Gut Microbiota and Inflammatory Changes

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Contributor: Simona Bungau

Intestinal microbiota have a series of beneficial effects on the normal development of the human organism, but disturbing the homeostasis between the gut bacteria and the immune response can lead to inflammatory changes.

Keywords: gut microbiota ; supplements ; inflammatory bowel disease ; Crohn's disease ; ulcerative colitis ; probiotics ; prebiotics ; fecal microbiota transplant ; phytochemicals

1. Introduction

Inflammatory bowel diseases (IBD) are chronic, immune-mediated conditions, which affect the gastrointestinal tract. The term IBD includes Crohn's disease (CD) and ulcerative colitis (UC), which both present an elaborate etiology and pathogenesis that has been insufficiently described and acknowledged. IBD occurrence has become more frequent over time, this being connected to industrial progress and innovation and related lifestyle changes. The prevalence of these diseases is higher in developed countries, where it can reach up to 4 cases per 1000 inhabitants [1].

Currently, the exact pathophysiological mechanism leading to IBD is not known, one of the hypotheses being the existence of an aggressive immune response to the intestinal microbiota in genetically predisposed individuals. This hypothesis is supported by recent studies that have identified susceptibility loci at or near genes involved in the innate or adaptive immune response to various germs [2][3][4][5]. However, there are numerous studies aimed at identifying non-immunosuppressive therapeutic agents with a possible benefit for patients with these conditions. These agents target microbial flora disorders that accompany IBD and include prebiotics, probiotics, antibiotics, or fecal microbial transplantation.

Thus, CD is characterized by the involvement of all the digestive tract layers, otherwise known as transmural damage, which can induce inflammation, strictures, or even fistulas. In the advanced stages, the mucosa acquires the appearance of cobblestone by the presence of linear ulcerations alternating with areas of normal mucosa [2][3][4]. The upper gastrointestinal tract may be more commonly involved in childhood CD forms [4]. Both patients with CD and those with UC can associate extraintestinal manifestations that may involve the eyes, skin, and bones, such as arthritis, ankylosing spondyloarthropathy, uveitis, aphthous stomatitis, or erythema nodosum [4].

This article centralizes the existing data provided by significant literature published between 1989 and 2021 and related to the pathophysiological and therapeutic implications of intestinal microflora in the development and evolution of IBD; moreover, it presents in detail the beneficial effects of probiotics, prebiotics, phytochemicals supplementation, or fecal transplant in improving the clinical parameters of IBD patients. In this regard, the authors searched the most well-known databases (i.e., MDPI, Scopus, ScienceDirect, Elsevier, Frontiers, etc.), using key words or combinations of them (i.e., gut microbiota; supplements; inflammatory bowel disease; Crohn's disease; ulcerative colitis; probiotics; prebiotics; fecal microbiota transplant; phytochemicals, etc.). As a result, 172 references were cited as supporting the statements in this work.

Moreover, the present research intends to provide specialists and patients with new information related to this topic and to make as accessible as possible the published data regarding the newest/latest therapies in the field; in addition, the most pertinent and relevant results obtained in medical practice were registered, focused on the optimization of the management of this pathology, both by examining the valuable scientific evidences and by presenting/respectively evaluating the modern ways of treating this disease.

2. Gut Microbiota in the Pathogenesis of IBD

There are currently numerous studies that have investigated the relationship between intestinal microflora and IBD [3]. According to existing data already published, the intestinal microflora of patients with IBD present an increase in the number of bacteria from theProteobacteria phylumfamily and a decrease in those from theFirmicutes phylumand

Bacterioides families, compared to normal individuals [4][6][7][8][9]. In addition, the diversity of bacterial microflora, known as α diversity, is lower in patients with intestinal inflammation [4][10][11]. There are data that show a reduction in microbial diversity in tissues with inflammatory changes, compared to those without inflammatory changes, even in the same patient [12].

A multicenter study that looked at more than 1000 fecal samples from children with CD has found an increase in the number of species from the Veillonellaceae, Pasteurellacaea, and Enterobacteriaceae families, and a decrease in those from the Bacteroidales, Erysipelotrichales, and Clostridioides families [13]. These changes have also been associated with disease status [13]. Another important observation of this study is the possibility of using the microbial profile of the rectal mucosa as a biomarker for the diagnosis of CD in its early stages [13].

From the patho-physiological point of view, the association of intestinal dysbiosis with the appearance of inflammatory changes can be explained by the increase in the number of bacteria with a proinflammatory role and the reduction of those with an anti-inflammatory role, compared to healthy individuals [8][9]. For example,Faecalibacterium prausnitzii,which is part of the Clostridium IV family, has demonstrated an anti-inflammatory role due to its production of butyrate. In patients with CD, there is a reduction in the number of bacteria from theFaecalibacterium prausnitziifamily, a reduction that has been correlated with the risk of relapse after surgical treatment among these patients [14][15].

Additionally, a reduction in the number ofBlautiafaecis,Roseburiainulinivorans,Ruminococcus torques, andClostridium lavalensehas been demonstrated in patients with CD [14][15]. ofFaecalibacterium prausnitziiwere observed, and the restoration of the population of this bacterial species after relapse is associated with the maintenance of clinical remission. They showed that the stimulation of peripheral blood mononuclear cells by this bacterium inhibits the production of inflammatory cytokines (Il-12, interferon gamma) and stimulates the production of IL-10 with an anti-inflammatory role [16] [17]. In patients with hereditary risk of IBD, a reduction ofRoseburiaspp.

These pathogenic bacteria have the ability to adhere to the intestinal epithelium, to alter intestinal permeability, and to induce intestinal inflammation by regulating the expression of inflammatory genes [18][19][20][21]. bacteria could lead to increased intestinal permeability (host-dependent zonulin secretion causes the impairment of the small intestine barrier function after bacterial exposure) [22]. Moreover, Arietta et al. confirmed that this alteration was associated with the development of colitis (reducing low intestinal permeability results in attenuated colitis in the IL10 gene-deficient mouse) In human models, Caviglia et al. showed that patients with IBD had increased intestinal permeability measured by serum zonulin compared to healthy subjects [23].

Another pathophysiological hypothesis incriminated in the development of IBD is the impairment of the production of metabolites by intestinal dysbiosis. An example is the decrease in the concentration of short-chain fatty acids (SCFAs) secondary to the production of butyrate byFaecalibacterium prausnitziior other species from Clostridium clusters IV, XIVa, or XVIII [15]. The consequence of this decrease in SCFAs is the alteration of regulatory T-cell differentiation and expression, and thus the growth of epithelial cells and the maintenance of intestinal homeostasis [24][25].

Besides the bacteria from the Enterobacteriacae family, another class of adherent and invasive bacteria in the mucosa of the digestive tract with a role in the development of IBD are those from the Fusobacteria family [25][26][27][28]. Their protective role is explained by the stimulation of the production of anti-inflammatory cytokines (like IL-10) and reduction of the production of inflammatory cytokines [29]. Ileal biopsies from CD patients have shown a reduction in the populations ofFaecalibacterium prausnitzii(which have an anti-inflammatory role), and an increase in the populations Another bacterium with a possible protective role against the development of IBD has been shown to beHelicobacter pylori[17][30].

3. Probiotics Effects in IBD

Probiotics are microorganisms capable of surviving in the acidic gastric environment. To this point, the existing data support the beneficial role of probiotics in the treatment of IBD [31][32].

The following mechanisms have been suggested for the action of probiotics: stimulation of anti-inflammatory cytokines production (IL-10, transforming growth factor beta (TGF β)), antimicrobial substances secretion, suppression of bacterial growths (thus antimicrobial role), induction of an immune response, immunomodulatory role, improvement of the epithelial barrier function, and suppression of T-cells proliferation [33][34][35][36].

In order for the microorganisms to be considered probiotic agents, they have to meet certain conditions:To survive in the pancreatic, biliary, and gastric acidic secretions, and thus to be viable when they reach the small and large intestines;To remain viable during transportation and storing;To lack toxic or other pathogenic effects on normal human structures;To

have beneficial effects for the host;To adhere to the intestinal epithelial cells;To stabilize the intestinal microbiota;To produce antimicrobial substances.

Intestinal microbiota have a series of beneficial effects on the normal development of the human organism, but disturbing the homeostasis between the gut bacteria and the immune response can lead to inflammatory changes [37][38][39].

The interaction between probiotic agents and intestinal epithelial cells leads to a decrease in the response of various pro-inflammatory stimuli [40]. This reaction can be explained by the inhibition of the degradation of the nuclear factor kB and also by the inhibition of the IkB/NF-kB pathway. Thus, nuclear translocation of NF-kB and the corresponding gene expression are prevented [41].

There is a study that used lining samples from CD patients to observe the existing differences between epithelial cells cultures with no probiotics and those treated with probiotic agents, such as non-pathogenE. colispecies,Lactobacillus casei DN-114001,Lactobacillus bulgaricus LB10,andLactobacillus crispatus, after 24 h. The following differences were detected: TNF-alpha release from the epithelial cells was significantly reduced in the cultures treated withLactobacillus casei DN-114001andLactobacillus bulgaricus, in opposition to the ones treated withLactobacillus crispatusandE. coli, where no important changes were noticed. In addition, in the cultures treated withLactobacillus casei DN-114001andLactobacillus bulgaricus, besides the TNF-alpha expression reduction in intraepithelial lymphocytes, a decrease of CD4 cells number could be noticed. Probiotics interact with immunocompetent cells by modulating pro-inflammatory cytokines' local production. The immune system mediators that are responsible for recognizing pathogenic agents are the toll-like receptors (TLRs) [42].

Summarizing the existing information so far, the main immune effects of this microorganism are as follows: Reduction of the activity of nuclear factor kappa B (NF-kB);Increase of the activity of natural killer cells (NK);Involvement in the maturation of dendritic cells;Stimulation of cytokines (IL 10) production;Activation of antigen presenting cells found in Peyer's plaques [43][44][45][46][47].

Most studies that have evaluated the role of probiotic agents in CD have demonstrated their effectiveness in maintaining the clinical remission of the disease.

A study conducted in 2000 showed thatSaccharomyces boulardiicombined with mesalazine reduce the recurrence rates in adults with CD [48]. Another study, conducted in 2013, compared two groups of patients: one group who underwent basic therapy withSaccharomyces boulardiiand another group who combined this with a placebo [49]. However, this study did not demonstrate significant efficacy ofSaccharomyces boulardiicompared to placebo, in terms of IBD recurrence rate (47.5% vs. 53.2%) [49].

An experiment in mice showed that supplementation withSaccharomyces boulardiileads to reduced intestinal permeability and secondary bacterial translocation. However, an immunomodulatory effect of this microorganism has been demonstrated, by increasing plasma levels of IL-10 and intestinal IgA secretion [50].

Another study of patients with CD showed a beneficial effect of using "Synergy 1", a product containingBifidobacterium longum, oligofructose, and inulin. The administration of this product for a period of six months led to the reduction of proinflammatory biomarkers such as TNF-α in the intestinal mucosa, reduction of the disease activity index, and also improvement of histological parameters [51].

evaluated in 2015 the efficacy of another probiotic agent, VSL#3 (containingBifidobacterium infantis,Bifidobacterium breve,Bifidobacterium longum,Streptococcus thermophilus,Lactobacillus paracasei,Lactobacillus acidophilus,Lactobacillus recarurus, in L.) in patients with CD undergoing surgical treatment (intestinal resection). Two groups were formed: the first group consisted of patients with CD who received VSL#3 immediately after surgery and maintained this treatment for a period of 365 days; the second group consisted of patients who received VSL#3 for a period of 275 days (from day 90 after surgery until day 365). Ninety days post-operatively, the two groups—the first of which had already been treated with VSL#3 for 90 days, while group 2 had not yet received VSL#3—underwent endoscopic investigation. According to this evaluation, group 1, who received VSL#3 immediately after surgery, had a lower level of pro-inflammatory cytokines (Il-8 and IL-1b) in the intestinal mucosa and a lower rate of recurrence compared to group 2, in whom VSL#3 treatment was initiated 90 days after surgery.

Two other studies evaluated the role of probiotic agents in inducing remission in patients with CD, demonstrating an improvement in the CDAI score among patients who received these microorganisms. However, these studies used different preparations and observed, in total, only 14 patients. The second study initially included 11 patients who were

randomized into two groups: the first group received a placebo agent, and the second group receivedLactobacillus rhamnosus GGin combination with antibiotics and steroids, over a period of one week. GGhas also been evaluated in children with DCI but has not shown additional benefit over placebo [52][53].

Other studies have evaluated the effectiveness ofLactobacillus johnsoniiandE. coli Nisslein maintaining IBD remission, but without showing significant benefit [54].

Current data suggest that the concomitant use of multiple microorganisms in patients with IBD leads to better results on disease progression than the use of a single microorganism. Many studies use higher than recommended doses, while other studies do not specify the dose used. Two special categories of patients are children and elderly patients, the data on the use of probiotics in IBD in these categories being much more limited. Thus, we need further studies to monitor the effective use of probiotics in IBD among these patients [55].

The effectiveness of combining probiotic agents with standard therapies has been evaluated in numerous studies and among patients with UC. One study, which included 244 patients with mild or moderate forms of UC, looked at the benefits of combiningSaccharomices boulardiiand VSL#3 with conventional therapy. According to this study, the products mentioned above did not significantly contribute to the improvement of the remission rates of the disease, but they proved a benefit in reducing disease activity [56].

Nevertheless, other studies have evaluated the effectiveness of VSL#3 in UC, with more promising results. Sood et al. demonstrated that combining VSL#3 with standard therapy over a 12-week period improves disease remission rates by reducing ulcerative colitis disease activity index (UCDAI) score by more than 50%. Another important conclusion was the improvement of lesions from the level of the colonic mucosa at the endoscopic evaluation in the group of patients who used VSL#3 [57].

The effectiveness of VSL#3 has also been proven among children with UC. Thus, a study that followed 29 children with UC over a 1-year period showed a significant improvement in the remission rate of the disease by combining VSL#3 with 5-aminosalicylic acid (5-ASA) and steroids therapy, compared with patients who combined placebo agents with this standard therapy (93% vs. 61%) [58]. Another study that looked at 18 children with UC reported both an improvement in histological scores and a reduction in the level of inflammatory markers by combining VSL#3 with standard therapy [59].

Another probiotic agent that has been shown to be effective in improving endoscopic and histological scores in patients with UC is bifidobacteria-fermented milk (a combination ofBifidobacterium strainsandLactobacillus acidophilus) However, a more recent study on 195 patients, describing a similar strategy of treatment (which included fermented milkcontaining B. breve+L. acidophilus), demonstrated no efficacy to cure or at least to maintain the UC remission [60]. In healthy volunteers, from a practical point of view,B. bifidumusage as single-strain-containing probiotic has been shown to be sufficient to enhace SCFAs levels in feces [61]. Taking into account all these data, however, the probiotic's protective role in both UC and/or CD remains insufficiently known.

Despite some discrepancies regarding the number of patients used in the studies mentioned above, the first study was the only one to confirm the increased number of Bifidobacteria in the feces of probiotics-treated patients and to perform endoscopic analysis.

In the literature there is, however, data suggesting that the use of certain microorganisms may even negatively influence the evolution of patients with IBD. For example, a Danish study comparatively followed two groups of patients with UC over a period of 7 weeks: the first group underwent the standard therapy ofE. coliwith foreign Nissle, and the second group, a placebo agent. The result was that the group of patients taking probiotic therapy had a higher dropout rate and an even lower rate of clinical remission. On the other hand, the rectal administration of Escherichia coli Nissle for proctitis or proctosigmoiditis did not demonstrate additional benefits over placebo [62].

Regarding rectally administered probiotic products,Lactobacillus reuteri ATCC 55730in combination with mesalamine has shown benefits in ameliorating mild to moderate forms of UC in children. The study which evaluated this biological compound looked at two groups of pediatric patients with mild or moderate forms of UC; the first group combinedLactobacillus reuteri ATCC55730with standard mesalamine therapy, and the second group combined placebo with mesalamine. At the end of the study, patients in the first group had both a better clinical response, objectified by reducing the Mayo Disease Activity Index [MDAI] by ≥2 compared to group 2 (100% vs. 53%), and a better remission rate, objectified by an MDAI score of <2.0 (31% vs. 0%) [63].

Among patients with mild to moderate forms of UC, a number of studies have compared the effectiveness of using probiotic agents with standard 5-ASA therapy. The probiotic agents evaluated wereEscherichia coli Nissle 1917,Bifidobacterium breve strain Yakult,Bifidobacterium breve,andSaccharomyces boulardii. These studies concluded

that the probiotic agents mentioned above have a similar efficacy to 5-ASA in maintaining clinical and histological remission in patients with mild to moderate forms of UC [64][65][66][67].

An important therapeutic target in the management of patients with IBD is the prevention of early relapses [68]. Thus, in 2010, a small study, which followed six patients with UC in remission, reported that maintenance treatment with 400 mg rifaximin and 500 mgSaccharomyces boulardiiled to the maintenance of clinical remission after three months of use. The conclusion was that this therapeutic combination might be useful in preventing early relapses in UC [69].

The main effects of different probiotics in IBD are summarized in**Figure 2**[67][70][71][72][73]. Dose, treatment duration, and efficiency of probiotic in UC and Crohn's disease are presented in**Table 1**.

4. Conclusions

In recent years, the importance of intestinal microflora in maintaining the normal functioning of the body has been more and more recognized. Numerous studies have looked at the potential therapeutic effects of the microorganisms that make up the intestinal microbiome in various diseases.

In IBD, therapies based on the intestinal microbiota have demonstrated great therapeutic potential. According to existing data, the benefits of these therapies can be mainly explained by the immunomodulatory effect. Compared to immunosuppressive treatments, the toxicity is much lower. The aim is to identify therapies based on the intestinal microbiome that can be used not only as adjuvants of immunosuppressive therapies, but also as therapies themselves. Moreover, there are data that suggest the potential of probiotics to reduce the risk of developing the disease among high-risk patients, such as those with a hereditary history of IBD [74].

The use of probiotic agents, especially those of the *Lactobacillus* and *Bifidobacterium* species, were reported to be effective in UC, which is why it represents an adjunctive treatment among these patients. Both the improvement of clinical signs and symptoms and the induction/maintenance of the remission period are the main proven benefits of probiotic agents in patients with UC. The mechanisms behind these results are the immunomodulatory and anti-inflammatory effects of probiotic agents. In other words, these products reduce the synthesis of pro-inflammatory mediators and increase the synthesis of anti-inflammatory mediators. Although these effects are potentially present in patients with CD, there are still insufficient data to support the use of probiotics as an adjunctive treatment in patients with CD [55].

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