

eBook on Inflammatory Bowel Disease

Chapter 2

The Gastrointestinal Disease to the Microbiota Treatment: From the Present to the Future

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1. Introduction

Chronic diseases, especially the inflammatory ones, share several characteristics to each other. In this chapter we will first discuss the main characteristics of inflammatory bowel diseases, afterwards the Metabolic Syndrome and associated diseases and, finally, intestinal mucositis that although is not a disease itself, produces an inflammatory process with serious problems related to the discontinuation of cancer treatment. Second, we will discuss the effects of probiotics on the control, prevention or attenuation of the symptoms of these diseases and, finally, we will discuss about the changes that these diseases cause in the microbiota, emphasizing the methods and results of the researches involving the microbiota and the next steps of scientific research to elucidate the mechanisms involved in the control of inflammatory processes through its manipulation.

2. Inflammatory Bowel Disease (IBD), Metabolic Syndrome and Intestinal Mucositis

2.1. IBD

Inflammatory Bowel Disease (IBD) is a term widely attributed to chronic and recurrent intestinal disorder characterized by severe inflammation which can lead to sometimes, irreversible impairment of the structure and function of the gastrointestinal tract (GIT). Several aspects such as the gut microbiota, external environmental factors, genetic predisposition and the host immunological responses have been related with the development of IBD [1,2]. Although the etiology is still unknown, scientific evidence that has been largely accepted to date indicates that IBD pathogenesis results from an anomalous immune response against the gut microbiota which is triggered by environmental factors in a genetically susceptible host [1,3].

IBD has been reported since the middle of the 20th century in the West and the emergence in developing countries in the last 25 years provides insight that this epidemiological shift is related to westernization of lifestyle and industrialization [4,5]. The IBDs worldwide incidence is increasing and still remains uncured, thus, they are considered a global public healthcare problem [6] once they reduce the quality of life, the capacity for work and increase disability of the population [7].

IBD is a heterogeneous disease with a wide range of phenotypic manifestations, being composed of two different disorders: Crohn's disease (CD) which can affect any part of the gastrointestinal tract and ulcerative colitis (UC) characterized by localized inflammation through the large intestine, which may lead to some complications such as stenosis and fistula [8,9].

Despite of the differences between CD and UC, distinguish and classify the IBDs could be a huge challenge, and it has been related to be critical for choosing the best clinical management [9,10]. The precise classification has many potential benefits, such as defining the disease's prognosis, properly advising the patient and deciding on the most appropriate form of treatment [11].

Considered a polygenic disorder, both CD and UC affect genetically susceptible individuals influenced by environmental factors and they are still poorly understood [12]. Studies with monozygotic twins have shown that they exhibit phenotypic concordance in 50-75% of CD patients, and a risk 800-fold greater of developing CD comparing to the remainder population [13]. On the other hand, phenotypic concordance of UC patients in monozygotic twins is less frequent (10-20%), indicating that genetic predisposition is stronger for CD than UC [13,14]. Nonetheless, the fact that genetic factors are responsible for only a part of the occurrence of these diseases provided compelling evidence that epigenetic factors can play

a crucial role in the development of IBD and explain the differences in disease expression in monozygotic twins [13,14].

2.2. Ulcerative colitis (UC)

Samuel Wilks (1824–1911) was the first to use the term “ulcerative colitis” in 1859 [15]. Nowadays, the UC is described as an idiopathic inflammatory disease that affects the colon section of the GIT, and causes a superficial continuous mucosal inflammation extending from the rectum to the proximal colon, commonly characterized by bloody diarrhea, tenesmus and abdominal pain with variable rates of relapse and remission [16] without sex predominance [7]. In addition, it has a bimodal age distribution afflicting adult aged 20–30 years with a second incidence peak between 50 and 80 years of age [17].

Even though the etiology of UC remains unclear, some evidence highlight the existence of an underlying autoimmune component [18,19]. Approximately 1/3 of patients with UC exhibit extraintestinal manifestations (EIM) involving multiple organs, sharing characteristics with other autoimmune diseases [20], and some of these manifestations could precede the development of colitis and the diagnosis of UC [21]. Furthermore, UC patients’ manifest elevated risk of developing colorectal cancer (CRC), and is estimated to be 2%, 8% and 18% after 10, 20 and 30 years of disease [22], respectively.

The UC diagnosis is performed by gathering data from clinical information, endoscopic biopsy, histological findings, and exclusion of other diagnoses and it is critical for choosing the appropriate treatment [20] which is made by medications, (thiopurines, corticosteroids and aminosalicylates) to control the inflammation, as well as to induce and maintain disease remission, improving quality of life and minimizing preventable related conditions, such as sexual functions, abdominal pain, unregulated defecation and the risk of colorectal cancer [23,24].

2.3. Crohn’s disease (CD)

CD was first fully described by Burrill B. Crohn (1884–1983), Leon Ginzburg (1898–1988) and Gordon D. Oppenheimer (1900–1974) in 1932 as a disease called “regional ileitis” because in studied cases the disease was limited to the terminal ileum [25]. The disease can affect any part of the gastrointestinal tract from mouth to anus, being defined as a transmural inflammation with complications such as strictures, abscesses and fistulas in a relapsing and remitting episodes [26, 27].

Despite of the CD be a worldwide affecting disease its prevalence and incidence in industrialized countries are higher than in developing ones. Nevertheless, in some developing countries of Asia and Africa the industrial development have increased the incidence of the

disease [28]. People of any age could be affected, from children to the elderly, but the incidence peak corresponds to people between 20 and 40 years old, with a smaller peak in adults aged between 50 to 60 years [27, 29], with women presenting a slight predominance (1.6:1) when compared to men disease cases [30].

CD diagnosis is usually a challenge based on a combination of symptoms, radiology, endoscopy, and histological criteria [31]. The conventional treatment is centered on strict control of diet associated to pharmacological therapy focused on corticosteroids and immunomodulatory drugs [32,33]. Surgical resection either obstructed areas or severely affected portions of the intestine is recommended for patients who have developed complications or who do not tolerate drug therapy [27]. Unluckily, the approach is not always completely effective and can lead to a fearful and irreversible “short bowel syndrome” [34]. However, recent advances in surgical intervention with techniques minimally invasive enables patients to faster recovery, remaining less time in hospitals with significant short and long-term patient benefits [35].

2.4. Metabolic Syndrome

Metabolic syndrome (MS) can be defined as a set of abnormalities, including glucose intolerance, hyperinsulinemia, hypertension as well as dyslipidemia with high concentrations of triacylglycerols and LDL and also with reduction in HDL levels [36,37]. Its diagnosis is important since MS triggers proinflammatory and prothrombotic processes [38]. These syndromes, such as insulin resistance and hypertension, are very often associated with abdominal fat accumulation and obesity [39-41].

According to National Centers for Environmental Prediction (NCEP) criteria, the prevalence of MS in the United States, increases from 6.7% in the population between 20 and 29 years to 43.5% in the population between 60 and 69 years old [42]. In addition, the increase in the number of cases of obesity and diabetes mellitus observed at the global level, led to an increase in the number of people with MS [36]. MS does not have a well-defined etiology, nonetheless there are certain characteristics and life habits that could contribute to its development as unbalanced diet, rich in refined carbohydrates with saturated fats and low in dietary fiber that leads to an increase in weight, lack of physical activity, smoking and also genetic predisposition [43]. Thus, overweight is considered the hallmark characteristic in people with MS [44-46].

The pathophysiology of MS is related to the effects of insulin resistance on the body. One of the factors most strongly associated with its development is the excess of free fatty acids in the circulation [47] that occurs in cases of obesity, since the deposits of triacylglycerols in the viscera presents a more accelerated turnover, and the presence of glucose in the portal system stimulates gluconeogenesis and increases glycemia and inhibits insulin clearance by the liver, causing hyperinsulinemia [36,47].

Metabolic disorders such as type 2 diabetes and obesity, as well as immune disorders such as IBDs are considered chronic diseases. In general, these diseases share common characteristics from the pathological point of view, since metabolic disorders present strong inflammatory responses and the inflammatory process is associated with several metabolic alterations. Since the early 2000s, these diseases constitute a public health problem at global level [48] and the increase in their prevalence can be directly associated with changes in the life habits of the general population [49-51].

The inflammatory process observed in MS is directly associated with increased oxidative stress. The reactive oxygen species (ROS) are capable of mediating symptoms of diabetes mellitus, such as the insulin and the decrease of its secretion, serving as precursors for the formation of LDLox (oxidized low-density lipoproteins), responsible for a large part of the development of atherosclerotic lesions, and the increase in circulating cholesterol fractions and glucose [52,53]. In addition, chronic diseases are directly related to changes in the intestinal microbiome [54,55] and they are also associated with elevated circulating levels of proinflammatory cytokines such as TNF and IL-6 [56].

2.5. Intestinal Mucositis

The GIT is directly affected by radiotherapy and chemotherapy during cancer treatment, being the intestinal mucositis one of the most frequent side effects caused by these agents. The 5-FU (5-Fluorouracil) and Irinotecan (CPT-11) are the most anti-tumor agents used in cancer treatment able to induce mucositis as a side effect [57-60].

The mucositis occurs due to non-selectivity of these drugs that, besides to destroy neoplastic cells, promote damage to healthy cells. The 5-FU causes cytotoxic damage mainly to the cells of the small intestine (duodenum, jejunum and ileum) by Thymidylate Synthase enzyme inactivation (essential for the synthesis of nucleotides) and, the incorporation of its metabolites into DNA/RNA of epithelial stem cells causes the inhibition on their function, intestinal cell proliferation and differentiation in enterocytes, Goblet cells and Paneth cells [61,62].

The prodrug irinotecan (CPT-11) inhibits the intestinal cells proliferation due to its active and toxic metabolite, the 7-ethyl-10-hydroxycamptothecin (SN-38), being capable to inactivate the DNA Topoisomerase I, a nuclear enzyme which relaxes torsionally strained DNA [59,63], leading to a replication blockage and activating the endonucleases that trigger DNA fragmentation and cellular apoptosis.

Intestinal mucositis is one of the most relevant gastrointestinal inflammatory conditions in humans, being a serious clinical issue. This gastrointestinal disorder is characterized by inflammation and alteration in intestinal epithelium architecture, such as cellular loss of

intestinal epithelial barrier and villi shortening, which reduces nutrients and water absorption [61, 64-66]. It is also observed polymorphonuclear cells infiltration (neutrophils, eosinophils and macrophages) [67-69], intestinal microbiota composition alteration [64,70,71] and intestinal permeability increased by rupture of the *tight junctions* proteins (paracellular pathway) [70,72]. These changes lead to a systemic translocation of harmful bacteria colonizing the gut, leading to secondary infections and promoting clinical debilitating symptoms such as diarrhea, abdominal pain, bleeding, fatigue, malnutrition, dehydration, electrolyte imbalance and infections [62,73], which affect the life quality of the patient and compromise both the duration and the efficacy of the treatment.

The pathology of mucositis involves a sequence of biological events, described by Sonis [62] in five phases: initiation, primary damage response, signal amplification, ulceration and healing.

The initiation phase of mucositis induced by 5-FU occurs right after administration of the drug, which after being metabolized is incorporated into the DNA/RNA molecules promoting structural changes and reactive oxygen species (ROS) production, consequently culminating in cell damage and death [59,62]. The initiation phase of irinotecan-induced mucositis is suggested to be related to the pharmacokinetics of the drug, that after metabolized is hydrolyzed by hepatic carboxylesterases to form SN-38. The drug or its metabolite SN-38 binds to topoisomerase I, forming a cleavable complex that causes the DNA damage and apoptosis [60].

These early damage lead to the activation of a number of pathways (primary response damage phase) involved in apoptotic death, oxidative stress, and inflammatory responses, such as the nuclear Kappa- β (NF- $\kappa\beta$) pathway [74]. This pathway is related to the activation of several inflammatory mediators, such as the IL-8, TNF- α , IL-6 and IL-1 β cytokines, COX-2 chemokine and iNOS enzyme, which play an important role in mucosal toxicity [70,75].

The presence of these pro-inflammatory mediators in intestinal mucosa, besides causing tissue damage, indirectly act on signal amplification by a positive feedback mechanism, activating pathways that increase the higher inflammatory mediators production, as well as oxidative stress. Consequently, the increase in production of these mediators initiates a cascade of inflammatory reactions, leading to matrix metalloproteinases activation, whose production culminates in additional tissue damage, exacerbating the lesion [62].

The progressive destruction of mucosal integrity culminates in ulceration phase. This is the most clinically significant phase due to deep and symptomatic lesions development, that are prone to pathogenic bacterial colonization and translocation, due to increased intestinal permeability [59,68,70]. These infections further stimulate the local inflammatory environment by metabolic products produced by colonizing microorganism and/or through

polymorphonuclear cells infiltration such as neutrophils, eosinophils and macrophages, extending mucosal damage [62].

Finally, about 3-4 days after the end of the chemotherapy treatment, there are a significant cell proliferation and differentiation, which leads to spontaneous mucosal restoration (**healing phase**) [62,76].

3. Probiotics Effects on IBD, MS and Intestinal Mucositis: an overview

The human GIT is highly colonized by bacterial communities [77], which live in a symbiotic relationship with the host in normal conditions. This homeostasis provides to the host a nutrient-rich habitat where microorganisms can play various beneficial functions, such as pathogen exclusion, production of essential metabolites (biotin, short chain fatty acids (SCFAs), vitamins [78-80], and contribute to the enteric and systemic immune system development and modulation [81].

Irritable bowel syndrome (IBS), colon cancer and IBD could be caused or aggravated by alterations in the intestinal microbiota [82]. This complex intestinal microbiome is highly influenced by diet, lifestyle, exposure to toxins and use of antibiotics, establishing a close relation between disease, health, immune system and modification in the microbiota [83]. Due to probiotics being capable to promote benefits to the host, several studies have related their use in prevention and treatment of several diseases, including inflammatory disorders [84].

Probiotic is a word derived from the Greek that meaning “for life.” The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) define probiotics as “Live microorganisms which when administered in adequate amounts confer a health benefit on the host” [85]. Their mechanism of action is still unclear. However, it is believed that the administration of probiotic may restore the homeostasis of the microbiota, raise levels of anti-inflammatory cytokines, adjust of immune response, increase the amount of antimicrobial products and improve gut physical barriers across increased epithelial junctions, intestinal permeability modification, and modulation of mucin production by the mucosa [86,87].

Studies related to the probiotics mechanisms shown that the lineages present high plasticity of mechanisms, as: i) promote the competitive adhesion to the mucosa and epithelium [88]; ii) regulation of the lymphoid immune system present in the intestine, either through cell recognition receptors or by the release of immunomodulatory peptides or metabolites by intestinal cells [89]; iii) reduction of lipid absorption and caloric intake by deconjugation of bile acids [90]; iv) induction of lipolysis [91]; v) induction of transcriptional activation of genes related to β -oxidation in the liver and muscle [92,93]; vi) improvement in glucose tolerance and insulin sensitivity by the production of SCFA and reduction of Lipopolysaccharides (LPS)

translocation [94-96]; vii) improvement the intestinal barrier function by the immunomodulation of intestinal immune cells [97]; viii) appetite regulation [98], among others.

Bennet and Brinkman were one of the first researchers to report an experiment correlation between the intestinal microbiota in the UC pathogenesis and the use of a treatment with replacing the colonic microbiota of a patient with UC with a microbiota from healthy donor; their results successfully induced the disease remission for at least 6 months without any medication [99]. Afterward, several studies were carried out using the probiotic intervention, varying the lineages, doses, duration and design of the experiments [100,101].

A combination treatment using oral gentamicin for 1-week cycle followed by non-pathogenic *E. coli* Nissle 1917 supplementation shown as effective as standard medication with mesalazine in preserving remission after a crisis of UC. No difference in the remission and relapse rates were observed, thus these results suggested that the relapsing course of UC is linked to the microbiota [102].

The VSL#3 probiotic preparation that has eight different probiotic bacteria, being 4 strains of *Lactobacillus* (*L. casei*, *L. plantarum*, *L. acidophilus*, *L. delbrueckii subsp. bulgaricus*), 3 strains of bifidobacteria (*B. longim*, *B. breve*, and *B. infantis*) and one of *Streptococcus salivarius subsp. Thermophiles* has been subject of many studies [103-106]. In a double-blind randomized controlled trial who underwent total proctocolectomy with ileal pouch-anal anastomosis (IPAA) for the management of UC, the oral administration of VSL#3 was evaluated. This treatment improved the IBD questionnaire score, in counterpoint with placebo group and it also was effective in the prevention of the onset of acute pouchitis [107]. This probiotic mixture was also tested in a 1-year pediatric study, double-blind, randomized, placebo-controlled trial. This study suggested either the efficacy and safety of this mixture, as well as the induction and maintenance of remission in children with active UC [108].

The 37,5% of Crohn's disease patients treated only with mesalamine in clinical remission manifested clinical relapses. However, patients treated with mesalamine plus the probiotic agent *Saccharomyces boulardii* presented a significative reduction in clinical relapses (6.25%) [109]. This probiotic was also able to improve the intestinal permeability in CD patients [110,111]. The *L. rhamnosus* GG administration shown improvement in the gut barrier function and clinical status in children with mildly to moderately active stable CD [112].

Our research group, in 2014, related the probiotic effect of *L. delbrueckii subsp. Lactis* CNRZ327 (Lb CNRZ327), a *Lactobacillus* strain isolated from cheese, either *in vitro* and *in vivo* anti-inflammatory effects. This dairy bacterium may be useful in the treatment or prevention of IBD (113) since was able to inhibit, *in vitro*, the TNF- α -induced by NF- κ B activation in intestinal epithelial cells by reducing I κ B phosphorylation and also attenuated the symptoms in a mouse colitis model induced by dextran sodium sulfate (DSS) [113].

An *in vitro* study analyzing the immunomodulatory effects of three *L. lactis* strains (NZ9000, MG1363 and NCDO2118) showed the potential anti-inflammatory effect of *L. lactis* NCDO 2118 since its strain was able to reduce IL-1 β -induced IL-8 secretion in Caco-2 cells [114]. Due to interesting *in vitro* results, the *L. lactis* NCDO 2118 strain was administered to C57BL/6 mice during the remission period of colitis induced by DSS. The oral treatment resulted in a slighter form of recurrent colitis, effect shown by the early increase in IL-6 and continuous IL-10 and Treg (CD4⁺) production in colonic tissue [114]. Another study performed by Souza and coworkers (2016) showed that preventive oral administration of *Escherichia coli* strain Nissle 1917 (EcN) in a murine model of colitis and the transplantation of fecal microbiota containing EcN in germ-free mice improves DSS-induced colitis by modifying inflammatory responsiveness to this agent.

The studies reported reinforce the idea that the intestinal microbiota plays an important role in triggering inflammatory bowel diseases and therefore are attractive targets for the control and treatment of these illnesses. However further studies are still needed to better elucidate the mechanism of action of probiotics and ensure their efficacy and safety [115].

Although the MS is developed due to different factors, such as life habits, genetic inheritance, among others, researches reveals that abdominal fat alone is not the predominant factor for the development of associated diseases of the metabolic syndrome, but rather than by an association between the inflammatory process observed in adipose tissue with a local metabolic dysfunction. This process was called metaflammation which modify the intestinal permeability allowing the translocation of bacterial proinflammatory components, such as lipopolysaccharides, to other tissues, leading to the development of insulin resistance in addition to the release of different inflammatory mediators by adipose tissue [116,117].

Among the environmental variables related to the development of comorbidities, the microbiota has been reported as one of a major impact factor [118].

Thus, to understand the microbiota compositions and behavior in these patients becomes essential to clarify the pathogenesis mechanisms, as well as to outline new treatment strategies [119]. Studies have shown the importance of the presence of the next generation probiotics (*F. prausnitzii*, *A. muciniphila* or *Clostridium* lineages), since low quantities of them developed an increase in the risk of developing immunometabolic diseases [120].

The probiotic use in attenuating symptoms of different inflammatory diseases is widely reported in the literature. Between commercial probiotics studied for treatment of these diseases, only a few products have been extensively tested in clinical trials in patients with MS, in order to demonstrate a palpable effect on weight loss, lipid metabolism, and reduction of inflammatory markers. Thus, it is suggested that the action of probiotics is species and lineage dependent which would explain the fact that probiotic treatment is effective for certain

diseases and not for others [121].

Performed studies with *Lactobacillus* strains shown the ability of this probiotic strains in reduction the lipid's accumulation in adipose tissues, as well as in induction of subexpression of lipogenic genes [122, 123]. Animals that received diets with high concentrations of lipids and then treated with *L. gasseri* SBT2050 had shown lower intestinal permeability and bacterial translocation, as well as reduction of inflammatory parameters, suggesting that this strain improves the intestinal barrier function [124-127]. In addition, *L. gasseri* BRN17 was studied to treat animals with MS caused by the carbohydrate-rich diets consumption. This strain reduced the accumulation of adipose tissue in mice, and it has a beneficial effect on weight loss [128-130]. An important approach done with associated probiotics (*Bifidobacteria*, *Lactobacilus* and *S. thermophilus*) for treatment of overweight patients showed an improvement in lipid profile, as well as insulin sensitivity [131].

Preclinical studies also demonstrate that the microbiota is directly related to the brain-gut axis either through the synthesis of SCFA or specific molecules that regulate both food intake and energy expenditure [132, 133]. In spite of promising results are being reported in the literature, deeper investigations need to be conducted in order to elucidate the best dose-response, as well as clarify if the beneficial effect becomes persistent or whether supplementation with these strains should be continuous to ensure treatment efficacy.

The alteration of the intestinal microbiota also has relevant role in intestinal mucositis progression [70, 134, 135]. Thus, the modulation of the microbiota in patients during cancer treatment, through the oral administration of probiotic bacteria, possibility a promising therapeutic strategy to minimize the symptoms of chemotherapy-induced mucositis. In this context, several studies conducted with probiotic microorganisms have demonstrated strain-dependent effects for prevention/treatment of experimental mucositis induced-chemotherapies.

Bifidobacterium infantis (1×10^9 CFU) administration improved the body weight, villus height, increased expression of proliferating cell nuclear antigen (PCNA), reduced expression of NF- κ B, pro-inflammatory factors (IL-1 β and TNF- α) and neutrophils infiltration in 5-FU inflamed animals (150mg/kg), being effective in reducing the symptoms of chemotherapy-induced intestinal mucositis in rats [136]. The protective effect of this strain was also investigated in a synergic colorectal cancer treatment model 5-FU (75mg/kg/3days)/Oxaliplatin (8mg/kg/3days), where was observed ameliorating the mucosal damage, decreasing in the Th1 and Th17 and increasing CD4⁺ CD25⁺ Foxp3⁺Tregs response [137].

Bifidobacterium bifidum G9-1 (10^9 CFU) also has ameliorative effect against 5-FU-induced intestinal mucositis (50mg/Kg/6 days). This probiotic prevented the reduction of small intestine length and body weight loss, attenuated also the shortening of villi and loss of

Goblet cells in the crypts, as well as neutrophils infiltration. The daily administration of this probiotic was able to reduce the pro-inflammatory factors such TNF- α and IL-1 β and inhibited significantly the effect of the 5-FU in the changes of microbiota composition, inducing either the decrease and the increase in the Firmicutes in the Bacteroidetes abundance respectively [138].

Saccharomyces boulardii (16×10^9 CFU) and *Lactobacillus acidophilus* (16×10^9 CFU) were able to reduce the inflammation and dysfunction of the gastrointestinal tract in intestinal mucositis induced by 5-FU (450mg/Kg). Both bacteria reduced significantly the concentration of pro-inflammatory cytokines such as TNF- α and IL-1 β , CXCL-8, CXCL-1 and neutrophils infiltration. Besides, these probiotics ameliorate the villus/crypt ratio, reduce delay in gastric emptying and increase the glutathione concentrations [139,140].

Yeung et al. [141] demonstrated that *Lactobacillus casei* variety *rhamnosus* (Lcr35) (1×10^7 CFU) or *Lactobacillus acidophilus* plus *Bifidobacterium bifidum* (LaBi) (1×10^7 CFU) attenuate the severity of intestinal mucositis induced by 5-FU treatment (30 mg/Kg/5 days) through the inhibition of proinflammatory cytokines such as TNF- α , IL-6, IL-1 β and IFN- γ , restoration of villus/crypt ratio and less Goblet cell degeneration. Thus, they were able to ameliorate the chemotherapy-induced intestinal mucositis. The protective effect of Lcr35 (1×10^7 CFU) also was demonstrated in FOLFOX (30 mg/Kg 5-FU/5 days; 10mg/kg Leucovorin/5 days and 1mg/Kg Oxaliplatin/5 days) chemotherapy regimen-induced intestinal injury in a syngeneic colorectal cancer model. The oral Lcr35 administration significantly attenuated diarrhea and improved diarrhea scores, restored the villus height-to-crypt depth ratio, decreased NF- κ B activity in the intestine and ameliorated mucositis by inhibition of the expression of pro-inflammatory cytokines (TNF- α , IFN- γ , IL-1 β and IL-6). Microbiota analyzes shown the capacity of this bacterium in regulation the gut microbiota composition, decreasing Firmicutes and increasing Bacteroidetes abundance [142].

The administration of DM#1 mixture, which contain *Bifidobacterium breve* DM8310, *Lactobacillus acidophilus* DM8302, *Lactobacillus casei* DM8121 and *Streptococcus thermophilus* DM8309 bacteria, shown the reduction of neutrophil infiltration, proinflammatory cytokine levels (IL-4, IL-6 and TNF- α) and intestinal permeability; it was also reported the restauration of the epithelium architecture and the homeostasis of mice which intestinal mucositis (5-FU 30mg/kg/5 days) [143].

Recently, the protective effect of probiotics against mucosal damage induced by 5-FU chemotherapy has been characterized in fermented products. These products serve as important delivery vehicles for probiotic bacteria, consequently create a very promising protective matrices for these bacteria, once it contributes to the survival and viability of probiotics during the passage through the gastrointestinal tract, and enhancing its therapeutics effects [144,145].

Using this approach Oh et al (2017) demonstrated that Mulberry leaf extract fermented with *Lactobacillus acidophilus* A4 (10^9 CFU) was effective at reducing the severity of intestinal mucositis caused 5-FU (150mg/kg). This treatment stimulated MUC2 and MUC5AC gene expression and mucin production with reduced IL-1 β expression and neutrophil infiltration in intestine epithelium [146].

Two studies were performed administrating fermented milk in a 5-FU mucositis mice model (300mg/kg). One used *Lactobacillus casei* BL23 and/or *Propionibacterium freudenreichii* CIRM-BIA138 plus 30% of whey protein isolate supplementation [147], and the other study used *Lactobacillus delbrueckii* CIDCA 133 (7.5×10^7 CFU) [68]. Both reports shown, respectively, the good effect of the fermented milk by the preservation of the intestinal epithelium architecture with prevention in the degeneration of Goblet cells [68,147], reduced polymorphonuclear cells infiltration (neutrophils and eosinophils), reduction in intestinal IgA secretion and in the intestinal permeability in the small bowel of inflamed animals [68].

The immunomodulatory/regulatory effects of probiotics against mucosal damage induced by chemotherapy drugs also has been enhanced with the use of prebiotic. The prebiotic compounds stimulate growth, activating metabolism and promote protection of bacteria beneficial to the host organism [148]. The oral administration of a symbiotic (Simbioflora[®]) (10^9 CFU) preparation containing *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus* and *Bifidobacterium lactis* plus 5,5g of fructooligosaccharide prebiotic (FOS) was able to attenuate body weight loss and increasing of intestinal permeability in mucositis model. The authors also demonstrated an increase in the intestinal mucus layer and production of extracellular factors as SCFA (acetate and butyrate), that could contribute to their immunomodulating activity and mucosal ulceration attenuation in small intestine of inflamed mice [149].

The probiotic-mixture (3.0×10^8 CFU) consisting of *S. thermophilus*, four strains of lactobacilli (*L. delbrueckii*, *L. casei*, *L. acidophilus*, and *L. plantarum*) and three species of Bifidobacterium (*B. longum*, *B. infantis* and *B. breve*) was effective in ameliorating mucositis through prevention of irinotecan (225mg/Kg)-induced diarrhea. VSL#3 was also able to prevent excess mucin secretion, to ameliorate the chemotherapy-induced weight loss, and to reduce intestinal apoptosis in intestinal crypts [150].

The administration of probiotics formulation, Colon Dophilus[™] (10×10^9 CFU) to 46 patients with colorectal cancer treated with irinotecan led to an incidence reduction both in severe diarrhea and in enterocolitis. This formulation contained lyophilized probiotic strains of *Bifidobacterium breve*, *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Streptococcus thermophilus*, *Lactobacillus brevis* and *Bifidobacterium infantis* enriched with

the prebiotic inulin, maltodextrin, magnesium stearate and ascorbic acid, proving to be safe and effective in reducing the severity of gastrointestinal toxicity caused by this chemotherapeutic agent [151].

In addition, it was demonstrated that the post-treatment with viable *Saccharomyces cerevisiae* UFMG A-905 (Sc-905) was able to protect mice against the damage caused by CPT-11 chemotherapy (75mg/kg/day during 3 days); reducing the weight loss, decreasing the intestinal permeability and jejunal lesions (villous shortening) was reported. Besides, this probiotic was capable to reduce the oxidative stress, prevented the decrease of goblet cells and stimulated the replication of cells in the intestinal crypts of mice with experimental Mucositis [152].

Recently, a study using the selenium-enriched *Bifidobacterium longum* (0.6 mg/Kg, 5×10^8 CFU) strain prevented irinotecan (75 mg/Kg/day, during 4 days)-induced intestinal mucositis in mice. The protective effect was related to decreased mortality of animals, weight loss and inflammation reduction, severity of diarrhea decreasing, intestinal shortening prevention and cytokines downregulation (TNF- α and IL-1 β) [153].

According to the above studies, the mechanisms related to the protective effects of probiotics in 5-FU-induced intestinal mucositis can be related to prevent pro-inflammatory cytokines production by inhibition of the NF- κ B pathway and the restoration of the Th17/Treg cells balance, inhibition oxidative stress due production of anti-oxidant compounds, immunomodulating activity by extracellular factors (e.g. butyrate and acetate) and microbiota regulation.

4. The Gut Microbiota: What We Already Know and What are the Next steps?

Approximately 100 trillion of bacteria live in a symbiotic relationship with the host in the human intestine [154,155] and plays a crucial role in the function and conservation of the gastrointestinal tract's health [156]. These bacteria are known as a 'metabolically active organ' has an active role in intestinal physiology and have effects in many host functions [157]. The primary colonization of the gut starts in the utero by the umbilical cord and placenta able to introduce maternal microbes to the fetus [158]. Moreover, the most important source of inoculum is the infant delivery (vaginal and cesarean) [159], added up maternal nutrition.

Enterococcus, *Streptococcus*, *Staphylococcus* and *Propionibacterium* are the firstly genera introduced into the newborn organism [160]. As during the firstly month the infant food is only milk, *Bifidobacteria* is the genera which can be abundant, highly adapted to process milk oligosaccharides [161]. The solid foods introduction changes the infant microbiota towards adult microbiota by the end of the first 3-5 years of life. The composition of gut microbiota is not stable, its change over time. Intestinal microbiome is more flexible in infancy and early

childhood, acquiring stability and similarity to a general population in adulthood, in the elderly the diversity is lower [162, 163]. Thus, the microbe's colonization of infant gastrointestinal is an indispensable process since the close relation between microbiota and host have relevant impact and also influence on health and disease of individuals.

The diversity in gut microbial has a fast boom during the infancy with bacteria, archaea, viruses and fungi [164]. The interpersonal variation between infant gut microbiomes could be caused by differences in immune responses to the colonizing microbes, random colonization events, changes in host behavior, as well as other aspects of host lifestyle [165,166]. Furthermore, its interpersonal variation in gut microbial diversity is greater between infants than between adults [167]. The enterotype (complete community structure) differs between individuals based on genetics, diet, body mass index, environmental and lifestyle factors, and demographic regions of living [168].

The gut colonization is very important, generally in early life, its required for full development and maturation of the immune system showing a principal role in its development and host tolerance to antigens, and it is also fundamental for an inflammatory response regardless of the stimulus. T regulatory cells (Tregs) has an essential role in maintaining the gastrointestinal homeostasis by suppression of responses to pathogenic bacteria [169] and food antigens [170]. Toll-like receptors signaling is one of the mechanisms suggested to keep the intestinal microbiota regulated [171]. The microbiota engages physiological functions, particularly metabolism, neurological and cognitive functions, as well as hematopoiesis, inflammation and immunity [172,173]. Other important role of microbiome is contributing to gut epithelial cell renewal and enteric immune system development [174].

The massive composition of microbiota is commensal bacteria, which have been difficult to culture, limiting their understanding. Nowadays, these limitations have been overcome by arrival of metagenomic sequencing approaches. These new techniques which associate the next generation sequencing of DNA with computational analysis either targeted, 16S rRNA hypervariable regions (V3, V4) or whole-genome with shotgun sequence reads have cited the diversity and abundance of microbes at different body sites in a culture-independent method [175,176].

The α and β diversity are two metrics from environmental microbial ecology useful to describe the complexity of microbiota; α diversity describes the richness (e.g., number of organisms and equality of distribution of them) in a specific sample, while β diversity defines the extent of absolute or relative overlap in shared taxa between samples [177].

Firmicutes (~65%) and Bacteroidetes (~25%) are the two predominate phyla in the gut microbiome of healthy human; the remaining bacteria species are distributed between the phyla Actinobacteria (e.g., *Bifidobacterium* spp.), Proteobacteria (e.g., *Escherichia coli*) and

Verrucomicrobia (e.g, *Akkermansia muciniphilia*), with a smaller presence of Fusobacteria and Cyanobacteria [178]. Archaea, fungi (e.g. mycobiota) and viruses (e.g. virome) also inhabit the human intestinal tract [179]. As the Bacteroidetes are involved in the breakdown of complex plant polysaccharides their metabolic activities could increase either directly or indirectly the SCFAs production [180]. Thus, diet rich in plant derivatives promotes a microbial community structure and metabolite production that is beneficial to the human host [164]. The understanding of the assembly, as well as the community composition of the microbiota is very useful due to the microbiome is involved in human health [181]. Many chronic pathologies are extremely related to gut microbiota either negatively or positively [181-184].

Throughout the GIT the species and the number of microorganisms is different, and each region of this organ has its own distinct microbiota [185], with specific functions, as protection against pathogenic microorganisms, metabolism of intestinal mucins, pancreatic enzymes, bilirubin and fatty acids production [186].

4.1. The Microbiota in Inflammation Bowel Disease (IBD)

Advances in metagenomics and metabolomics have shown the importance of considering and understanding the functional characteristics of the intestinal microbiome in IBD. Individuals with IBD-related genes are more probable to display related microbiome modifications, although not showing phenotypic IBD characteristics [187]. The commensal microbiota besides to maintain homeostasis, protects against diseases which contributes in the maturation of immune system; thus, its instability in initial steps of life could have effect on immune disturb and, consequently, contribute to microbe-induced immunopathologies in adulthood [188].

There are compelling evidences about the extremely connection between intestinal microbiota and the pathogenesis of Crohn's disease (CD) and Ulcerative Colitis (UC). However, it's unclear if the tissue injury is the result of an abnormal immune response to a normal microbiota or a normal immune response which reacts to an abnormal microbiota. Both issues have been related, and it was reported that immune deficits can change the microbiota toward one with a colitogenic capacity; therefore, the gut microbiota provides an environmental risk factor for inflammatory bowel disease in susceptible individual [189]. Thus, the question of whether gut dysbiosis observed in IBD is a cause or consequence of the disease remains unresolved.

The complex ecosystem between human host and gut microbes offers a symbiotic relationship able to protect against pathogens invasion due to the nutrient competition, as well as epithelial binding site [190] besides fermenting indigestible food substances [191]

Intestinal dysbiosis may contribute to the pathogenesis of IBD by loss of "health-

promoting” or potential gain of “pathobionts” (microorganisms which become pathologic in the scenery of a specific environmental stimulus, for example individuals genetically susceptible) [81]. The microbiota balance could be affected by factors like host genetics, antibiotic treatment, intestinal inflammation or diet. Both dietary and bacterial antigens are the most common kinds of luminal antigens, thus, intestinal inflammation could be started by an abnormal response to the gastrointestinal microbiota and consequently dietary constituents, as well as their metabolites could alter the mucosal barrier function [192-194].

IBD are highly related with modifications in the gut microbiome. Researches shown that not any a single microorganism is involved in the gut dysbiosis. In IBD, the typical microbiome changes including a significant decrease in Bacteroidetes and Firmicutes genera [195,196], besides a specific reduction in microorganisms with anti-inflammatory proprieties such as *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii* [197] with full *Enterobacteriaceae* enrichment [195, 198] and also a reduction in overall alpha bacterial diversity, consequently in IBD patients existed a disbalance between protective and harmful intestinal bacteria.

The gut microbiota of these individuals has huge interindividual variations, being difficult to find specific biomarkers to diagnose the IBD. Nevertheless, the absence of specific taxon in the intestinal microbiome could serve as biomarker for this disease [199]. The DNA sequencing could contribute to deduce the microorganism’s abundance, even though taxon abundance does not exactly correlates with metabolic activity. Small changes in DNA relative abundance in these organisms may reveal significant impact on disease imposed by large changes in metabolic activity. Consequently, important attention must also be given to metagenomics and metatranscriptomics approaches because in spite of some taxon to be appear elevated in abundance, their metabolic activity may show a different trend [200]. Even more, some taxon well represented by their relative abundance in the metagenomics data were closely undetectable in the metatranscriptomics data [201].

Fungi, archaea, and viruses are important microorganisms to be point out in gut microbiome of IBD patients; as in bacteria, some recent reports, shown the closely relation existing between fungal and virome diversity in IBD. The new sequencing technologies open broad pathway to evaluate these overlooked microorganisms in the IBD gut microbiome [199]. Thus, the complex network of interactions between host-microbiota, bacteria-bacteria, and inter-kingdom must be kept to avoid the generation of any dysbiosis able to generate substantial impact on homeostasis.

Host genetics, immunology, environmental factors (age, diet, antibiotic exposure), and the gut microbiome are some aspects involved in IBD development. As IBD presents quiescent disease interspersed with outbreaks of disease activity current operational approaches focus

on reducing the inflammatory process in patients with active disease, and trying to preserve remission in individuals with dormant/inactive disease by using drug therapy [16,202] which could associate with adverse events [203]. To counteract this fact, the possible handling of the enteric microbiota, could be interest as a new field to be explored.

Dietary interventions including the exclusive enteral nutrition (EEN) are very effective inducing remission in children and adolescents with active Crohn's disease in 80-85% of patients [204, 205]. This approach has impact on the mainly components of the IBD paradigm: intestinal microbiome, mucosal integrity and the immune system. The modulation of intestinal microbiome might include the use of probiotics, prebiotics, antibiotics, as well as the Fecal Microbial Transplantation (FMT). However, the permanent change of the microbiome requires continuous supplementation of these therapies [206].

Antibiotics with broad spectrum have been explored as a primary therapy. They are able to decrease the luminal bacteria concentration, producing changes in microbial composition promoting the beneficial bacteria and decreasing bacterial tissue invasion [207].

The probiotic uses as IBD treatment have been extensively studied also in induction and maintenance of remission, either by wild type or recombinant strains [113, 115, 208, 209]. However, further studies are required to elucidate the anti-inflammatory mechanism of probiotics in IBD [3]. Prebiotics as Inulin and FOS are substances able to change the metabolome of the intestinal microbiota, promoting the growing of beneficial microorganisms as *Bifidobacterium* and *Lactobacillus* spp. [210]. FOS, specifically, induces immunoregulatory dendritic cell (DC) responses which are able to reduce disease activity in patients with Crohn's disease [211]. The production of SCFA as acetate, propionate and butyrate is performed by colonic bacteria from fermentable fiber. SCFA are able to increase gene expression, histone acetylation and modulate cell proliferation, and the immune response [212]. These compounds are able to prevent/reduce intestinal inflammation [213], as well as varying the intestinal microbiome.

In 1989 was done the first FMT in a patient with UC. After one week of the transplant, the patient entered a medication-free remission [99]. This is a therapeutic method by infusing fecal suspension from a healthy individual into the gastrointestinal tract [214]. This approach has been reported as effective in treatment of *Clostridium difficile* infection and IBD [214, 215]. After the transferred microbiota by healthy donor is observed an alteration in the intestinal microbiota, that could restore the microbial diversity lost in IBD. Although it's a promising strategy, additional studies are indispensable, especially in the selection of an appropriate stool donor, which is the key factor in FMT success [216].

4.2. The Microbiota in Metabolic Syndrome

In order, to create a consensus to characterize a person who has metabolic syndrome, in 2010, the International Diabetes Federation (IDF), in collaboration with the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI), the World Heart Federation, the International Atherosclerosis Society (IAS), and the International Association for the Study of Obesity (IASO) established that metabolic syndrome exists when a person manifest central obesity following any two of these subsequent features: reduced HDL cholesterol, elevated blood pressure or blood glucose abnormalities (elevated fasting plasma glucose, previously diagnosed type 2 diabetes, or glucose intolerance), and elevated triglycerides [217]. Also, elevated plasma proinflammatory markers, prothrombic state, and vascular dysfunction, are parameters that could be related to metabolic syndrome.

The emergency of metabolic abnormalities has arised great interest in scientific understandings either in mechanisms by which are developed, and also in their etiology, and pathogenesis. In pathological circumstances the delivery of specific proinflammatory signals from gram-negative bacteria coming from gut microbiota develop the metabolic endotoxemia [218]. Thus, in this report was related by the first time that the etiology of obesity has a strong relation between gut microbiota, metabolic endotoxemia, insulin resistance and also with the innate immune system. Another important point to be highlighted is that the microbiota impacts on the regulation of the energy metabolism and fat storage. One study developed with male humans donors with metabolic syndrome which received small intestine infusion of microbiota from slim donors. After six weeks of this infusion the patients presented significant increase in insulin sensitivity, as well as the intestinal microbiota diversity and producing butyrate [219]. Thus, all these results suggest that the gut microbiota is a key component and verified that intestinal microbiota could be handle to develop novel therapeutic conditions/agents able to increase insulin sensitivity in patients with metabolic syndrome.

4.3. The Microbiota in Intestinal Mucositis

The intestinal microbiota composition has been changed by chemotherapeutics and radiotherapy action [71,220], consequently its functions have been modified. In addition, multiple host pro-inflammatory and apoptotic pathways are activated by chemotherapy, therefore the gut microbiota are central to the mucositis pathogenesis [76] and the absence of intestinal microbiota is related with a reduction in Goblet cells [221].

Therefore, lower mucus production makes this environment vulnerable to commensal bacteria; leading to lower production of SCFAs, and subsequently lower anti-inflammatory effect by the microbiota [222] and retarding in the regeneration of the intestinal epithelium [223].

Chemotherapeutic agents trigger changes in the microbiome that compromise energy metabolism, cause inflammation, and cause the adverse events, as well as poor quality of life

of patients undergoing treatment. Thus, the intestinal microbiota has active participation of chemotherapy-induced mucositis. As related in a previous section, the Irinotecan (CPT-11) widely used to treat colorectal and pancreatic cancer is administrated as a pro-drug which is metabolized to SN38 (active chemotherapeutic agent), afterward in the liver is glucuronidated and excreted into gastrointestinal tract, where becomes susceptible to be processed by different bacterial enzymes as β -glucuronidase. Thus, the microbiota directly metabolizes chemotherapeutic medication and may produce toxic secondary metabolites, harmful to the health of the host [224].

The effect of CPT-11 on the intestinal microbiota composition in rats has been explored and revealed great changes in its composition; the 16S rRNA analyses (300mg/kg of Irinotecan) shown significant increase in β -glucuronidase producing bacterium (*E. coli*) with significant decrease in *Bifidobacterium spp.* and *Lactobacillus spp.*, which do not produce β -glucuronidase [71,225]. This drug also increased the abundance of Clostridial clusters XI and Enterobacteriaceae, both potentially pathogenic, thus, shown the disruption in the intestinal microbiota [226].

It was also observed global reduction in microbial abundance with 13-fold less of anaerobes and 296-fold less of Streptococci, with a relative increase of Bacteroides in rats treated with the antimetabolite methotrexate, being this change in microbiota composition associated with diarrhea and villous length reduction [227]. This drug is used in clinical practice for the treatment of neoplasia, psoriasis, rheumatoid arthritis and Crohn's diseases and can lead to inhibition of the synthesis of purines and pyrimidines necessities for nucleic acid synthesis [228].

5-FU administration can cause a disruption in the community structure of gut microbiota, usually reducing the richness and abundance of Operational Taxonomic Unit (OTUs) [134]. This drug reduces the overall abundance of important phyla which participates in regular microbial metabolism [70]. When the mucositis was induced in BALB/c mice by 5-FU administration (3x 50mg/kg) the sequencing of 16rRNA V3-V4 region of gut microbiota showed lower richness and diversity in the bacterial community. 5-FU treatment shown decrease in the relative abundance of Firmicutes, Proteobacteria, and Cyanobacteria at phyla level in feces, while the abundance of Verrucomicrobia was increased [70]. These results are in agreement with Carvalho et al., 2018 [229] report, which evaluated the intestinal microbiota in inflamed animals with 5-FU (300mg/kg) and it was observed that the Actinobacteria abundance significantly decreased while the number of Verrucomicrobia increased. Thus, the chemotherapy drugs contributed to the disturbance of gut microbiota and consequently to induce mucositis.

Chemotherapy treatment induces important disturb in the gut intestinal microbiota, consequently there are many alterations in microbiome functions which could contribute to the

damage caused by chemotherapeutic agents intensifying the situation, by compromising the diversity in the gut microbiota. The intestinal microbiota is a complex microbial community whose symbiotic relationship with the host organism is critical for gut homeostasis and colonization resistance against intestinal pathogens. Thus, in the not-distant future, manipulation of gut microbiota could be a vital component for the development of personalized and effective anticancer therapy.

5. Conclusion

The gut microbiota plays an important role in maintaining human health. As we discussed in this chapter, different strains of probiotic bacteria could help in maintaining the gut homeostasis through the microbiota manipulation, performing a number of beneficial functions to the host. The new technical approaches as metagenomics analyses have contributed considerably to elucidate microbiomes (including the human microbiome) and the complex relationships between microbes and their hosts, as well as it has developed progressive understanding of the gut microbiota composition and its activity to health and disease phenotypes such as mucositis, IBD among others diseases that affect directly or indirectly the gastrointestinal tract. In addition, the identification of the microbiota and its use in intestinal dysbiosis associated with host genetics may restore effective communication between the host and its target microbiota (eubiosis). Thus, better knowledge of the gut microbiota using modern techniques could help to develop specific and personalized strategies in future clinical trials.

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