for the induction and maintenance of remission in adults with pouchitis.*

Author, year	Study design	Number of patients	Regimen	Rationale of the use of the probiotics employed	Duration (months)	Outcomes
Gionchetti et al., 2003	Randomized controlled trial	40	VSL#3 versus placebo	-Reduction of secretion of TNF-a and interferon-γ. - Improvement of the colonic barrier function. -Inhibition of Salmonella Dublin invasion into T-84 cells. -Convertion of linoleic acid into conjugated linoleic acid. -Inhibition of TNF-a-induced IL-8 secretion, mitogen-activated protein kinase activation and NF-kB activation in HT-29 cells (CpG DNA). - Upregulation of mucin expression.	12	Significant reduction in the onset of acute pouchitis with probiotic group in evaluating the prophylaxis
Gionchetti et al., 2000	Randomized controlled trial	40	VSL#3 versus placebo	- Reduction of secretion of TNF-a and interferon-γ. - Improvement of the colonic barrier function. - Inhibition of Salmonella Dublin invasion into T-84 cells. - Convertion of linoleic acid into conjugated linoleic acid. - Inhibition of TNF-a-induced IL-8 secretion, mitogen-activated protein kinase activation and NF-kB activation in HT-29 cells (CpG DNA). - Upregulation of mucin expression.	9	Significant decrease in relapse in the probiotic group in evaluating the maintenance of remission
Kuisma et al., 2003	Randomized controlled trial	20	Lactobacillus GG versus placebo	- Inhibition of apoptosis of intestinal epithelial cells. - Decreased translocation of commensal bacteria via the mesenteric lymph nodes and liver.	3	No difference in disease activity in evaluating the maintenance of remission
Mimura et al., 2004	Randomized controlled trial	36	VSL#3 versus placebo	-Reduction of secretion of TNF-a and interferon-γ. - Improvement of the colonic barrier function. - Inhibition of Salmonella Dublin invasion into T-84 cells. - Convertion of linoleic acid into conjugated linoleic acid. - Inhibition of TNF-a-induced IL-8 secretion,.mitogen-activated protein kinase activation and NF-kB activation in HT-29 cells (CpG DNA). - Upregulation of mucin expression.	12	Significantly decreased relapse in the probiotic group in evaluating the maintenance of remission

cells of mucosal glands but not always in connective tissue cells of lamina propria, where only HSP60 or, less often, HSP10 was found; cells typical of inflammation were significantly more abundant in CD and UC than in negative controls. Since chaperonins are key factors in the activation of the immune system leading to inflammation, we propose that they play a central role in the pathogenesis of the two diseases, which, consequently, ought to be studied as chaperonopathies (43). Furthermore, it is intriguing the recent discovery of genetic polymorphisms (involving pattern recognition receptor genes) related to serum levels of anti-microbial antibodies in CD patients but not in negative controls (44); on the basis of this finding it could be postulated an abnormal immunological response to alteration of microbiota in patients carrying these polymorphisms.

Probiotics

Probiotics are microorganisms that have beneficial properties for the host (45). Several mechanisms of action of probiotics relative to prevention and treatment of IBD have been reported, such as antimicrobial activity and suppression of bacterial growth, immunomodulation and initiation of an immune response, improvement of intestinal barrier

function, suppression of human T-cell proliferation (46-50), and modulation of pain perception (51-55). Probiotics have also been found to induce their effect by means of their DNA, as shown by experiments using probiotic DNA (56-58) and subcutaneous administration of probiotic DNA (59). Derived originally from cultured food, especially dairy products, this group includes *Lactobacillus species*, *Bifidobacterium species*, *E. coli Nissle 1917* (a nonpathogenic *E. coli* strain), *Saccharomyces boulardii*, *Clostridium butyricum*, *VSL#3* and *Lactococcus lactis* genetically engineered to secrete IL-10.

Probiotics in Crohn's Disease

The literature on the induction and maintenance of remission in CD is heterogeneous and difficult to interpret. The reasons for such heterogeneity are several, as the different probiotics (and doses) used, the differences in study duration, the features of the included patients, and the measured endpoints.

In the setting of inducing remission, in a pivotal study performed by Schultz and coworkers, with only 11 patients, probiotics provided no additional benefit to steroids and antibiotics in inducing remission (60). Subsequently, Fujimori and coworkers, in an open-label study with 10 patients who were refractory to standard therapies (prednisolone and aminosalicylates), tried a combination of probiotics (*Bifidobacterium breve, Bifidobacterium longum,* and *Lactobacillus casei*) and a prebiotic (psyllium) simultaneously. A complete response was found in 6 of 10 patients without any adverse events (61).

In the paediatric setting, Gupta and coworkers used, in an open-label trial, *Lactobacillus rhamnosus* GG (62). In this study, 3 of the 4 children were reported to have improved Pediatric Crohn's Disease Index (PCDAI) scores or serial determinations over the 6 months of the trial. Specifics with regards to ESR or CRP are not reported even if the ESR is a component of the PCDAI (63).

The placebo-controlled trial, using *Lactobacillus rhamnosus* GG was the sole study included in a Cochrane review, performed by Butterworth and coworkers, concerning the efficacy of probiotic supplementation for the induction of remission in CD that met the inclusion criteria of being a randomized controlled trials of participants with CD whose disease was active at the time of entry into the study (64). In the study 11 patients were enrolled; four of 5 patients in the probiotic group achieved remission compared to 5 of 6 in the placebo group (OR 0.80; 95% CI 0.04 to 17.20). Thus, this one small study did not show that probiotics had any effect in treating active CD (64). More controlled studies have been performed on the maintenance of remission in adults with CD, but in general these studies fail to show any benefit of probiotic administration (55).

Initial randomized trials in CD were reported with probiotics used as sole maintenance therapy following corticosteroid therapy (65) or in combination with lower does of 5-aminosalicylate therapy compared to controls for maintenance therapy in those already in remission (66). Subsequent trials have focused on *Lactobacillus rhamnosus* strain GG for maintenance therapy following induction of remission with corticosteroids (60) and maintenance of remission with probiotic used as additional maintenance therapy (67).

In the largest maintenance trial to date, Bousvaros and coworkers (68) also reported no difference in the proportion of those developing relapse on *Lactobacillus rhamnosus* strain GG 2 × 10 (10) CFU/day (31%; 12 of 39) or placebo (17%; 6 of 36, $p = 0.18$) (60).

Data are also more robust on the prevention of relapse following surgical intervention, but again probiotics fail to prevent endoscopic and clinical recurrence.

The meta-analysis performed by Doherty and coworkers (69) of the effects of probiotics as a class suggested that their effect was not different from placebo. The relative risk of clinical recurrence with any probiotic relative to placebo ($n = 213$) was 1.41 (95% CI 0.59 to 3.36), any endoscopic recurrence ($n = 333$) was 0.98 (95% CI 0.74 to 1.29) and severe endoscopic recurrence ($n = 213$) was 0.96 (95% CI 0.58 to 1.59) (64). Table I shows the outcomes of controlled trials of probiotics for the induction and maintenance of remission in adult with CD.

Probiotics in Ulcerative Colitis

Although various probiotic species have shown promising in the treatment of UC, given the small number of patients in these studies and the risk associated with probiotics, two systematic reviews

have concluded that there is insufficient evidence to support the use of probiotics for the induction or maintenance of remission in ulcerative colitis (70, 71, 102).

In fact, several trials have been published examining probiotics in the induction and remission of UC, however, only few of these are randomized controlled trials (RCTs). Most are with different probiotic formulations and overall have been performed in a relatively small number of patients. For induction of remission, the first and largest controlled trial to date performed by Remnacken and coworkers showed no additional efficacy of *Escherichia coli Nissle 1917* than steroids, mesalamine, and antibiotics (63).

Three additional trials, all small in number of patients, and of short duration of therapy and with variable standard of care, showed improvement in various measures of disease activity and even cytokine profiles (72-74, 55), whereas the rectal administration of *Escherichia Coli 1917 Nissle* enema has equivocal results (75). It was showed that the combination of VSL#3™ plus balsalazide was slightly more effective than balsalazide or mesalamine alone, in a randomized trial of patients with acute mild-to-moderate UC (75). Similar results were found in the paediatric setting by Miele and coworkers (76).

Further evidences show that VSL#3™ could induce remission and reduce disease activity in patients with mild-to-moderate active ulcerative colitis (77-80). A randomized trial of 77 patients, performed by Sood and coworkers, proved that VSL#3™ was more effective than placebo in improving the ulcerative colitis disease activity index (UCDAI) by 50 percent at week 6 (33% versus 10%) and inducing remission at week 12 (43% versus 16%) (77). Another randomized trial by Tursi and coworkers examined 144 patients who were receiving a 5-ASA, azathioprine, and/or methotrexate (80). This trial also showed that patients receiving VSL#3™were more likely to have at least a 50% decrease in UCDAI at week 8 compared with patients receiving placebo (63% versus 41 %), with a similar increase in remission rate in the VSL#3™group (48% versus 32%), even if histologic scores were not significantly improved with VSL#3™ therapy.

With regard to maintenance of UC remission, probiotics have been tested in a larger number of patients. One trial by Kruis and colleagues tested *Escherichia coli Nissle 1917* and found no difference in relapse rates in patients on a probiotic versus mesalamine (81). A trial by Zocco and colleagues also found no difference in relapse rates at 6 or 12 months when comparing *Lactobacillus GG* with mesalamine with a combination of the two (82). Those patients who took the probiotic did appear to have a longer time to relapse (55).

However, a small double-blind placebo controlled trial showed no significant difference in the rates of maintenance of remission in patients with left sided UC randomized to 52 weeks of *Lactobacillus basilicus La-5* and *Bifidobacterium animalis subsp. Lactis Bb-12* or placebo (relapse rate 75% versus 92%) (65).

All of these studies support the idea that probiotics may be as effective as mesalamine in maintaining remission, almost in the short-term trial. Table I shows the outcomes of controlled trials of probiotics for the induction and maintenance of remission in adult with UC.

Probiotics in pouchitis

With regard to the surgical treatment of UC and familial adenomatous polyposis, proctocolectomy with ileal pouch-anal anastomosis (IPAA) is the favored alternative to proctocolectomy with permanent ileostomy, since it preserves intestinal continuity and sphincter function and removes almost all of colorectal mucosa. After proctocolectomy with IPAA, pouchitis or acute and chronic inflammation of the ileal reservoir is the most frequent long-term complication of this operation, occurring in up to 20% of patients at 1 year. Studies of the microflora in the pouch have revealed deficiency of streptococcal species (83, 55).

Most patients will develop this problem in the short term (within the first year) and antibiotics may be considered an effective form of therapy in many (84, 85) but for those that do not respond other forms of therapy are required (85). For some, antibiotics improve the pouchitis but there is a relapsing course of the pouchitis following the discontinuation of antibiotics. As antibiotics can provide relief for most with pouchitis, a basic assumption has been

the importance of the microbiota of the pouch in the development and chronicity of pouchitis. Thus, alteration of the microbiota by addition of probiotics was considered (65). In fact, the strongest evidence for the use of probiotics in IBD is in prevention and treatment of pouchitis (86-89).

Trials about the treatment of mild/moderate pouchitis are few, with small numbers of adult patients. Kuisma and coworkers (87) recruited 20 patients (10 intervention arm) for a trial of *Lactobacillus rhamnosus GG* 2×10^{10} CFU/day for 3 months. Patients with chronic, active pouchitis were excluded. The Pouchitis Disease Activity Index (90) was utilized for evaluation of clinical effect. Prior to study entry, the mean PDAI was in the mild range (8.0±0.8) and there was no difference following the intervention period with clinical response (defined as a PDAI score reduction of ≥3) occurring in 1/10 (10%) patients in the probiotic group and 0/10 (0%) patients in the placebo group (10% *vs* 0%, P=0.32).

In an open-label trial of 51 UC patients post ileal pouch-anal anastomosis, performed by Laake and coworkers, and using a fermented milk product with a blend of probiotic strains (*Lactobacillus acidophilus* strain La5 + *Bifidobacterium lactis* strain Bb12) containing 5×10^{10} CFU/day (91) however, there was a reported improvement in endoscopic evaluation. In another open label trial by Gionchetti and coworkers, twenty-three consecutive patients with mild pouchitis as defined using Pouchitis Disease Activity Index (scores 7-12) were treated with 3.6×1012 CFU/day of VSL#3™ for 4 weeks (92). Sixteen of 23 patients (69%) with mild pouchitis were in remission after treatment and the median total Pouchitis Disease Activity Index scores reported before therapy improved following therapy (10 *vs* 4, P<0.01). Thus, there is limited evidence for a role of probiotics as monotherapy for mild to moderate pouchitis at the present time.

Two trials have studied whether there is an advantage to initiate probiotics immediately following ileal pouch-anal anastomosis to evaluate the eventual delay in onset of development of pouchitis. Gionchetti and coworkers performed a placebo-controlled trial (89) where, at the end of one year, 2 of 20 (10%) of the patients in the intervention arm had developed colitis as determined compared to 8 of 20 (40%, no episodes 80% *vs* 60%, P=0.03) of the control arm participants using the PDAI with endoscopy. The Peto odds ratio for prevention of pouchitis by VSL#3™ compared with placebo was 4.76, 95% CI 1.16 to 19.56 (93).

The other randomized trial or probiotics also studied VSL#3™ in an open-label design performed by Pronio and coworkers, that compared the probiotic to no treatment over a 12 month period (94), where none of the 16 patients in the group administered probiotic compared to one of 12 (8.3%, no pouchitis 100% *vs* 92%, p =0.24) developed pouchitis.

Small controlled trials have also suggested that at least one probiotic preparation (VSL#3™) may be effective in prevention of recurrent pouchitis after antibiotic induction of remission.

The first randomized trial addressed to evaluate this outcome, performed in the year 2000 by Gionchetti and coworkers, included 40 patients with a history of chronic, relapsing pouchitis who were placed into clinical and endoscopic remission with broad spectrum antibiotics (88). Patients were randomly assigned to VSL#3™ 6 g/day or placebo. After nine months of daily treatment, significantly fewer patients in the probiotic group had experienced a relapse (15% *vs* 100%). Within 3 months of stopping treatment, all patients in the probiotic group had relapsed. Interestingly, fecal *Lactobacillus* and *Bifidobacteria* concentrations returned to pretreatment levels within one month after therapy withdrawal, indicating that permanent colonization with the probiotic species did not occur.

A similar result was noted in another European trial of VSL#3™ that also evaluating the prevention of recurrence of pouchitis in relapsing or chronic pouchitis patients (86). Remission of the pouchitis was induced in these participants by administering 4 weeks of a combination of antibiotics (metronidazole + ciprofloxacin) that was followed by either VSL#3™ or a placebo. In the treatment group remission was maintained in 17 of 20 (85%) but only 1 of 16 (6%, p<0.0001) on placebo. The pooled Peto odds ratio for these two studies for the combined rate of maintenance of remission with probiotic bacteria compared to placebo (97% *versus* 3%, P<0.0001) was 25.39 (95% CI 10.37 to 62.17). The number needed to treat with oral probiotic therapy to prevent one additional relapse was 2 (92).

A third study included 40 consecutive patients

who underwent IPAA for ulcerative colitis (89). Patients were randomly assigned to receive VSL#3™ 3 g/day or placebo immediately after ileostomy closure for one year. Patients receiving the probiotic had significantly fewer episodes of pouchitis (10% versus 40%); furthermore, probiotic treatment was also associated with significant improvement in quality of life, compared to placebo.

In contrast, an open label trial by Shen and colleagues (95) reported lesser responses. In their trial, 31 subjects were prescribed a 2-week treatment of a single antibiotic (ciprofloxacin) followed by VSL#3™. Also in contrast to the other studies, the VSL#3™ was bought by patients rather than be supplied through the study. Probiotic therapy was stopped by 9 of 31 (29%) seven weeks into therapy and 25 of 31 (81%) by 8 months had discontinued the probiotic because of failure to prevent pouchitis (n=23) or side effects of the probiotic administration (n=2). Only 6 of 31 (19%) did not develop clinical evidence of pouchitis by the end of the 8-month trial period. Even among these 6 subjects endoscopy revealed some level of pouch inflammation. In this trial (95), there was a single antibiotic administered and endoscopy was not performed prior to probiotic administration to ensure pouch inflammation had completely resolved.

A recent clinical practice guideline on management of pouchitis (96) has suggested that for those patients with prompt recurrence of pouchitis following antibiotic usage or having multiple recurrences of pouchitis despite antibiotics either VSL#3™ or chronic use of antibiotics but does not suggest probiotics for acute treatment of pouchitis. Table III shows the outcomes of controlled trials of probiotics for the induction and maintenance of remission in adult with pouchitis.

3.4 Probiotics in extraintestinal manifestations of IBD

3.4.1 Arthralgia: Karimi and coworkers performed an open label trial where 16 patients with either CD or UC completed a 3-month trial of ingesting 9×10^{11} CFU/day of VSL#3™ to assess whether there was a clinical improvement in arthralgia (97). Participants had quiescent IBD at entry and no clinical or laboratory evidence of arthritis, were not taking non-steroidal anti-inflammatory medications and other medications were unchanged. An improvement in peripheral but not axial arthralgia was reported using an articular index score (65).

3.4.2 Spondylarthropathy: In an interesting internet-based randomized control trial (98) of probiotic in patients with spondylarthropathy (7% of patients were IBD patients), Brophy and coworkers aimed to determine whether an internet-based trial of a complementary and alternative medicine could fulfill the revised CONSORT (Consolidated Standards of Reporting Trials) statement quality checklist for reporting of RCTs. However a secondary aim was to study the effect of probiotics on improving well-being. Well-being was measured by self-assessment using a visual analogue scale and 96 of 147 (65%) of people randomized to receive a blend probiotic completed a 3-month trial. No statistically or clinically significant difference between placebo and probiotic groups in terms of global well-being was found in this study (98).

3.4.3 Sclerosing Cholangitis: Vleggaar and coworkers randomized fourteen patients with concurrent IBD to treatment with a blend probiotic (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus salivarius*, *Lactobacillus lactis*, *Bifidobacterium bifidum and Bifidobacterium lactis*; total daily dose of 1010 CFU/day) or placebo during 3 months in a double-blind crossover design that included a 1-month washout period (99). The subjects remained on their ursodeoxycholic acid. The results of this study showed no evidence of a beneficial effect of the probiotics on PSC-related symptoms, serum liver biochemistry or liver function.

CONCLUSIONS AND PERSPECTIVES

Many concerns remain about the use of probiotics, such as the optimal number of colony forming units delivered into the gut, the survival of administered probiotics as they transit into the gut, the best method of delivery (e.g. yogurt versus milk), the difference in the action of probiotics related to the age of the patients, the use of combination or single probiotic preparation, and the optimal duration of the therapy. Other questions and concerns have been raised, however, about the safety of probiotic administration in the setting of a severe illness: for example, worsening of Crohn's disease (CD) in

patients taking some probiotic formulations (100) or exacerbation of indomethacin- induced enteropathy in animal models by *Lactobacillus GG* had been observed (101). As rare as these complications appear to be, probiotic safety profile needs to be specifically studied, particularly in hospitalized patients.

In conclusion, considering the available data arising from the scientific literature:

– In IBD, probiotics could have a promising therapeutic role, associated to conventional drugs.

– in UC, a benefit of probiotics remains unproven, even if *Escherichia coli Nissle 1917* shows promising in maintaining remission and could be considered an alternative in patients intolerant or resistant to 5-ASA preparations;

– in pouchitis, small controlled trials suggest a benefit from VSL#3 in the primary and secondary prevention of pouchitis;

Well-designed randomized control clinical trials are necessary in order to understand the undoubted role of these agents in the management of gut physiology in health and disease.

REFERENCES

1. Fava F, Danese S. Intestinal microbiota in inflammatory bowel disease: friends of foe? World J Gastroenterol 2011; 17(5):557-66.
2. Sartor RB. Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. Nat Clin Pract Gastroenterol Hepatol 2006; 3:390-407.
3. Danese S, Fiocchi C. Etiopathogenesis of inflammatory bowel diseases. World J Gastroenterol 2006; 12:4807-12.
4. Kirsner JB. Historical review: the historical basis of the idiopathic inflammatory bowel disease. Inflamm Bowel Dis 1995; 1:2-26.
5. Qin X. Etiology of inflammatory bowel disease: A unified hypothesis. World J Gastroenterol 2012; 18(15):1708-22.
6. Bassler A. The etiology and treatment of chronic ulcerative colitis (non-specific). Am J Dig Dis 1949; 16:275-85.
7. De Dombal FT. Ulcerative colitis: definition, historical background, aetiology, diagnosis, natural history and local complications. Postgrad Med J 1968; 44:684-92.
8. Korzenik JR, Dieckgraefe BK. Is Crohn's disease an immu-nodeficiency? A hypothesis suggesting possible early events in the pathogenesis of Crohn's disease. Dig Dis Sci 2000; 45:1121-29.
9. Weinstock JV, Elliott DE. Helminths and the IBD hygiene hypothesis. Inflamm Bowel Dis 2009; 15:128-33.
10. Sartor RB. Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. Nat Clin Pract Gastroenterol Hepatol 2006; 3:390-407.
11. Fiocchi C. Future of IBD pathogenesis: how much work is left to do? Inflamm Bowel Dis 2008; 14(2):S145-S147.
12. Kaser A, Zeissig S, Blumberg RS. Inflammatory bowel disease. Annu Rev Immunol 2010; 28:573-621.
13. Greenberg GR. Antibiotics should be used as first-line therapy for Crohn's disease. Inflamm Bowel Dis 2004; 10:318-20.
14. Sellon RK, Tonkonogy S, Schultz M, Dieleman LA, Grenther W, Balish E, Rennick DM, Sartor RB. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infect Immun 1998; 66:5224-31.
15. Tomasello G, Bellavia M, Damiano G, Gioviale MG, Lo Monte AI. Possible relation between gut microflora composition and oncogenic risk: is stimulation of inflammation the one ring of connection? Reviews in Medical Microbiology 2012; 23(3):52-7.
16. Elson CO, Cong Y, McCracken VJ, Dimmitt RA, Lorenz RG, Weaver CT. Experimental models of inflammatory bowel disease reveal innate, adaptive, and regulatory mechanisms of host dialogue with the microbiota. Immunol Rev 2005; 206:260-76.
17. Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell 2005; 122:107-18.
18. Rovedatti L, Kudo T, Biancheri P, et al. Differential regulation of interleukin 17 and interferon gamma production in inflammatory bowel disease. Gut 2009; 58:1629-36.
19. Brand S. Crohn's disease: Th1, Th17 or both? The change of a paradigm: new immunological and genetic insights implicate Th17 cells in the

pathogenesis of Crohn's disease. Gut 2009; 58:1152-67.
20. Tomasello G, Bellavia M, Palumbo VD, Gioviale MC, Damiani P, Lo Monte AI. From gut microflora imbalance to mycobacteria infection: is there a relationship with chronic intestinal inflammatory diseases? Ann Ital Chir 2011; 82(5):361-8.
21. Ivanov II, de Llanos Frutos R, Manel N, et al. Specific microbiota direct the differentiation of Th17 cells in the mucosa of the small intestine. Cell Host Microbe 2008; 4:337-49.
22. Bellavia M, Damiano G, Gioviale MC, Palumbo VD, Cacciabaudo F, Buscemi G, Lo Monte AI. Abnormal expansion of segmented filamentous bacteria in the gut: a role in pathogenesis of chronic inflammatory intestinal diseases? Reviews in Medical Microbiology 2011; 22:45-7.
23. Ivanov II, Atarashi K, Manel N, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell 2009; 139:485-98.
24. O'Mahony C, Scully P, O'Mahony D, et al. Commensal-induced regulatory T cells mediate protection against pathogen-stimulated NF-kB activation. PLoS Pathog 2008; 4:e1000112.
25. Takayama N, Vosshenrich CAJ, Lesjean-Pottier S, et al. Microbial flora drives interleukin 22 production in intestinal NKp46Rcells that provide innate mucosal immune defense. Immunity 2008; 29:958-70.
26. Takayama T, Kamada N, Chinen H, et al. Imbalance of NKp44RNKp46- and NKp44- NKp46Rnatural killer cells in the intestinal mucosa of patients with Crohn's disease. Gastroenterology 2010; 139:882-92.
27. Cash HL, Whitham CV, Behrendt CL, Hooper LV. Symbiotic bacteria direct expression of an intestinal bactericidal lectin. Science 2006; 313:1126-30.
28. Ogawa H, Fukushima K, Naito H, et al. Increased expression of HIP/PAP and regenerating gene III in human inflammatory bowel disease and a murine bacterial reconstitution model. Inflamm Bowel Dis 2003; 9:162-70.
29. van Eden W, Thole JER, van der Zee R. Cloning of the mycobacterial epitope recognized by T lymphocytes in adjuvant arthritis. Nature 1988; 331:171-3.
30. Cobelens PM, Heijnen CJ, Nieuwenhuis EE, et al. Treatment of adjuvant-induced arthritis by oral administration of mycobacterial HSP 65 during disease. Arthritis Rheum 2000; 43:2694-02.
31. Birk OS, Douek DC, Elias D, et al. A role of HSP 60 in autoimmune diabetes: analysis in a transgenic model. Proc Natl Acad Sci USA 1996; 93:1032-7.
32. Raz I, Elias D, Avron A, Tamir M, Metzger M, Cohen IR. b-Cell function in new-onset type 1 diabetes and immunomodulation with a heat-shock protein peptide (Dia-Pep277): a randomized, double-blind, phase II trial. Lancet 2001; 358:1749-53.
33. Maron R, Sukhova G, Faria A-M, et al. Mucosal administration of heat shock protein-65 decreases atherosclerosis and inflammation in aortic arch of low-density lipoprotein receptor-deficient mice. Circulation 2002; 106:1708-15.
34. Rha YH, Taube C, Haczku A, et al. Effect of microbial heat shock proteins on airway inflammation and hyperresponsiveness. J Immunol 2002; 169:5300-7.
35. van Puijvelde GHM, van Es T, van Wanrooij EJA, et al. Induction of oral tolerance to HSP60 or an HSP60-peptide activates T cell regulation and reduces atherosclerosis. Arterioscler Thromb Vasc Biol 2007; 27:2677-83.
36. Ohue R, Hashimoto K, Nakamoto M, et al. Bacterial heat shock protein 60, GroEL, can induce the conversion of naı¨ve T cells into a CD4RCD25R Foxp3-expressing phenotype. J Innate Immun 2011; 3:605-13.
37. Arvans DL, Vavricka SR, Ren H, et al. Luminal bacterial flora determines physiological expression of intestinal epithelial cytoprotective heat shock proteins 25 and 72. Am J Physiol Gastrointest Liver Physiol 2005; 288:G696-G704.
38. Otani S, Otaka M, Jin M, et al. Effects of preinduction of heat shock proteins on acetic acidinduced colitis in rats. Dig Dis Sci 1997; 42:833-46.
39. Petrof EO, Kojima K, Ropeleski MJ, et al. Probiotics inhibit nuclear factor-kappaB and induce heat shock proteins in colonic epithelial cells through proteasome inhibition. Gastroenterology 2004; 127:1474-87.
40. Preville X, Gaestel M, Arrigo AP. Phosphorylation is not essential for protection of L929 cells by HSP25 against H2O2-mediated disruption actin cytoskeleton, a protection which appears related to the redox change mediated by HSP25. Cell Stress

Chaperones 1998; 3:177-87.
41. Sasahara H, Otaka M, Itoh S, et al. Effect of preinduction of heat-shock proteins on acetic acid-induced small intestinal lesions in rats. Dig Dis Sci 1998; 43:2117-30.
42. Tomasello G, Sciumé C, Rappa F, et al. Hsp10, Hsp70, and Hsp90 immunohistochemical levels change in ulcerative colitis after therapy. Eur J Histochem. 2011; 24;55(4):e38.
43. Rodolico V, Tomasello G, Zerilli M, et al. Hsp60 and Hsp10 increase in colon mucosa of Crohn's disease and ulcerative colitis. Cell Stress Chaperones 2010; 15(6):877-84.
44. Henckaerts L, Pierik M, Joossens M, Ferrante M, Rutgeerts P, Vermeire S. Mutations in pattern recognition receptor genes modulate seroreactivity to microbial antigens in patients with inflammatory bowel disease. Gut 2007; 56(11):1536-42.
45. Damaskos D, Kolios G.Probiotics and prebiotics in inflammatory bowel disease: microflora 'on the scope'. Br J Clin Pharmacol 2008; 65(4):453-67.
46. Nerstedt A, Nilsson EC, Ohlson K, Hakansson J, Thomas SL, Lowenadler B, Svensson UK, Mahlapuu M. Administration of Lactobacillus evokes coordinated changes in the intestinal expression profile of genes regulating energy homeostasis and immune phenotype in mice. Br J Nutr 2007; 97:1117-27.
47. Rioux KP, Fedorak RN. Probiotics in the treatment of inflammatory bowel disease. J Clin Gastroenterol 2006; 40:260-3.
48. Barbosa TM, Serra CR, La Ragione RM,Woodward MJ, Henriques AO. Screening for bacillus isolates in the broiler gastrointestinal tract. Appl Environ Microbiol 2005; 71:968-78.
49. Resta-Lenert S, Barrett KE. Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive Escherichia coli (EIEC). Gut 2003; 52:988-97.
50. Peluso I, Fina D, Caruso R, et al. Lactobacillus paracasei subsp: paracasei B21060 suppresses human T-cell proliferation. Infect Immun 2007; 75:1730-7.
51. Rousseaux C, Thuru X, Gelot A, et al. Lactobacillus acidophilus modulates intestinal pain and induces opioid and cannabinoid receptors. Nat Med 2007; 13:35.
52. Inohara N, Ogura Y, Fontalba A, Gutierrez O, Pons F, Crespo J, et al. Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. J Biol Chem 2003; 278:55095512.
53. Kobayashi KS, Chamaillard M, Ogura Y, Henegariu O, Inohara N, Nunez G, et al. Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. Science 2005; 307:731734.
54. Sartor, R.B. Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics. Gastroenterology 2004; 126:1620-33.
55. Verna EC, Lucak S.Use of probiotics in gastrointestinal disorders: what to recommend? Ther Adv Gastroenterol 2010; 3(5):307-19
56. Rachmilewitz D, Katakura K, Karmeli F, et al. Toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis. Gastroenterology 2004; 126:520-8.
57. Jijon H, Backer J, Diaz H, Yeung H, Thiel D, McKaigney C, De Simone C, Madsen K. DNA from probiotic bacteria modulates murine and human epithelial and immune function. Gastroenterology 2004; 126:1358-73.
58. Lee J, Rachmilewitz D, Raz E. Homeostatic effects of TLR9 signaling in experimental colitis. Ann NY Acad Sci 2006; 1072:351-5.
59. Sheil B, McCarthy J, O'Mahony L, et al. Is the mucosal route of administration essential for probiotic function? Subcutaneous administration is associated with attenuation of murine colitis and arthritis. Gut 2004; 53:694-700.
60. Schultz M, Timmer A, Herfarth HH, Sartor RB, Vanderhoof JA, and Rath HC. Lactobacillus GG in inducing and maintaining remission of Crohn's disease. BMC Gastroenterol 2004; 4:5.
61. Fujimori S, Tatsuguchi A, Gudis K, Kishida T, Mitsui K, Ehara A, et al. High dose probiotic and prebiotic cotherapy for remission induction of active Crohn's disease. J Gastroenterol Hepatol 2007;22:11991204.
62. Gupta P, Andrew H, Kirschner BS, Guandalini S. Is Lactobacillus GG helpful in children with Crohn's disease? Results of a preliminary, open-label study. J Pediatr Gastroenterol Nutr 2000; 31:453-57.
63. Turner D, Griffiths AM, Walters TD, et al. Appraisal of the Pediatric Crohn's Disease Activity Index

(PCDAI) on four prospectively collected datasets: recommended cutoff values and clinimetric properties. Am J Gastroenterol 2010; 105:2085-92.
64. Butterworth, A.D.; Thomas, A.G.; Akobeng, A.K. Probiotics for induction of remission in Crohn's disease. Cochrane Database Syst Rev 2008; 3:CD006634.
65. Mack DR. Probiotics in Inflammatory Bowel Diseases and Associated Conditions. Nutrients 2011; 3:245-64.
66. Malchow H. Crohn's disease and Escherichia coli: A new approach in therapy to maintain remission of colonic Crohn's disease. J Clin Gastroenterol 1997, 25, 653-8.
67. Guslandi M, Mezzi G, Sorghi M, Testoni PA. Saccharomyces boulardii in maintenance treatment of Crohn's disease. Dig. Dis. Sci. 2000; 45:1462-4.
68. Bousvaros A, Guandalini S, Baldassano RN, et al. A randomized, double-blind trial of Lactobacillus GG versus placebo in addition to standard maintenance therapy for children with Crohn's disease. Inflamm Bowel Dis 2005; 11:833-9.
69. Doherty G, Bennett G, Patil S, Cheifetz A, Moss AC. Interventions for prevention of post-operative recurrence of Crohn's disease. Cochrane Database Syst Rev 2009; 4:CD006873.
70. Mallon P, McKay D, Kirk S, Gardiner K. Probiotics for induction of remission in ulcerative colitis. Cochrane Database Syst Rev 2007; CD005573.
71. Naidoo K, Gordon M, Fagbemi AO, et al. Probiotics for maintenance of remission in ulcerative colitis. Cochrane Database Syst Rev 2011; CD007443.
72. Furrie E, Macfarlane S, Kennedy A, et al. Synbiotic therapy (Bifidobacterium longum/Synergy 1) initiatesresolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. Gut 2005; 54:242249.
73. Kato K, Mizuno S, Umesaki Y, et al. Randomized placebo-controlled trial assessing the effect of bifidobacteria-fermented milk on active ulcerative colitis. Aliment Pharmacol Ther 2004; 20:1133-41.
74. Tursi A, Brandimarte G, Giorgetti GM, Forti G, Modeo ME, Gigliobianco A. Low-dose balsalazide plus a high-potency probiotic preparation is more effective than balsalazide alone or mesalazine in the treatment of acute mild-to-moderate ulcerative colitis. Med Sci Monit 2004; 10:PI126PI131.
75. Shen B, Brzezinski A, Fazio VW, Remzi FH, Achkar JP, Bennett AE, Sherman K, Lashner BA. Maintenance therapy with a probiotic in antibiotic-dependent pouchitis: experience in clinical practice. Aliment Pharmacol Ther; 2005; 22:721-28.
76. Miele E, Pascarella F, Giannetti E, et al. Effect of a probiotic preparation (VSL#3) on induction and maintenance of remission in children with ulcerative colitis. Am J Gastroenterol 2009; 104:437.
77. Sood A, Midha V, Makharia GK, et al. The probiotic preparation, VSL#3 induces remission in patients with mild-to-moderately active ulcerative colitis. Clin Gastroenterol Hepatol 2009;7:1202.
78. Bibiloni R, Fedorak RN, Tannock GW, et al. VSL#3 probiotic-mixture induces remission in patients with active ulcerative colitis. Am J Gastroenterol 2005; 100:1539.
79. Huynh HQ, deBruyn J, Guan L, et al. Probiotic preparation VSL#3 induces remission in children with mild-to-moderate acute ulcerative colitis: a pilot study. Inflamm Bowel Dis 2009; 15:760.
80. Tursi A, Brandimarte G, Papa A, et al. Treatment of relapsing mild-to-moderate ulcerative colitis with the probiotic VSL#3 as adjunctive to a standard pharmaceutical treatment: a double-blind, randomized, placebo-controlled study. Am J Gastroenterol 2010; 105:2218.
81. Kruis W, Fric P, Pokrotnieks J, Lukas M, Fixa B, Kascak M, et al. Maintaining remission of ulcerative colitis with the probiotic Escherichia coli Nissle 1917 is as effective as with standard mesalazine. Gut 2004;53: 16171623.
82. Zocco MA, dal Verme LZ, Cremonini, Piscaglia AC, Nista EC, Candelli M. Efficacy of Lactobacillus GG in maintaining remission of ulcerative colitis. Aliment Pharmacol Ther 2006; 23:15671574.
83. Komanduri S, Gillevet PM, Sikaroodi M, Mutlu E, Keshavarzian A. Dysbiosis in pouchitis: evidence of unique microfloral patterns in pouch inflammation. Clin Gastroenterol Hepatol 2007; 5:352360.
84. Elahi B, Nikfar S, Derakhshani S, Vafaie M, Adollahi M. On the benefit of probiotics in the management of pouchitis in patients underwent ileal pouch anal anastomosis: A meta-analysis of controlled clinical trials. Dig Dis Sci 2008;53:1278-84.

85. Pardi DS, D'Haens G, Shen B, Campbell S, Gionchetti, P. Clinical guidelines for the management of pouchitis. Inflamm Bowel. Dis 2009;15:1424-31.
86. Mimura T, Rizzello F, Helwig U, Poggioli G, Schreiber S, Talbot IC, et al. Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. Gut 2004; 53:108114.
87. Kuisma J, Mentula S, Jarvinen H, Kahri A, Saxelin M, Farkkila M. Effect of Lactobacillus rhamnosus GG on ileal pouch inflammation and microbial flora. Aliment Pharmacol Ther 2003; 17:509515.
88. Gionchetti P, Rizzello F, Venturi A, Brigidi P, Matteuzzi D, Bazzocchi G. Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. Gastroenterology 2000; 119: 305309.
89. Gionchetti P, Rizzello F, Helwig U, Venturi A, Lammers KM, Brigidi P. Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. Gastroenterology 2003; 124:12021209.
90. Sandborn, WJ, Tremaine WJ, Batts KP, Pemberton JH, Philips SF. Pouchitis following ileal pouch-anal anastomosis: a pouchitis disease activity index. Mayo Clin. Proc. 1994;69:409–15.
91. Laake KO, Bjorneklett A, Aamodt G, Aabakken L, Jacobsen M, Bakka A, Vatn MH. Outcome of four weeks' intervention with probiotics on symptoms and endoscopic appearance after surgical reconstruction with a J-configuration ileal-pouch-anal-anastomosis in ulcerative colitis. Scand. J. Gastroenterol. 2005; 40:43–51.
92. Gionchetti P, Rizzello F, Morselli C, Poggioli G, Tambasco R, Calabrese C, Brigidi P, Vitali B, Straforini,G, Campieri M. High-dose probiotics for the treatment of active pouchitis. Dis. Colon Rectum 2007; 50;2075-2078.
93. Holubar SD, Cima RR, Sandborn WJ, Pardi DS. Treatment and prevention of pouchitis after ileal pouch-anal anastomosis for chronic ulcerative colitis. Cochrane Database Syst Rev 2010; 6:CD001176.
94. Pronio A, Montesani C, Butteroni C, Vecchione S, Mumolo G, Vestri A, Vitolo, D, Boirivant M. Probiotic administration in patients with ileal pouch-anal anastomosis for ulcerative colitis is associated with expansion of mucosal regulatory cells. Inflamm Bowel Dis 2008; 14: 662-8.
95. Shen B, Brzezinski A, Fazio VW, Remzi FH, Achkar JP, Bennett AE, Sherman K Lashner BA. Maintenance therapy with a probiotic in antibiotic-dependent pouchitis: experience in clinical practice. Aliment Pharmacol Ther 2005; 22:721-28.
96. Pardi DS, D'Haens G, Shen B, Campbell S, Gionchetti P. Clinical guidelines for the management of pouchitis. Inflamm. Bowel Dis 2009; 15:1424-31.
97. Karimi O, Pena S, van Bodegraven AA. Probiotics (VSL#3) in arthralgia in patients with ulcerative colitis and Crohn's disease: A pilot study. Drugs Today 2005; 41:453-9.
98. Brophy S; Burrows CL Brooks, C.; Gravenor, M.B.; Siebert, S.; Allen, S.J. Internet-based randomized controlled trials for the evaluation of complementary and alternative medicines: probiotics in spondylarthropathy. BMC Musculoskelet Disord 2008; 9:4.
99. Vleggaar FP, Monkelbaan JF, van Erpecum KJ. Probiotics in primary sclerosing cholangitis: A randomized placebo-controlled crossover pilot study. Eur J Gastroenterol Hepatol 2008; 20:688-92.
100. Rolfe, V.E., Fortun, P.J., Hawkey, C.J. And Bath-Hextall, F. Probiotics for maintenance of remission in Crohn's disease. Cochrane Database Syst Rev 2006; 4:CD004826.
101. Kamil, R., Geier, M.S., Butler, R.N. and Howarth, G.S. Lactobacillus rhamnosus GG exacerbates intestinal ulceration in a model of indomethacin induced enteropathy. Dig Dis Sci 2007; 52:12471252.
102. Whelan K, Quigley EM. Probiotics in the management of irritable bowel syndrome and inflammatory bowel disease. Curr Opin gastroenterol 2013; 29(2):184-9.