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Manipulation of Intestinal Flora as a Way to Treat Crohn's Disease: The Role of Probiotics, Prebiotics and Antibiotics

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1. Introduction

Crohn's disease (CD) is a chronic gastrointestinal disorder which, together with ulcerative colitis, is known as Inflammatory Bowel Disease (IBD). CD is characterized by transmural inflammation of the entire gastrointestinal tract, from oral cavity to anus. The most common symptoms are chronic diarrhea and abdominal pain, which are often accompanied by anorexia, malaise, weight loss, fever and extra-intestinal manifestations. The latter can involve almost every organ system (Macfarlane et al., 2009). CD can cause the formation of strictures, abscesses or fistulas. For that reason, surgical resection in CD patients is very common.

Regardless of the vast amount of data gathered on the etiology and the course of CD, the actual cause is still unknown. The interplay of various factors is blamed for the disease outbreak. These factors include genetic predisposition, environmental influences (e.g. smoking), imbalance in immune response and changes in intestinal flora. Given the complexity of the disease, some factors may be the consequence of others.

Genetic predisposition has received the most research attention in recent years. Family studies have shown that more than 50% concordance of CD can be expected in monozygotic twins. These findings indicate that the pathogenesis of CD is not based only on one gene but on a polygenic risk profile (Vavricka & Rogler, 2009). In 2001, two independent groups of researchers discovered one of the most important susceptibility genes for CD, a gene for NOD2/CARD15 (Hugot et al., 2001; Ogura et al., 2001). Its mutation is present in about one third of all CD patients. Patients with mutated NOD2/CARD15 variants could have a deficient response to bacterial LPS which could lead to the development of CD (Ogura et al., 2001). Several other genes that can contribute to susceptibility to CD have been identified. These include IBD5, IL23R, ATG16L1, Chr5p13.1, Chr5q33.1 and Chr10q21.1 (Xavier & Podolsky, 2007). They are involved in the maintenance of barrier function and regulation of innate and adaptive immunity.

There are several environmental factors that have been predicted to be involved in the development of CD. The single most important environmental risk factor for the development of CD is tobacco smoking. Further environmental risk factors are related to living in urban areas of developed western countries with stressful lifestyle. There is also some evidence that diet could influence the risk for CD. Uptake of fatty acids and sugar,

which has increased in the last decades in developed countries, has been linked to an increase in the incidence of CD (Hanauer, 2006).

Dysregulated immune response and exaggerated response to exogenous factors, including the intestinal microbiota, is also an important contributor to CD. Immune response at the intestinal mucosa is mediated by epithelial and mucosal immune cells. They recognize molecules of bacterial origin via Toll-like and NOD-like receptors. Receptor binding leads to activation of the NF-κB pathway, which is a crucial regulator of the inflammatory response. In epithelial Paneth cells, NF-κB activation leads to the production of defensins. In antigen presenting cells, activation of NF-κB leads, with the involvement of procaspase 1, to the production of proinflammatory cytokines, which stimulate further inflammation events (Xavier & Podolsky, 2007). Increased expression of Th1 cytokines, mainly IFNγ, IL-12, TNFα, IL-1β and IL-6, has been considered fundamental in maintaining the inflammation. Other cytokines, such as IL-8, IL-18 and IL-23 have also been involved (Dharmani & Chadee, 2008).

Even though the actual role of bacteria in the pathogenesis of CD is still unknown, the differences in the composition of intestinal flora between healthy individuals and CD patients are today a well established fact. They have recently been confirmed by the metagenomic sequencing of the intestinal microbiome (Manichanh et al., 2006; Qin et al., 2010). The difference is characterized by the reduction of species diversity from the phylum *Firmicutes* and an increase in the concentration of enterobacteriaceae, especially *Escherichia coli*. Interestingly, no significant differences in flora composition were observed between inflamed and non-inflamed intestinal mucosa of CD patients (Vasquez et al., 2007). CD is often associated with the presence of specific microorganisms (adherent and invasive *E. coli*, *Mycobacterium avium* subsp. *paratuberculosis*; described below) and lack of others (*Faecalibacterium prausnitzii*). *F. prausnitzii* is a member of *Firmicutes* which has been observed to be present in lower quantities in patients with endoscopic recurrence of CD six months after the surgery, in comparison to patients that were still in remission after surgery. Anti-inflammatory effects of *F. prausnitzii* have been demonstrated in *in vitro* and *in vivo* mouse models (Sokol et al., 2008). Taken together, it has been hypothesized that either a single pathogen, increased mucosal permeability, or imbalance between "good" and "bad" bacteria (dysbiosis) contribute to the CD onset.

The hypothesis of a single pathogen is based on the assumption that *M. avium* subsp. *paratuberculosis* (MAP) could be the causative agent of CD, and has been supported by a considerable body of evidence. Investigators were able to isolate MAP from the inflamed tissue and peripheral blood of CD patients, detect anti-MAP antibodies and also prove its presence using molecular analysis (De Hertogh et al., 2008; Macfarlane et al., 2009). However, this was not enough to prove MAP as causative agent of CD, especially since treatment with drugs against MAP had no effect on the improvement of CD. Several other bacteria have also been associated with CD pathogenesis. These include *Listeria monocytogenes*, *Pseudomonas maltophilia*, *M. kansasii*, *Bacteroides fragilis* and adherent invasive *E. coli* (AIEC) (De Hertogh et al., 2008; Macfarlane et al., 2009). The latter is a specific pathovar that has been shown to colonize intestinal mucosa of 36.4% of patients with ileal CD. It is able to replicate in macrophages and cause the release of large amounts of TNFα (Rolhion & Darfeuille-Michaud, 2007). There is also evidence that AIEC plays an important role in the formation of granulomas which are a histological characteristic of CD. Colonization with AIEC was recently linked to the previously mentioned NOD2/CARD15 mutation, (Barnich & Darfeuille-Michaud, 2007; Rolhion & Darfeuille-Michaud, 2007).

A second hypothesis is based on an increased permeability of intestinal mucosa which can cause translocation of bacteria and their metabolites from the gastrointestinal tract to mesenteric lymph nodes and other internal organs (De Hertogh et al., 2008). Occurrence of such bacterial translocation in patients with CD after surgery has been documented and has caused systemic inflammatory septic response. *E. coli*, *Enteroccoccus* ssp, *Bact. fragilis*, and *Klebsiella pneumoniae* were among the bacterial species which translocated to the greatest extent (Takesue et al., 2002).

A third, dysbiosis theory suggests a broken equilibrium between "good" and "bad" intestinal bacteria as a cause of CD (Tamboli et al., 2004). The hypothesis is based on a number of studies of faecal and mucosal associated microflora which were found to differ between CD patients and healthy individuals (reviewed by Tamboli et al., (2004)). The data obtained with classic bacteriological culturing techniques was recently substantiated by metagenomic sequencing (Qin et al., 2010). Faecal microflora from patients with CD contains decreased numbers of butyrate producing *Firmicutes*, especially the *Clostridium leptum* group (Manichanh et al., 2006). Ribosomal DNA sequence analysis of mucosa associated bacteria in patients with CD has shown increased levels of facultative bacteria (e. g. *E. coli*) in colonic mucosa. In small intestinal mucosa, decreased levels of *C. leptum* and *Prevotella nigrescens* subgroups were observed, as well as increased level of the *Ruminococcus gnavus* subgroup (Prindiville et al., 2006). These results speak in favor of the dysbiosis theory and make the single pathogen theory less likely.

The aim of this chapter is to introduce ways of interference with the diversity and abundance of intestinal bacteria, which are important in CD pathogenesis. Clinical efficacy of various strategies will also be presented.

2. Probiotics

The Food and Agriculture Organization and the World Health Organization have defined probiotics as "live microorganisms which, when administered in adequate amounts as part of food, confer a health benefit on the host". Probiotics usually belong to the genera *Lactobacillus* or *Bifidobacterium.* These are gram positive bacteria with fermentative metabolism, in which lactic acid is a major product. They are obligatory or facultative anaerobes and are non-motile. Among lactobacilli, strains with probiotic properties are *Lb. acidophilus*, including strain LA-5, *Lb. crispatus*, *Lb. johnsonii* LA1, *Lb. gasseri* PA16, *Lb. casei*, *Lb. paracasei,* strains *"shirota"* and *"defensis"*, *Lb. rhamnosus* GG, *Lb. reuteri* and *Lb. plantarum.* Among bifidobacteria, *B. longum,* strains BB536 and SP07/3, *B. bifidum* MF20/5, *B. infantis*, *B. animalis, B. adolescentis* and *B. breve*, have been considered probiotic. Some other bacteria and yeasts can also have probiotic properties. These include *Enterococcus faecalis, Streptococcus thermophilus, Propionibacteria, E. coli* Nissle 1917 and yeast *Saccharomyces boulardii* (de Vrese & Schrezenmeir 2008). Probiotics have many potential beneficial health effects, which are more or less well documented. The effective use of probiotics for significant improvement of lactose digestibility, childhood infectious gastroenteritis, diarrhea associated with either antibiotics, rotavirus infection, or chemotherapy, as well as traveller's diarrhea has been well documented (Schrezenmeir & de Vrese, 2001; Walker et al., 2006). Probiotics can stimulate humoral or cellular immune systems. They can cause a decrease in unfavourable metabolites (e. g. ammonium) in the colon. There are fewer reports of other possibilities of the use of probiotics for health improvement, which are limited to a

specific probiotic strain, or to a combination of two or more strains (Schrezenmeir & de Vrese, 2001; Walker et al., 2006; Thomas & Greer, 2010).

Due to the implication of intestinal bacteria in the CD, probiotics were also suggested as a possible treatment for CD. Among probiotics, *Lb. rhamnosus* GG, *Lb. johnsonii* LA1, *B. lactis*, *Str. thermophilus*, *E. coli* Nissle 1917 and yeast *S. boulardii* have been tested for the treatment of CD.

2.1 Mechanism of action

Several mechanisms by which probiotics exert beneficial effects in treatment of CD have been suggested (Boirivant & Strober, 2007; Dharmani & Chadee, 2008; Guandalini, 2010; Ng et al., 2009). Probiotics are supposed to improve the epithelial cell barrier function, decrease the load of "bad" bacteria by direct or indirect antibacterial effects or exert direct effects on epithelial and immune cells by, among other things, affecting their cytokine expression profiles. These effects are intertwined to a large extent and the contribution of an individual effect is therefore hard to establish. Besides, an individual probiotic bacterium is probably not capable of exerting the entire spectrum of activities. Instead, it is more likely to be responsible for a specific effect.

2.1.1 Effects on epithelial cell barrier function

The layer of epithelial cells that lines the intestinal tract constitutes a physical barrier that prevents intestinal bacteria from entering into the organism. The mix of probiotic organisms, VSL#3, was able to normalize barrier integrity in IL-10 deficient mice, as shown by the measurements of several parameters (conductivity, mannitol flux) on excised tissue (Madsen et al., 2001). Barrier function is reinforced by the layer of mucus, which is secreted by goblet epithelial cells. It has been shown that some probiotic bacteria are capable of modifying the expression of proteins that are involved in mucin production. *Lb. plantarum* 299v was able to increase MUC2 and MUC3 mRNA expression in the epithelial cell line (Mack et al., 1999), while VSL#3 and *E. coli* Nissle 1917 increased MUC2, MUC3 and MUC5AC protein expression (Otte & Podolsky, 2004). The epithelial layer is also responsible for the active transport of nutrients, electrolytes and water. Impaired ability for reabsorption of sodium and water from the distal colon leads to diarrhea that usually accompanies CD (Zeissig et al., 2007). *Str. thermophilus* and *Lb. acidophilus* were able to increase trans-epithelial resistance, but also alleviate electrolyte transport by increasing chloride secretion (Resta-Lenert & Barrett, 2009). Probiotics were also reported to influence the maintenance of tight junction proteins by influencing the cytoskeleton architecture. VSL#3 prevented the redistribution of tight junction protein ZO-1 (Otte & Podolsky, 2004) and *Lb. acidophilus* was able to prevent the rearrangement of F-actin upon exposure to pathogenic *E. coli* (Liévin-Le Moal et al., 2002). Probiotics can even prevent apoptosis of epithelial cells, as was shown for *Lb. rhamnosus* GG, which was able to activate anti-apoptotic and inhibit pro-apoptotic proteins (Yan & Polk, 2002).

2.1.2 Antibacterial effects

Probiotics influence the composition of intestinal flora by competing for the available space (e. g. available binding sites for adhesion) and for available essential nutrients. They can also exert antimicrobial effects by either direct or indirect means. Direct means include productions of inhibitory substances like bacteriocins, hydrogen peroxide and organic acids.

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Bacteriocins are diverse group of low molecular weight peptides, usually produced by strains from genus *Lactobacillus*, and by some other lactic acid bacteria, including *Lactoccocus lactis*. Bacteriocins have antimicrobial activity against several bacteria, but are especially effective against Gram positive bacteria. Similar substances are produced in probiotic strains of *Bifidobacteria*, and are active against both Gram negative and Gram positive bacteria (Guandalini, 2010). Organic acids such as lactic acid, propionic acid and butyric acid are produced in fermentative metabolism of probiotics and are responsible for a decrease in pH in the gastrointestinal tract, which is harmful to acid sensitive intestinal bacteria. Probiotics can also exert indirect antibacterial effects, by stimulating the production of defensins. Defensins are human endogenous cationic antimicrobial peptides with antimicrobial activity against Gram negative and Gram positive bacteria. They are secreted by specialized epithelial Paneth cells. Deficiency in defensins could play an important role in the pathogenesis of colonic and ileal CD (Wehkamp et al., 2009). Low expression of β-defensin occurs in patients with colonic CD and affects the structure of colonic mucosa. In patients with ileal CD, decreased level of Paneth cells α-defensin has been observed and these patients also had the NOD2 mutation. These results suggest that there is a connection between NOD2 function and expression of α-defensin and that this connection has an important implication in the development of ileal CD (Wehkamp et al., 2009).

2.1.3 Immunomodulative effects on epithelial and immune cells

Probiotic bacteria can modulate the activity of epithelial and immune cells including dendritic cells, monocytes, macrophages, T cells, B cells and NK cells. The immune response is mediated by pattern recognition receptors, such as toll-like receptors, which recognize specific bacterial features, termed pathogen-associated molecular patterns. These include peptidoglycan, lipopolysaccharides, flagellin and DNA variants, such as unmethylated CpG motifs. Probiotic bacteria can induce regulatory T cells by stimulating the dendritic cells to produce anti-inflammatory cytokines IL-10 and TGFβ, as was demonstrated with the treatment with VSL#3 (Hart et al., 2004) and with several other bifidobacterial strains (Young et al., 2004). The activation of regulatory T cells can be initiated by direct binding of probiotics to dendritic cells via the DC-SIGN molecule (Smits et al., 2005). This can cause the diversion of the immune response toward noninflammatory tolerogenic pattern. Probiotics can downregulate the Th1 response by inhibition of the production of proinflammatory cytokines by dendritic cells, including the production of IL-12, TNFα and IFNγ. Downregulation of the production of TNFα was demonstrated in *ex vivo* treatment of intestinal tissue from CD patients with probiotics (Borruel et al., 2002). Probiotics can also decrease T cell proliferation and their production of IL-2, IL-4 and IL-10 cytokines, as demonstrated with *Lb. rhamnosus*. This increased T cell hypo-responsiveness was also induced *in vivo* in CD patients and in healthy individuals (Braat et al., 2004). Involvement of different mechanisms of action is highlighted by the fact that some strains of lactobacilli not only downregulate, but also upregulate the production of IL-12 (Mohamadzadeh et al., 2005) and switch towards the Th1 response.

2.2 Clinical trials with probiotics for the treatment of CD

Probiotics have been tested for their ability to treat CD in several clinical trial settings. Some basic data on clinical trials is summarized in Table 1. Some probiotic strains or mixtures of strains (*E. coli* Nissle 1917, *S. boulardii*, VSL#3, *Lb. rhamnosus* GG and *Lb. johnsonii* LA1) were

tested in more than one clinical trial. Almost all the trials were designed to be randomized and placebo controlled, and more than half were double blind. Four studies were designed for the maintenance of remission in CD and five for the prevention of postoperative recurrence of CD. One study was designed for the treatment of one of the common extra-intestinal manifestations of CD, arthralgia, and one for evaluation of the impact of intestinal permeability in patients with CD.

Author	Year	Probiotics and dose	n	Study design	Study duration	Study purpose	Outcomes
Malchow	1997	*E. coli* Nissle 1917 5×10^{10}/day	28	Randomized, placebo controlled, single center	12 months	Maintenance of remission of colonic CD	Better outcome in probiotic group, not significant
Guslandi	2000	*S. boulardii* 1g/day	32	Randomized, single center	6 months	Maintenance of remission of CD	Significant difference between probiotic and mesalazine group
Campieri	2000	VSL#3 6g/day + rifaximin 1,8 g/day	40	Randomized, controlled	12 months	Prevention of postoperative recurrence of CD	Efficacy of combination of antibiotic and probiotic
Prantera	2002	*Lb. rhamnosus* GG 12×10^9 CFU/day	45	Randomized, double blind, placebo controlled, single center	12 months	Prevention of recurrence of CD after surgery	No difference
Schultz	2004	*Lb. rhamnosus* GG 2×10^9 CFU/day	11	Randomized, double blind, placebo controlled, single center	6 months	Maintenance of remission of CD	No difference, small number of patients
Bousvaros	2005	*Lb. rhamnosus* GG 2×10^9 CFU/day + 295 mg inulin/day	75	Randomized, double blind, placebo controlled, multicenter	24 months	Maintenance of remission of CD in children	No difference
Karimi	2005	VSL#3 450 bilion bacteria twice a day	29	Open labeled, pilot study	3 months	Treatment of arthralgia in patients with ulcerative colitis and CD	Significant improvement

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Marteau	2006	*Lb. johnsonii* LA1 2×10^9 CFU twice a day	98	Randomized, double blind, placebo controlled, multicenter	6 months	Prevention of postoperative recurrence of CD	No difference
Van Gossum	2007	*Lb. johnsonii* LA1 1×10^{10} CFU/day	70	Randomized, double blind, placebo controlled, multicenter	3 months	Prevention of postoperative recurrence of CD	No difference
Garcia Vilela	2008	*S. boulardii*	34	Randomized, placebo controlled	3 months	Influence on intestinal permeability of patients with CD	Improvement of intestinal permeability
Madsen	2008	VSL#3 900 billion bacteria twice a day	120	Randomized, double blind, placebo controlled, multicenter	3 months	Prevention of postoperative recurrence of CD	Less endoscopic recurrence comparing to placebo

Table 1. Clinical studies investigating the effects of probiotics in the maintenance of remission in CD or in the prevention of postoperative recurrence of CD (adapted from (Isaacs & Herfarth, 2008)).

For maintenance of remission of the CD, 28 patients with active colonic CD were treated with synthetic corticosteroid prednisone and non-pathogenic probiotic *E. coli* strain Nissle 1917, or with placebo (Malchow, 1997). From the results of remission rate we can conclude that a similar proportion of patients entered in remission in the two groups. More patients from the placebo group (63.6%) had relapse, when compared with the probiotic group, in which 33.3% of patients relapsed after 12 months of treatment. Even though these results were not statistically significant, a better outcome was reached in the probiotic group, which indicated some beneficial effect of *E. coli* Nissle 1917 on the maintenance of remission in colonic CD. Because of the small number of participants in this study, the authors suggested that larger study should be performed.

Guslandi et al. investigated possible therapeutic benefits of non-pathogenic yeast *S. boulardii* in the maintenance of remission in CD (Guslandi et al., 2000). In this study, 32 patients with CD in clinical remission were divided randomly into two groups. The first group was treated with mesalamine two times a day and with *S. boulardii* once a day. The second group was treated only with mesalamine three times a day. The study was ended after 6 months of treatment. In the mesalamine group, 6 of 16 (37.5%) patients had clinical relapse after 6 months while, in the group treated with mesalamine and *S. boulardi*, only 1 patient in 16 (6.25%) had clinical relapse. This difference is statistically significant and demonstrates that *S. boulardii*, in combination with anti-inflammatory drug, could have beneficial effects in the maintenance of remission in CD.

The efficacy of *S. boulardii* in the treatment of CD was again evaluated in a study of the influence of *S. boulardii* on intestinal permeability in patients with CD in remission (Garcia

Vilela et al., 2008). The study was carried out on 34 patients who received either placebo or *S. boulardii* for 3 months. Additionally, patients received standard therapy with corticosteroids, anti-inflammatory drugs or antibiotics. Intestinal permeability was evaluated by measuring lactulose/mannitol ratio. The ratio was 0.005 +/- 0.0037 in healthy volunteers, and 0.021 +/- 0.01 in patients with CD. After 3 months of treatment, lactulose/mannitol ratio increased by 0.004 +/- 0.010 in the placebo group and decreased by 0.008 +/- 0.006 in the *S. boulardii* group. These results demonstrated that *S. boulardii*, in combination with standard therapy for CD, could improve intestinal integrity in patients with CD.

Probiotic *Lb. rhamnosus* GG was also tested for the ability to maintain the remission of CD (Schultz et al., 2004). Probiotic therapy with *Lb. rhamnosus* GG was tested in eleven patients with moderate to active CD. Patients were first treated with antibiotics for two weeks, followed by probiotic therapy for 6 months. Relapse occurred in 2 of 5 patients in the probiotic group, as well as in 2 of 6 patients in the placebo group. Two patients from each group achieved and maintained remission. No difference between probiotic and placebo group was thus observed and no benefit of *Lb. rhamnosus* GG could be reported. Small sample size was suggested as a reason why no difference was observed.

More than 70% of CD patients will undergo at least one surgery in their life, and recurrence of CD in patients after surgical intestinal resection is very common (Marteau et al., 2006). Endoscopic recurrence of CD in patients is usually observed within one year after surgery and is followed by clinical recurrence. Around one quarter of patients will require further surgery in the following years, if no postoperative treatment of the disease will take place (Doherty et al., 2009). Prevention of postoperative CD is therefore another important target of probiotic treatment. This approach was first tested with probiotic mixture VSL#3 in combination with antibiotic rifaximin in a randomized controlled clinical trial with 28 patients (Campieri et al., 2000). Half of the patients received rifaximin dose for the first three months, which was followed by probiotic dose for the next 9 months. The control group received 4 g of mesalamine per day for 12 months. Probiotic mixture VSL#3 contains 4 strains of lactobacilli, 3 strains of bifidobacteria and *Str. thermophilus*. No side effects were reported during the study. At the end of the treatment, 4 of 20 patients had endoscopic recurrence, while in the placebo group, 8 patients had endoscopic recurrence. The authors concluded that the combination of VSL#3 and rifaximin is effective in preventing postoperative endoscopic recurrence of CD. The effectiveness of probiotic mixture VSL#3 alone in preventing postoperative recurrence of CD was later confirmed in a larger randomized, double blind, placebo controlled multicenter study on 120 patients (Madsen et al., 2008). Patients receiving VSL#3 for 3 months showed less endoscopic recurrence (9,3% of patients) compared with placebo group (15,7% recurrence). Results were substantiated with measurements of mucosal pro-inflammatory cytokine levels.

Beside VSL#3, which is a mixture of probiotic strains, some *Lactobacillus* strains were tested by themselves for the ability to prevent postoperative recurrence of CD. Probiotic strain *Lactobacillus rhamnosus* GG was tested for prevention of postoperative recurrence in a randomized double blind and placebo controlled study (Prantera et al., 2002). 23 patients received *Lb. rhamnosus* GG and 22 received placebo daily for one year. No side effects were reported, however 13 patients, from the two groups, did not finish the study. At the end of the study, 15 patients from *Lb. rhamnosus* GG group and 17 patients from the placebo group showed clinical remission. Among the patients with clinical remission, 9 of 15 patients in the probiotic group, and 6 of 17 patients in the placebo group had endoscopic recurrence of CD.

Clinical recurrence was observed in 3 patients from the *Lactobacillu*s GG group and in two from the placebo group. No benefit of *Lactobacillu*s *rhamnosus* GG in the postoperative treatment of CD could thus be observed. It was suggested that the study should be considered as a pilot one because of small number of patients, different demographic and different disease background.

Lb. johnsonii LA1 is another *Lactobacillus* strain that was used in clinical trials for the prevention of postoperative CD. The first double blind, placebo controlled clinical trial with *Lb. johnsonii* LA1 was reported in 2006 (Marteau et al., 2006). 98 patients that underwent ileocolonic, colonic or ileal CD surgery were involved. Patients were administered $2x10^9$ colony forming units (CFU) *Lb. johnsonii* LA1 or placebo twice a day for 6 months. Endoscopic recurrence was observed in 49% of patients in the probiotic group and in 64% of patients in the placebo group. Clinical recurrence was noted in four patients in the probiotic group and in three in the placebo group. Results were not statistically significant and beneficial effects of *Lb. johnsonii* LA1 on postoperative recurrence in patients with CD could not be established.

A second clinical trial of the effectiveness of probiotic *Lb. johnsonii* LA1 in preventing endoscopic postoperative recurrence of CD was conducted with 70 patients that underwent curative ileo-caecal resection (Van Gossum et al., 2007). Clinical trial was designed as a randomized, double blind, placebo controlled multicenter study. Patients were treated either with $1x10^{10}$ CFU *Lb. johnsonii* LA1 daily or with placebo for 12 weeks. No difference in measured parameters or in clinical relapse between placebo group and *Lb. johnsonii* group was observed. Clinical relapse was observed in 4 patients in *Lb. johnsonii* group and in 2 patients in the placebo group after 12 weeks of treatment.

Some limited research has also been done on the use of probiotics in pediatric CD. A small pilot study was conducted with *Lb. rhamnosus* GG in children with active CD (Gupta et al., 2000). Four patients were involved in the study, two with ileocolonic CD and two with gastrocolonic CD. Report has shown significant improvement after treatment with 10^{10} CFU of probiotic. Patients were also receiving standard therapy for CD. This study was later followed by a randomized, double blind, placebo controlled trial with *Lb. rhamnosus* GG strain which was given in addition to standard therapy for the maintenance of remission in children with CD (Bousvaros et al., 2005). 75 children with CD in remission were involved in the study for the period of two years. Results of the study have shown no significant difference between probiotic and placebo group in the time of relapse of CD. These results substantiated the results of a previous study on adults which also showed the ineffectiveness of *Lb. rhamnosus* GG in maintaining remission in CD. Because the effectiveness of probiotics in the treatment of CD was not established, treatment of CD with probiotics is not recommended for children (Thomas & Greer, 2010).

Extra-intestinal manifestations like arthralgia and arthritis are common complications of CD. They are reported to occur in 10-35% of patients. Since beneficial effects of probiotics were observed in the treatment of ulcerative colitis and sometimes of CD, the treatment of arthralgia with probiotics mixture VSL#3 was also suggested (Karimi et al., 2005). Of 29 patients with IBD involved in the study, only 16 patients ended the 3 months study and 9 of them were CD patients. After three months of treatment, the status of arthralgia differed between patients with peripheral arthralgia and axial arthralgia. Only patients with peripheral arthralgia reported improvements in their general well being and joint complaints. There was no relapse reported in patients after 3 months of treatment. The results of the study were shown for all IBD patients together. The results for patients with CD therefore cannot be discerned from those of patients with ulcerative colitis. From that it

can only be concluded that probiotic mixture VSL#3 had beneficial effects on arthralgia in patients with IBD, and possibly also in the subgroup of patients with CD.

2.3 Recombinant probiotic bacteria for the treatment of CD

Health effects of probiotic bacteria can be strengthened by genetic engineering which enables incorporation of new, defined traits into the existing bacterial repertoire. Recombinant bacteria can be used as a vector for the delivery of therapeutic proteins to the intestinal mucosa. Potential synergistic effects between existing probiotic and introduced therapeutic properties can be envisaged. Genetically modified probiotics will enable the selection of the desired protein, selection of the localization of protein expression (intracellular, secreted) and selection of the conditions required for the induction of the expression. It may also be possible to obtain a long term effect with colonizing strains (Marteau et al., 2009).

To date, the focus has been on lactic acid bacterium *Lactococcus lactis*. It does not have a probiotic status and does not colonize the intestine, but is well studied and is readily amenable for genetic modification. *L. lactis* strains with the ability to produce anti-inflammatory cytokine IL-10 or to bind pro-inflammatory cytokine TNFα have been constructed.

Genetically modified *L. lactis* secreting cytokine IL-10 was engineered and tested in two murine colitis mouse models (Steidler et al., 2000). Results of intragastric administration of L. lactis secreting IL-10 showed 50% reduction in DSS-induced colitis and prevention of colitis in IL-10 knockout mice. In further research, the authors presented genetically modified *L. lactis*, which was engineered by replacing the thymidylate synthase gene *thyA* with synthetic human IL-10 gene (Steidler et al., 2003). This recombinant bacterium (*LL-Thy12*) was able to produce human IL-10 and was not able to survive in the environment without thymidine or thymine. This enabled the biological containment of the bacterium and markedly decreased the safety concerns. Such a strain is appropriate for human use and the effectiveness of *LL-Thy12* was investigated in a phase I trial on patients with CD (Braat et al., 2006). Results of the trial have demonstrated safety of the strain. Only minor adverse effects were noted and some beneficial effects on the disease activity were observed.

Two recent studies have introduced another approach for the treatment of intestinal inflammations by binding the pro-inflammatory cytokine TNFα. The first study used engineered *L. lactis* which secreted TNFα-neutralizing nanobody (camelid heavy chain fragment). Its efficacy was demonstrated in two mouse models of colitis (Vandenbroucke et al., 2010). In another study, different binding TNFα-binding molecule was applied. TNFα-binding affibody was expressed and immobilized on the surface of engineered *L. lactis* (Ravnikar et al., 2010).

As noted earlier, besides IL-10, transforming growth factor beta (TGFβ) has often been described as an anti-inflammatory cytokine in CD. Commensal gut Gram-negative bacterium *Bact. ovatus* was engineered to express TGFβ₁ under the control of xylan. Xylan is a dietary fiber which is important for the safety of this system. Results of the study have shown significant improvement of acute colitis in mice (Hamady et al., 2010).

Initial studies of the concept of recombinant probiotic bacteria have shown promising results, but were mostly obtained in the animal studies of the treatment of ulcerative colitis. Further research will show whether recombinant probiotics have the capacity to be considered as an alternative in the treatment of CD.

multicenter, randomized, double-blind, placebo-controlled study to prevent postoperative recurrence of CD (Chermesh et al., 2007). Synbiotic 2000 is composed of 4 probiotics (10^{10} *Pediacoccus pentoseceus*, 10^{10} *Lb. raffinolactis*, 10^{10} *Lb. paracasei* subsp. *paracasei* 19, and 10^{10} *Lb. plantarum* 2362) and 4 prebiotics (2.5 g β-glucans, 2.5 g inulin, 2.5 g pectin and 2.5 g resistant starch). The clinical trial involved 30 patients with CD who were treated, after surgery, either with Synbiotic 2000 or with placebo once a day for up to 24 months. Only 9 patients completed the study, 7 from Synbiotic 2000 group and 2 from placebo group. No difference between the two groups in clinical, laboratory and endoscopic results could be confirmed and it was concluded that Synbiotic 2000 is not effective in preventing postsurgical recurrence of CD (Chermesh et al., 2007). Because of relatively small group of patients involved in this clinical trial more research was suggested, involving a larger setup and different synbiotic mixtures.

Another synbiotic therapy was reported in a clinical trial with 35 patients with active CD (Steed et al., 2010). The synbiotic product in this instance contained 2×10^{11} CFU of probiotic bacterium *B. longum* and 6 g of prebiotic Synergy 1, which is the commercial name for oligofructose-enriched inulin. Patients had received synbiotic or placebo twice a day for six months. Treatment with synbiotic improved clinical symptoms and histological score in treated patients with active CD. A significant reduction of TNFα expression in patient treated with synbiotic was observed after three months, however after six months, the reduction was no longer significant. A beneficial effect of synbiotic could also be observed in the significant increase of the level of bifidobacteria in treated patients.

4. Antibiotics

The use of antibiotics in the primary treatment of CD is again based on the theory of involvement of bacteria in the pathogenesis of the disease (Lal & Steinhart, 2006). CD could be caused by an aggressive immune response to antigens in the gut of genetically susceptible individuals (Prantera, 2009). Antibiotics in CD have been used for the nonspecific reduction of intestinal bacterial load. In clinical trials, several antibiotics were used for the treatment of CD (Prantera, 2009):

- antibiotics with antimycobacterial activity (clofazimin, clarithromycin, rifabutin)
- metronidazole (active against anaerobic bacteria)
- ciprofloxacin (active against *E. coli)*
- rifaximin.

Antibiotics have shown some effectiveness in the treatment of CD, mostly in reducing post-operative recurrence of CD. Their benefits however have to be balanced by the side effects they cause (Doherty et al., 2010).

4.1 Antimycobacterial therapy

The earlier described single pathogen hypothesis suggests *M. avium* subsp. *paratuberculosis* as a causative agent for CD. For that reason, antibiotics against mycobacteria have been tested in the treatment of CD. In 2007, a prospective, parallel, placebo-controlled, double-blind, randomized clinical trial in patients with active CD was reported. The authors used the combination of clarithromycin, rifabutin, and clofazimine for two years (Selby et al., 2007). They concluded that this combination of antibiotics against *M. paratuberculosis* showed no benefits in the treatment of CD. This could serve as another piece of evidence

against the single pathogen hypothesis with *M. avium* subsp. *paratubercolosis*. Nevertheless, further studies should be performed to confirm or disprove these findings.

4.2 Metronidazole and ciprofloxacin

Metronidazole and ciprofloxacin are the most popular antimicrobial drugs used in the treatment of CD. They exert antimicrobial activity against Gram negative and Gram positive bacteria (Dharmani & Chadee, 2008). Metronidazole is a nitromidazole antibiotic, and ciprofloxacin belongs to a group of fluoroquinolone antibiotics. Both antibiotics, alone or in combination, were used in several clinical trials for active CD treatment. Antibiotics have been effective in preventing the remission of CD; however their benefits have to be balanced by the side effects they cause. One of the reports describes the use of a combination of metronidazole and ciprofloxacin therapy in patients with active ileal or colonic CD. The therapy was well tolerated and showed beneficial effects with symptomatic improvement in patients with active CD, especially in the colon (Greenbloom et al., 1998). Only five of 72 patients required withdrawal of the therapy because of side effects. The authors speculated that only metronidazole is to be held responsible for side effects (e. g. neuropathy, nausea and anorexia), as those have already been reported in previous studies on metronidazole treatment of CD (Greenbloom et al., 1998; Prantera et al., 1996).

4.3 Rifaximin

Rifaximin is a rifamycin-based antibiotic with a broad spectrum of antimicrobial activity. This covers Gram positive and Gram negative bacteria, and includes both aerobic and anaerobic bacteria. Rifaximin is poorly absorbed from the gastrointestinal tract and therefore has less systemic effects. It has an excellent safety profile, minimal drug interaction, and negligible impact on the intestinal microbiome. For those reasons, it has a great potential in the treatment of gastrointestinal diseases, including active CD. Rifaximin is approved in more than 30 countries for a variety of gastrointestinal disorders, including the treatment of traveler's diarrhea, caused by noninvasive diarreagenic *E. coli* (Koo & DuPont, 2010).

In recent years, several studies have reported rifaximin effectiveness in the treatment of CD. In 2006, a multicenter, double blind, randomized, placebo-controlled study was reported. Rifaximin gastroresistant granules (800 mg once a day or 800 mg twice a day) were given orally to 83 patients with mildly to moderately active CD for 12 weeks (Prantera et al., 2006). Rifaximin gastroresistant granules were designed specifically for CD treatment. They were coated with a co-polymer, which was designed to by-pass the stomach and dissolve in the duodenum-jejunum, thereby concentrating the active rifaximin in the small intestine. In the study, the clinical remission was achieved in 52% of the group who received rifaximin twice a day, in 32% of the group who received rifaximin once a day, and in 33% of the group who received a placebo dose twice a day. In comparison to the placebo group, the rifaximin group was superior in inducing clinical remission of mildly to moderately active CD, although the difference was not statistically significant. Nevertheless, the results were encouraging and a larger randomized placebo-controlled study is justified.

In 2008, Shafran and Burgunder reported the treatment of five patients with newly diagnosed mild CD with 400 mg rifaximin dose twice daily for 3 months. This was the patients' first therapy without prior biologic or immunomodulatory treatment. The therapy was successful in 3 patients and substantial endoscopic and clinical improvements were observed. The authors suggested that rifaximin therapy could be an effective first-line treatment in patients with small intestinal CD (Shafran & Burgunder, 2008).

The same authors performed a retrospective analysis of 68 patients with CD, who were treated with rifaximin between 2001 and 2005. Patients had CD localized to the small or large intestine, and 56% of patients had previously undergone surgery. Most of the patients were treated with rifaximin dose 600 mg/day. Almost half of them were also receiving steroids, and some of them were also treated with anti-inflammatory agents, biologics (e.g. infliximab), antidiarrheal agents, and immunomodulators. The retrospective analysis showed that clinical remission was achieved in 65% of the patients with CD. Interestingly, this percentage was a little higher in patients who were not treated with steroids. Among the patients who received only rifaximin therapy, 67% achieved remission. These findings again substantiate the use of rifaximin alone for the induction of remission in CD (Shafran & Burgunder, 2010).

Several studies on the efficacy of rifaximin in CD have shown that it has a good potential in CD therapy, either as a first or second line treatment. Nevertheless, further research of rifaximin efficacy in patients with CD is required. Among other things, its precise mechanism of action in CD should be clarified.

4.4 Antibiotics in the prevention of post-operative CD

Antibiotics are most frequently applied for the prevention of post-operative recurrence of CD (Doherty et al., 2009; Doherty et al., 2010; Lal & Steinhart, 2006). Rutgeerts and co-workers published two double-blind randomized studies of the efficacy of nitroimidazole antibiotics in the prevention of postoperative CD (Rutgeerts et al., 2005; Rutgeerts et al., 1995). In the first study, one week after resection surgery, 30 patients started to receive antibiotic metronidazole at a dose of 20 mg/kg body weight once a day for a period of three months (Rutgeerts et al., 1995). Another 30 patients received placebo. Nine patients did not finish the study, two in the placebo group and seven in metronidazole group. The reasons for the latter patients were gastrointestinal intolerance, acute paranoia, polyneuropathy, suture leak, and the lack of compliance. After the end of the therapy, patients were observed every 6 month for the period of three years. After a 3 month therapy, 21 of 28 (75%) patients in the placebo group had recurrent lesion in neoterminal ileum, while this only happened to 12 of 23 (52%) patients in the metronidazole group. Metronidazole significantly reduced endoscopic recurrence, which was observed in 3 of 23 (13%) patients in metronidazole group and in 12 of 28 (43%) patients in the placebo group. Statistically significant reduction of clinical recurrence was observed only after one year. The reduction was no longer significant two and three years after surgery, but the benefit of metronidazole treatment was still present. This study showed that metronidazole was effective in preventing early severe recurrence of CD. However, several side effects occurred in the metronidazole group, including gastrointestinal intolerance, metallic taste, leucopenia, paraesthesias of the limbs, abnormal liver function, polyneuropathy, and psychosis. Because of high rates of adverse effects with metronidazole, a second study for the prevention of postoperative CD was performed with antibiotic ornidazole (Rutgeerts et al., 2005). Ornidazole is also a nitroimidazole antibiotic, but supposedly causes fewer side effects than metronidazole. 80 patients after ileocolonic resection were included. Half of the patients received 500 mg of ornidazole twice daily and half received a placebo for 12 months. After the end of the treatment, clinical recurrence manifested in 3 of 38 (7.8%) patients in the ornidazol group and in 15 of 40 (37.5%) patients in the placebo group. Additionally, the results of clinical recurrence were measured two and three years after surgery. After two years, 11 of 37

(29.7%) patients had clinical recurrence in the ornidazol group, and 18 of 40 (45%) patients in placebo group. After 3 years, 17 of 37 (45.9%) patients had clinical recurrence in the ornidazol group and 19 of 40 (45.5%) patients in the placebo group. Similar to metronidazol, ornidazol has shown efficacy in preventing postoperative clinical recurrence of CD, but has also caused numerous side effects. To alleviate these, the authors suggested the use of a smaller dose (500 mg/day) of ornidazol in postoperative therapy of CD.

When antibiotics are used for preventing postoperative recurrence of CD, they are often combined with other therapies for CD, including immunomodulators, probiotics, steroids, and others. The studies of combinations of antibiotics with probiotics were presented in the Chapter 2.2. The combination of antibiotic metronidazole and immunomodulator azathioprine was studied in a placebo-controlled randomized trial to prevent postoperative recurrence of CD (D'Haens et al., 2008). It was performed on 81 CD patients after curative ileal or ileocolonic resection. The first group of 40 patients received 500 mg metronidazole three times per day and 100-150 mg azathioprine once per day for a period of three months. The second group of 41 patients received metronidazole without azathioprine. After three months, the metronidazole treatment was discontinued, but the study continued for another nine months with azathioprine treatment only (the first group received azathioprine and the second group placebo). 32 patients from the first group and 29 patients from the placebo group reached the end of the study. Endoscopic recurrence was measured three and twelve months after the beginning of the study. After 3 months, significant endoscopic recurrence occurred in 12 of 35 (34.3%) patients in the first group, and in 20 of 38 (52.6%) patients in the placebo group. After twelve months, significant endoscopic recurrence occurred in 14 of 32 (43.7%) patients in the first group and in 20 of 29 (69%) patients in the placebo group. Also after twelve months, inflammatory lesions were not observed in seven patients in the first group and only in one patient in the placebo group. There was no clinical recurrence in patients after three months of surgery. Twelve months after surgery, 10 clinical recurrences were observed, three in the first group and seven in the placebo group. Some adverse effects occurred in both groups, but overall the treatment was well tolerated. It can be concluded that the combination of antibiotic with immunomodulator is superior to antibiotic alone in preventing postoperative recurrence of CD. Because of the shorter period of antibiotic treatment, the number of side effects in patients was lower than in previous studies. Nevertheless, further studies on larger number of patients are needed to support this approach.

5. Conclusion

Human intestinal bacteria are an important factor in human health, as well as in the pathogenesis of intestinal disorders, including CD. However, none of the proposed mechanisms of action can alone sufficiently explain the disease occurrence. Bacterial components are undoubtedly involved in the activation of abnormal autoimmune responses which further trigger the disease symptoms. The whole picture is however more complex with more active bacterial involvement.

Probiotics are an obvious choice for influencing the intestinal microbiome. Probiotics have shown some promising initial results in the treatment of CD. However, in general, they were not found to be superior to placebo in increasing the time of remission of CD or reducing post-operative recurrence in controlled clinical trials. Two notable exceptions

preserve hope for probiotics in the treatment of CD. Yeast *S. boulardii* was shown to be more effective than melsalazine in maintaining remission of CD (Guslandi et al., 2000) and improving intestinal permeability in CD patients (Van Gossum et al., 2007). Probiotic mixture VSL#3 was successful in the treatment of CD-induced arthralgia (Karimi et al., 2005) and in the prevention of postoperative recurrence of CD (Madsen et al., 2008). These studies offer support for the commonly asserted claim that further trials are justified.

There are several reasons that could explain the failure of other clinical trials and speak in favor of probiotics. The overall number of clinical trials is low, as well as the number of participants. Trials were designed in different ways, had different goals and measured different outcomes. Despite the abundance of bacteria with attributed probiotic properties, just a few were used in clinical trials. Additionally, there were differences in doses and regimens. Future trials should broaden the spectrum of probiotic bacteria in the treatment. Probiotics are not alike and exert effects by different mechanisms. The growing number of probiotic bacteria, however, clearly prevents systematic testing of all of them. To overcome this difficulty two strategies can be foreseen.

The first includes testing of larger combinations of bacterial strains. Individual bacteria in the mixture would have different effects and could well act synergistically. This view can be supported by the success of probiotic mixture VSL#3 relative to individual bacteria *Lb. rhamnosus* GG and *Lb. johnsonii* LA1. The ultimate example of that approach would be a fecal transplant from healthy donors to patients. This has been successfully applied in the treatment of *C. difficile* infection. It was also shown that the changes in the composition of a recipient's intestinal flora were long-lasting. Recipients' intestinal flora resembled the composition of the donor's flora even after 24 weeks (Grehan et al., 2010). This approach is not without risks, as transfer of potential pathogens is difficult to exclude.

The second approach would necessitate better understanding of the disease etiology and modes of action of various bacteria. This would require the use of better animal models of CD. The majority of the studies in mice were performed on models of intestinal inflammation which resemble that of ulcerative colitis, with inflammatory changes limited to the colon. Inflammation was achieved with chemicals or genetic knock-out, which only weakly reflect the origin and course of CD in humans. Two mouse models of CD have been described, TNF ΔARE and SAMP1/YitFc (Pizarro et al., 2003). They should be applied in the search of a single probiotic strain with the desired properties.

Recombinant probiotics have reasonable potential for the treatment of CD in the future. They combine the safety of probiotics with a defined mechanism of action. However, solution of the remaining safety concerns and further human clinical trials will be needed before they can be recommended.

Antibiotics constitute the most straightforward approach for interfering with the intestinal microbiome by lowering the bacterial content in a mostly unspecific fashion. The approach has shown clinical efficacy, but at a price of quite severe adverse effects. Rifaximin could assume greater importance in the future since it is a poorly absorbed, broad-spectrum antibiotic with fewer side effects.

6. Acknowledgment

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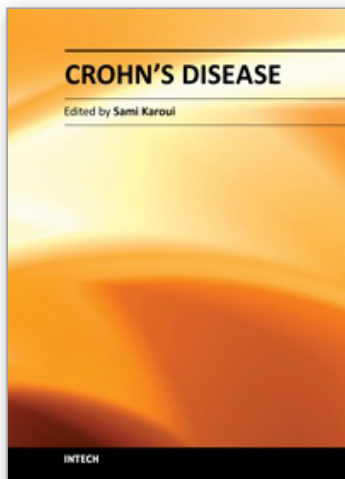
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In this book, several important points regarding Crohn's disease are discussed. In the first section, we focus on etiopathogeny of Crohn's disease and the recent advances in our overall understanding of the disease - specifically, the role of the gut epithelium, alterations of the epithelial crypts, and the roles of the different cytokines in the pathophysiology of Crohn's disease. In the second section, a diagnosis of Crohn's disease is discussed. Another particular area of focus is in the diagnosis of intestinal tuberculosis, and the role of mycobacterium avium in Crohn's disease. In the third and final section, the management of Crohn's disease is discussed, with a focus on recent evidence-based medicine recommendations.

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