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Review article

Exploring the relationship between *Faecalibacterium duncaniae* and *Escherichia coli* in inflammatory bowel disease (IBD): Insights and implications

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ABSTRACT

Inflammatory bowel disease (IBD) is a group of disorders characterized by an inflammation of the gastrointestinal tract (GIT) and represents a major social and economic burden. Despite ongoing research into the etiology and pathophysiology of this multifactorial disease, treatment options remain limited. From this perspective, the gut microbiota has emerged as a potential player in the pathogenesis of IBD, and animal and human studies support this hypothesis. Indeed, the human gut is one of the most complex ecological communities (composed of 10^{13}-10^{14} microorganisms) that plays a critical role in human health by influencing normal physiology and disease susceptibility through its collective metabolic activities and host interactions. In addition, live probiotic bacteria present in some food products (which transit through the GIT) have been shown to interact with the host immune system and confer several health benefits. The aim of this review is to provide an overview of the link between *Faecalibacterium duncaniae* and *Escherichia coli* and IBD, highlighting the main areas of research in this field. An ecological perspective on the gut microbiota may offer new insights for the development of clinical therapies targeting this bacterial community to improve human health.

1. Gut microbiota

The human gut microbiota, which includes bacteria, archea, protozoans [1] and viruses [2], represents the entire microbial population residing inside the human body. The number of these microorganisms is really remarkable: about 100 trillion (10^{14}) microorganisms reside in the gastrointestinal tract (GIT) alone [3]. This is almost 3 times the total number of cells in the entire human body, which has been estimated at 3.72×10^{13} [4]. From a physiological point of view, the microbiota constitutes approximately 2% of the body mass of an adult, which is comparable to the size of the human brain or liver [5]. This has led researchers to refer to the microbiota as the "forgotten" organ [6,7]. These diverse and numerous microorganisms play an essential role in many bodily processes by providing nutrients to the host, metabolizing indigestible compounds, aiding in defense against colonization by opportunistic pathogens, and possessing immunomodulatory properties [8].

By using next generation DNA sequencing technologies and metagenomic analysis, researchers have shown that the gut microbiota in vertebrates is composed of approximately 500–1000 different bacterial species, with the dominant phyla *Bacteroidetes* and *Firmicutes* accounting for 98% of the total [9–11]. Notably, the number of genes in the gut microbiota is about 100 times greater than that of the human genome, suggesting a co-evolutionary relationship [11].

The microbiota can be considered a dynamic ecological community, influenced by numerous interactions between microbial species and human host cells, as well as the external environment [12]. Maintaining a dynamic equilibrium of the microbiota is crucial for overall health, but this balance can be disrupted by various factors such as environmental conditions, external stimuli like antibiotics, illness, stress, aging, unhealthy dietary habits, and lifestyle choices [13]. These disturbances often lead to microbial imbalances known as dysbiosis, which have direct associations with various pathological conditions [14]. In May 2023, a Pubmed search for articles with the keywords "microbiota," "dysbiosis," and "disease" led a total of 10,281 articles published to date, providing strong evidence that gut microbiota is closely related to several specific disease, such as, autism spectrum disorders [15], cardiovascular diseases [16], diarrhea [17], alcoholic liver disease [18],

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acute-on-chronic liver failure [19], colorectal adenomas [20], arthritis [21], lung diseases [22], autoimmune diseases [23], lupus erythematosus [24], coeliac disease [25], irritable bowel syndrome (IBS) [26], and inflammatory bowel diseases (IBD) [27]. It should be noted that the latter diseases (*ie.* IBD) alone account for 1853 citations.

Therefore, the aim of the present review is to highlight the role of the gut microbiota in the development of IBD and current strategies to treat or prevent them.

2. Inflammatory bowel diseases

Inflammatory bowel disease (IBD) is a group of diseases characterized by a chronic and relapsing inflammation of the GIT, with Crohn's disease (CD) and ulcerative colitis (UC) being the most common forms. The exact etiology of these pathologies remains unknown, although it has been suggested that an abnormal immune tolerance to the gut microbiota may lead to chronic intestinal inflammation and damages in genetically-predisposed hosts [28]. However, whether the immune dysfunction leading to intestinal inflammation is related to the normal microbiota or to a normal immune response to an altered microbiota remains unclear. Recent research has emphasized the significance of modifications in the gut microbiota in the development of IBD [29] and proposed that gut microbiota changes in IBD patients may be a consequence of environmental factors [30]. In Fig. 1 we summarize the composition of the gut bacterial microbiota of IBD patients versus healthy volunteers.

In this context, in a recent study, the mucosal microbiota of patients with quiescent CD, their healthy siblings, and unrelated healthy controls was evaluated using 16 S rRNA gene pyrosequencing [31]. The study showed that the diversity of core microbiota in both CD patients and their healthy siblings had a distinct microbial community compared to healthy controls, characterized by reduced microbial diversity and a decrease in specific bacteria.

as *Faecalibacterium duncaniae* (formerly known as *F. prausnitzii* [32–34]) which is known to have anti-inflammatory properties [35] (see below). In addition, previous research has also reported that both patients with inactive CD and their healthy siblings, present immune abnormalities associated with CD, such as predominance of memory T cells and increased naïve CD4 T cell β7 integrin expression, compared to healthy controls [36]. While these results support the theory that microbiological [31] and immune [36] processes are involved in CD pathogenesis, the shared dysbiosis between CD patients and their healthy siblings, requires a nuanced interpretation. Firstly, the similarity in microbiota composition may be indicative of shared genetic or environmental factors influencing the microbiome, but it does not necessarily imply a direct causative relationship. The multifactorial nature of CD, involving genetic predisposition, environmental triggers, and complex immune responses, suggests that dysbiosis alone may not be the sole determinant of disease manifestation. Additionally, the dynamic nature of the microbiome and the variability in disease expression among affected individuals highlight the need for longitudinal studies to track changes over time. While healthy siblings may have shared a similar microbiome composition with CD patients, this doesn't guarantee that they will not develop CD in the future. Longitudinal studies following healthy siblings over an extended period would be crucial to understand how their microbiome composition evolves and whether any changes correlate with the onset or prevention of CD. It's also plausible that healthy siblings possess protective factors or exhibit different immune responses that prevent CD development despite sharing a dysbiotic microbiome with their affected siblings.

In addition, a meta-analysis and a systematic review of the literature found: i) a decrease of *F. duncaniae* in IBD patients, in particular in CD patients with ileal involvement, compared to healthy controls [37] and ii) that the steady increase of *F. duncaniae* after relapse in UC patients is related to disease remission [38].

Faecalibacterium duncaniae and *E. coli* have been proposed as valuable biomarkers for phenotypic classification in IBD patients [39]. CD patients exhibit lower *F. duncaniae* counts and higher *E. coli* counts, not only compared with healthy controls, but also compared to IBS patients. The *F. duncaniae-E. coli* (F-E) index effectively discriminated between healthy controls, CD and UC patients, and even between different disease phenotypes.

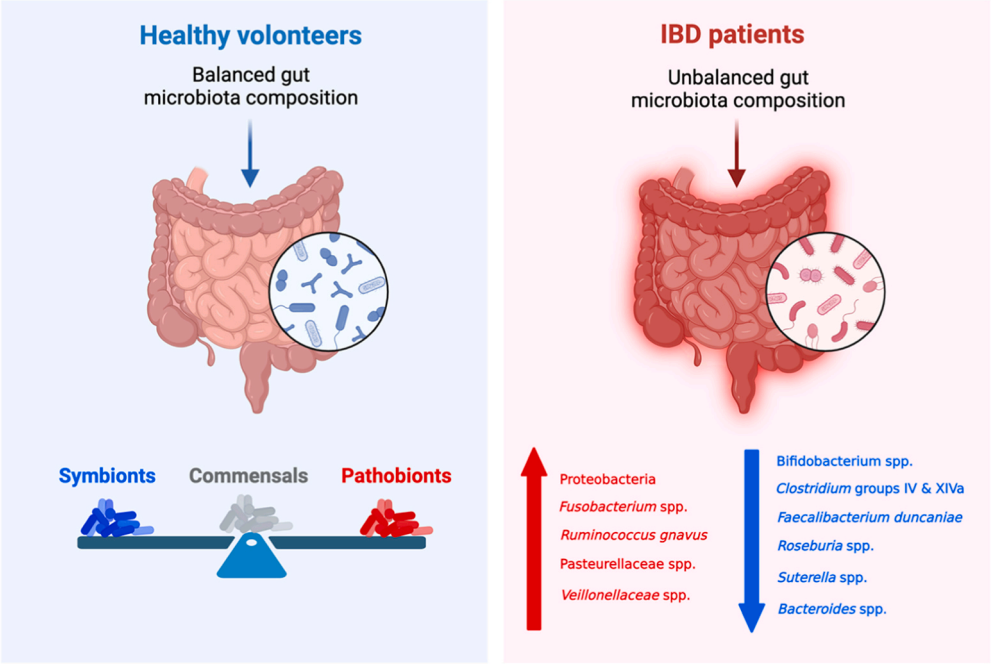

Fig. 1. Composition of the human gut bacterial microbiota (at the level of keystone gut microbiota species) of IBD patients versus healthy volunteers. This figure was created with Biorender (agreement number: GQ264AN65V).

3. Pro-inflammatory role of adherent-invasive *Escherichia coli* (AIEC) in IBD

Biopsies from patients with ileal involvement of CD, 36.4% show the presence of adherent-invasive *E. coli* (AIEC) while the prevalence in biopsies from non-IBD controls is 6.2% [40]. A recent article also discusses the role of *E. coli*, in particular the AIEC pathotype, in the etiopathogenesis of CD [41]. The overgrowth of AIEC in the intestine may result from inflammation, leading to dysbiosis and proliferation of this microorganism. Conversely, AIEC has been shown to induce changes in the gut microbiota components, including higher levels of bioactive LPS and flagellin, loss of microbial diversity, and alterations in bacterial species composition, which activated chronic inflammation, particularly in susceptible hosts lacking the flagellin receptor TLR5 [42].

In this context, AIEC strain LF82 was used to explore the different mechanisms of action of this bacterium. *Escherichia coli* LF82 was able to translocate through M cells of Peyer's patches due the interaction of type 1 pili and long polar fimbriae with GP2, a protein present in the surface of M cells [43]. The involvement of IbeA invasin in *E. coli* LF82 was explored using a mutant strain inactivated at the *ibeA* locus [44]. Invasins, a class of proteins associated with the penetration of pathogens into host cells, play a crucial role in facilitating pathogen entry during the initial stages of infection [45]. The study found that the IbeA invasin of AIEC mediates interaction with intestinal epithelia and macrophages, which is highly relevant in the context of CD and the increasing evidence supporting the association of an imbalanced microbiota with CD development. Gram-negative bacteria, including AIEC, have been reported to be enriched in CD patients, and understanding the virulence factors contributing to AIEC's pathogenicity is crucial. The study identified IbeA as a novel virulence determinant in AIEC, contributing to the invasion of intestinal epithelial cells and survival within macrophages [44]. Although AIEC colonization in a mouse model was not significantly affected by the absence of IbeA, it was observed that IbeA played a role in increased pathology in the ilea and ceca, possibly through enhanced IFN-γ secretion. These results underscore the complexity of AIEC interactions with the host, involving multiple virulence factors such as type 1 pili, flagella, and long polar fimbriae, in addition to IbeA. Furthermore, *E. coli* LF82 induced increased production of reactive oxygen species (ROS) in cultured epithelial T84 cells, inhibited mucin gene expression, and increased the expression of the chemotactic cytokine IL-8. These characteristics play a major role in the maintenance of inflammatory injuries in CD [46]. The persistence of AIEC in the gut, its ability to induce inflammation, and the correlation between AIEC colonization and pathology provide valuable insights into the mechanisms that may contribute to the perpetuation of inflammation in CD.

The ability of monocyte-derived macrophages from CD patients to control AIEC internalization was recently analyzed [47]. Higher levels of AIEC were internalized within monocyte-derived macrophages from CD patients than monocyte-derived macrophages from healthy controls or UC patients. In addition, monocyte-derived macrophages from CD patients were incapable to limit intracellular replication of AIEC and the persistence of this infection resulted in increased secretion of IL-6 and TNF-α compared to the infection with non-pathogenic *E. coli*. It was also demonstrated that the infection with AIEC reduced the expression of proteins necessary for autophagy in intestinal epithelial cells by up-regulating microRNAs [48].

4. Potential role of anti-α-Gal antibodies in IBD

Understanding the association between the occurrence of anti-α-Gal antibodies and different bacteria is crucial for shedding light on the intricate interplay between the immune system and gut microbiota. This relationship has far-reaching implications, as it involves immune defense against infections and the development of chronic inflammatory diseases like IBD. One intriguing mechanism potentially contributing to IBD severity is the induction of pro-inflammatory anti-α-Gal antibodies by *E. coli*, and other bacteria present in the GIT.

In this context, it is essential to note that inactivation of the α-1,3-galactosyltransferase gene (*ggta1*) in old world monkeys, apes, and humans resulted in an almost unique ability of this group of primates to produce high antibody titers against the glycan Galα1–3Galβ1–4GlcNAc-R (α-Gal) [49]. Gut microbiota bacteria induce anti-α-Gal immunoglobulins (Ig) of the isotypes IgM and IgG, which are widely expressed in humans [50], fish [51–53] and birds [54–56], and at high levels, these Igs protect against malaria [57], tuberculosis [51–53], ectoparasite infestation [58,59], and bacterial sepsis [60]. However, the evolutionary advantage of enhanced resistance to infections through anti-α-Gal antibodies comes with trade-offs. For instance, the ability of humans to produce these antibodies has been associated with disorders like red meat allergy induced by tick bites [59,61], and chronic inflammatory diseases such as multiple sclerosis [62], and IBD [63].

Recent research has uncovered the presence of α-1,3-galactosyltransferase genes, different from *ggta1*, in 193 species and strains of bacteria within the human gut microbiota [64]. Among these bacteria are members of the Enterobacteriaceae family (genus *Escherichia*), and Lactobacillaceae (genera *Pediococcus*, and *Lactobacillus*) family. Notably, *Lactobacillus* strains were found to have α-Gal on their surfaces [65], but their oral administration in the form of fermented milk containing *Lacticaseibacillus casei* failed to elicit anti-α-Gal antibodies in humans [66]. Furthermore, oral administration of *Lactobacillus brevis* (strain LBH1073), *Agrilactobacillus composti* (strain LBH1073), *Lacticaseibacillus paracasei* (strain LBH1073) reduced significantly the production of anti-α-Gal IgM and did not affect the levels of anti-α-Gal IgG in mice [65]. In contrast, oral administration of *E. coli* strain O86:B7, a bacterium with high α-Gal content [56,57], recapitulates the etiology of anti-α-Gal IgM production in mice [67], chicken [68], turkeys [55] and humans [69]. This insight into bacterial influences on pro-inflammatory anti-α-Gal antibodies production in response to *E. coli* antigenic stimulation raises the question of how these antibodies and their isotypes, particularly IgG, affect immune mechanisms, engagement of macrophages, and potentially contribute to the severity of conditions like IBD.

The immunological mechanisms underlying these potential effects are intriguing. IgM antibodies activate the complement cascade upon binding to α-Gal on invading pathogens [57], while the absence of α-Gal from IgG-associated glycans increases IgG effector function by enhancing IgG-Fc gamma receptor (FcγR) binding [60]. Mucosal IgG was found to drive intestinal inflammation in UC via a mechanism associated with enhanced engagement of FcγR on local macrophages [70], and structural variations in IgG-associated glycans altering the IgG effector function have been linked with IBD [71]. These results suggest that a compositional imbalance of the microbiota towards bacteria enhancing production of mucosal anti-α-Gal IgG (*eg. Escherichia*) may contribute to enhanced IgG effector function, engagement of macrophages and IBD severity.

Although mucosal anti-α-Gal IgG may have a role in IBD severity, a longitudinal study conducted by Mangold et al. [63] found no significant changes in the serum levels of α-Gal specific IgG, IgM, IgD, and IgA in healthy individuals over time. Notably, CD patients showed a significant increase of anti-α-Gal IgA compared with control subjects [63]. The pro-inflammatory function of mucosal anti-α-Gal IgA was demonstrated in an avian model of aspergillosis [55]. Specifically, when *E. coli* O86:B7 was orally administered to turkeys, it led to reduced levels of anti-α-Gal IgA in the lung tissue, correlating with a decreased occurrence of lung granulomas in response to *Aspergillus fumigatus* infection. In contrast, such an effect was not observed after oral administration of *E. coli* BL21 [55], a bacterium with lower α-Gal content [56]. This suggests that anti-α-Gal IgA antibodies may trigger lung inflammation, while their potential inflammatory role in the digestive tract remains unclear.

Moreover, the same study revealed that oral administration of *E. coli* BL21 in turkeys resulted in the upregulation of cytokines, including IL-10, IFN-γ, and IL-6 expression in the ceca [55]. Ceca are paired blind sacs arising from the junction of the ileum and colon, often extending

alongside the ileum in birds [72]. Notably, the only significant increase observed in cecal cytokine gene expression after oral administration of *E. coli* O86:B7 was for IL-2; other tested cytokine genes, such as IL-10, IFN-γ, and IL-6, did not exhibit significant changes [55]. Collectively, these findings indicate that the expression of pro-inflammatory cytokines in the mucosa and the regulation of anti-α-Gal IgA by *E. coli* are dependent on the bacterial strain. Future studies should delve into the role of different E. coli strains with varying α-Gal levels in the induction of pro-inflammatory cytokines and anti-α-Gal IgA in the human intestine, particularly concerning their relationship with IBD severity.

Additionally, it is intriguing that mucosal anti-α-Gal IgA plays a role in shaping microbiota composition through microbiota-specific IgA responses [73]. It was reported that intestinal bacteria that showed high coating with IgA increased the susceptibility to colitis in germ-free mice. This suggests that IgA coating may identify commensal bacteria with inflammatory properties that contribute to the intestinal disease [74]. Following this line of investigation, it was demonstrated that IgA-coated bacteria were increased in a mouse model of colitis [75]. These authors also evaluated IgA+ and IgA- bacteria from stool of patients with IBD in a mouse model of colitis induced with dextran sodium sulfate (DSS). Mice transplanted with the IgA+ bacterial strains showed exacerbation of intestinal inflammation, which was different to the mice, transplanted with IgA-bacteria that showed less pronounced symptoms. These findings were not observed in germ free mice without inflammation demonstrating that high IgA coated bacteria could worsen inflammation in patients suffering IBD through the modulation of the microbiota. Further studies could explore a potential link between α-Gal-specific IgA+ bacterial strains and exacerbation of intestinal inflammation in IBD models.

5. Anti-inflammatory role of *F. duncaniae* in IBD

On the other hand, the ability of several strains of *F. duncaniae*, a commensal bacterium present in the core of the gut microbiota of the healthy individuals, to protect against inflammation has been explored using in vitro and in vivo assays. Thus, the supernatant (SN) of a culture of *F. duncaniae* strain A2–165 exerted a stronger anti-inflammatory effect than the bacterium itself [35]. In addition, in a 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced rat colitis model, the SN of *F. duncaniae* strain A2–165 increase IL-10 and IL-12 levels and decrease IL-17 levels in both plasma and colonic mucosa [35]. The changes in interleukin levels suggest that *F. duncaniae* has a significant impact on the immune response and inflammation. IL-10 is known for its anti-inflammatory properties, and an increase in its levels often indicates a regulatory response to suppress inflammation [76,77]. IL-12, although involved in the activation of immune responses, can also contribute to anti-inflammatory effects [78,79]. Conversely, there is a decrease in the pro-inflammatory cytokine IL-17. IL-17 is associated with promoting inflammation and is often elevated in inflammatory conditions [80]. Therefore, the observed changes in cytokine levels suggest that *F. duncaniae* has a modulatory effect on the immune response, suppressing pro-inflammatory signals (IL-17) while promoting anti-inflammatory responses (IL-10 and IL-12). This modulation is indicative of the potential protective role of *F. duncaniae* against inflammation.

Moreover, administration of *F. duncaniae* strain A2–165 or its SN decreased the severity of colitis in two mouse models (severe and moderate chronic colitis) [81]. The anti-inflammatory effects of *F. duncaniae* and its SN were also demonstrated in a chronic low-grade inflammation model in mice by decreasing the intestinal permeability, the colonic levels of the cytokines IL-6, INF-γ, IL-4 and IL-22 and serotonin levels [82]. The amelioration of TNBS-induced colitis in mice by *F. duncaniae* and its SN was previously attributed to the induction of *Foxp3* and Treg response [83].

Concerning the potential mechanism of anti-inflammatory effect of this commensal bacterium, recent data suggest that butyrate (a short-chain fatty acid, SCFA) produced in high amounts by it, could be the main effector. Indeed, butyrate produced by *F. duncaniae* (present in large amounts in its SN) increases the expression of beta-catenin binding antagonist 3 (*Dact3*) gene in TNF-α-stimulated HT-29 cells [84]. *Dact3* acts as a negative regulator of the Wnt/JNK inflammatory signaling pathway and may be one of the host effectors responsible for the anti-inflammatory effects of *F. duncaniae*, since its silencing causes a partial loss of the anti-inflammatory effects exerted by *F. duncaniae* [84]. Furthermore, it is noteworthy that *F. duncaniae* also produces several bioactive molecules that affect inflammation and intestinal barrier function, such as shikimic and salicylic acids [85] and a microbial anti-inflammatory molecule (MAM) [86]. Taken together, these findings underscore the complexity of the immunomodulatory mechanisms of *F. duncaniae* and suggest the involvement of multiple factors, in addition to butyrate, in its observed anti-inflammatory properties.

6. An overview of current therapeutic approaches to treat IBD

There is currently no cure for IBD, and treatment strategies are aimed at inducing and maintaining remission, reducing the frequency and severity of flare-ups, and improving patients' quality of life. Within the current treatments to treat IBD we can cite a number of drugs such as aminosalicylates [87], corticosteroids [88], immunomodulators [89] and biologics [90]. Immunosuppressive therapies have long been used to treat IBD patients. However, due to the chronic nature of the disease and the ongoing need for treatment, there is a need to explore non-immunosuppressive therapies that offer a more favorable risk-benefit profile. Among these, there is growing interest in therapies targeting gut microbiota dysbiosis, as they hold promise for improving outcomes and reducing side effects.

7. Fecal microbiota transplantation

One of these therapies targeting gut microbiota dysbiosis is fecal microbiota transplantation (FMT), which is aimed at correcting the dysbiosis found in the gut microbiota. The theoretical basis for using FMT lies in the idea that introducing a healthy donor's fecal material into the recipient's gastrointestinal tract will restore a more balanced and diverse microbial community, potentially alleviating symptoms and promoting recovery [91,92].

The effectiveness of FMT in the treatment of CD and UC remains an area of ongoing research with variable outcomes. While some studies have suggested potential benefits of FMT in modulating the gut microbiota and reducing inflammation in both conditions [93,94], the results have not been consistently positive across all trials [95]. For example, a recent study reported that a cohort of patients with active UC and without infectious diarrhea responded well to FMT [93]. Notably, patients in the FMT group had a higher remission rate, with nearly 25% achieving remission, compared to the 5% remission rate observed in patients who received the placebo. It was also shown that stool from patients receiving FMT had more microbial diversity compared with baseline, than that of patients from placebo group [93]. FMT led to a significant shift in microbiota composition, resulting in increased diversity in the treatment group compared to the placebo at week 6 versus baseline. Additionally, the microbiota of FMT-treated patients exhibited greater similarity to their respective donors than a control fecal sample. Despite a trend indicating that responders had microbiota more similar to donor B with a significant enrichment for the family *Lachnospiraceae* and the genera *Ruminococcus*, particularly associated with successful FMT, this trend did not achieve statistical significance.

Additionally, a case report showed that a patient with CD, who previously failed to control his disease with immunosuppressant therapies, achieved clinical, endoscopic, and histologic remission after a single FMT [94]. However, a recent meta-analysis [95], encompassing twelve studies and 550 participants with FMT administered through various methods, found that for UC, FMT may enhance induction rates of

clinical and endoscopic remission compared to control, although the certainty of evidence was low. Uncertainty prevailed regarding the risk of any adverse events with FMT in UC, and evidence on serious adverse events and improvement in quality of life was very uncertain. Concerning the maintenance of remission in UC, the evidence was highly uncertain. Notably, none of the included studies investigated FMT for remission induction in CD. Only one study, with 21 participants, reported data on FMT for maintenance of remission in CD, but the evidence was weak. The authors concluded that while FMT may increase the proportion of people achieving remission in active UC, further studies are needed to address its efficacy and safety in both UC and CD, including its potential for maintenance of remission in the long term for both conditions [95].

Even with these and other successful results of FMT (especially when other therapies did not work), there are several concerns that should be addressed before this technique is generalized in clinical practice [91]. Firstly, safety issues arise due to the transfer of live microorganisms, posing a risk of transmitting infectious agents. To mitigate this risk, rigorous screening and testing protocols for donor stool are essential. Another concern is the lack of standardized procedures for FMT, encompassing donor screening, stool processing, and administration methods. Standardization is crucial to ensure consistency and reproducibility across different clinical settings. The long-term effects of FMT remain poorly understood, with limited data on the persistence of transplanted microbiota and associated risks over time, necessitating further research [96]. Efficacy varies among individuals and conditions, prompting ongoing investigations to identify specific patient populations that benefit most and factors influencing treatment response [92]. Ethical considerations, including donor anonymity and commercialization risks, require attention, and regulatory frameworks for FMT are still evolving [97], underscoring the importance of upholding ethical standards and ensuring patient safety in its clinical implementation. A synthetic assembly of specific fecal microorganisms grown in vitro is also a promising therapeutic approach for IBD [98].

8. Microbial ecosystem therapeutics

Defined microbial ecosystems, known as microbial ecosystem therapeutics (MET), aim to reintroduce beneficial microbes or microbial communities to treat dysbiosis by promoting a more balanced and diverse ecosystem [99], [100]. They target microbial diversity, recognizing that a healthy gut is characterized by a diverse and balanced microbial community [101] [102]. The MET aim to reintroduce or enhance this diversity, promoting a more resilient and adaptable gut ecosystem. Additionally, MET approaches use specific microbial strains [99], [100], for a precise and controlled approach to address distinct aspects of gut health.

Mixtures of defined microbes derived from stool are being developed as therapeutics for the treatment of *Clostridioides difficile* infection (CDI). The idea behind these mixtures is to restore the intestinal microbiota and cure recurrent CDI. Unlike FMT, which involves transferring whole stool from a donor, these mixtures have a known and controlled composition and do not require human donors, which ensures safety, consistency, and regulatory compliance, making it a promising avenue for tailored interventions in gut health.

One study reported on six patients with recurrent CDI who received either FMT or a mixture of 10 intestinal bacterial species isolated from human feces [99]. The 10-strain mixture, consisting of different strains of *Clostridia*, *Bacteroides*, *E. coli*, *Streptococcus faecalis*, and *Peptostreptococcus productus*, was administered to four patients. The response to the mixture was positive, with patients becoming asymptomatic and testing negative for *C. difficile* toxin within 24 h [99]. In a more recent study from 2013, researchers used a modified continuous culture chemostat system to isolate 33 non-pathogenic strains of bacteria from the stool of a healthy donor [100]. These strains were characterized, banked, and then reconstituted as a synthetic mixture called "RePOOPulate." Two patients with recurrent CDI were treated with this mixture, resulting in both patients being cured and remaining symptom-free during the 6-month follow-up. Colonization resistance, a key aspect of gut health, refers to the ability of the resident gut microbiota to prevent the establishment of potentially harmful microorganisms [103–106]. Disruptions in the microbiota's balance, often induced by antibiotics, can lead to infections such as *C. difficile*-associated colitis, underscoring the critical role of colonization resistance in preventing pathogenic overgrowth [107]. By increasing microbial diversity, MET may enhance colonization resistance, fortifying the gut against the establishment of harmful microbes like *C. difficile*.

The use of MET offers a potential alternative to FMT for recurrent CDI. These studies highlight the potential of using mixtures of defined microbes to treat CDI by reestablishing a healthy intestinal microbiota composition without the need for whole stool transplantation. The importance of this approach lies in its potential to provide more targeted and tailored interventions for specific conditions, minimizing variability and optimizing therapeutic outcomes. By focusing on defined microbial mixtures, researchers and healthcare professionals can develop treatments that are safer, more predictable, and potentially applicable to a broader range of individuals. The approach aligns with the growing interest in precision medicine and personalized therapies for various health conditions.

9. Reducing human anti-α-Gal antibody activity

As explained above, anti-α-Gal antibodies could contribute to chronic inflammatory diseases including IBD [63]. In this case, the approach of reducing human anti-α-Gal antibody binding to mammalian cells by polyacrylamide-based or polylysine-based [108,109] α-Gal--glycoconjugates could potentially be used to reduce the risk of IBD associated with pro-inflammatory anti-α-Gal antibodies. This approach focuses on developing synthetic α-Gal epitope polymers that can inhibit the binding of anti-α-Gal antibodies to mammalian cells. By reducing the binding of these antibodies, it may be possible to mitigate their pro-inflammatory effects and potentially lower the risk or severity of IBD.

Synthetic α-Gal epitope polymers could potentially interfere with the interaction between anti-α-Gal antibodies and α-Gal epitopes present on gut bacteria by competitively inhibiting the binding sites of these antibodies. By blocking or reducing this binding, the pro-inflammatory effects triggered by these antibodies could be diminished, potentially lowering the severity of IBD. Additionally, since mucosal anti-α-Gal IgA shapes the composition of the microbiota, the synthetic α-Gal epitope polymers could also be investigated for their potential to modulate the interaction between anti-α-Gal IgA and IgA-coated bacteria in the gut. By reducing the binding of IgA to specific bacterial strains associated with intestinal inflammation, these polymers might help mitigate the exacerbation of IBD.

While the specific application of the synthetic polymers in reducing the risk of IBD has not been studied, the concept of interfering with anti-α-Gal antibody binding holds promise for modulating the immune response and potentially mitigating inflammation associated with IBD. Further research would be needed to evaluate the effectiveness and safety of these synthetic α-Gal epitope polymers in the context of IBD and their potential as a therapeutic approach.

10. Probiotics

Probiotics represent another possibility to exert beneficial effects on microbial dysbiosis and may also provide other properties, such as immunomodulatory capabilities, and could be used for treatment or prevention or to maintain remission in patients suffering from IBD. Probiotics are defined as *"live microorganisms that, when administered in adequate amounts, confer a health benefit to the host"* [110]. Most probiotics belong to the group of bifidobacteria and lactic acid bacteria

(LAB), within which the groups of lactococci, lactobacilli and enterococci are the most studied [111]. It should be noted that LAB represent a very heterogeneous group of microorganisms that are present in the normal diet of many people and may also form part (only some species) of the microbiota (mainly in the gastrointestinal and urogenital tract) of the host. Thus, beneficial balance of the intestinal microbiota and immunomodulatory properties are the most studied effects associated with various benefits that have been attributed to probiotics.

Currently, most populations tend to consume foods that in addition to their nutritional values can offer some benefits that improve their overall well-being, and many products containing probiotic microorganisms are available worldwide to meet this demand. Also, probiotics can be included in medicinal products that are prescribe to certain patients. The effects of probiotics and fermented products containing beneficial microorganisms on intestinal disorders have been the most extensively studied considering that these microorganisms enter the organism orally and can positively modulate the intestinal microbiota and the gut immune system. Inflammatory bowel diseases constitute pathologies for which there are many reports about the beneficial use of probiotic microorganisms.

The use of experimental animal models has permitted the understanding of mechanisms by which probiotics can exert their positive effects on the host. The value of these models is the insight they can provide into the complex, multi-faceted processes and mechanisms that can result in gut inflammation development. However, the application of probiotics against IBD needs to be ultimately tested in human clinical trials.

11. In this section the focus will be placed on the effect of probiotics against IBD through their capacity to stabilize the gut microbiota

LAB and other probiotic microorganisms can counteract inflammatory processes in the gut by balancing positively the microbial environment and the permeability of the intestinal barrier, by enhancing the degradation of enteral antigens and altering their immunogenicity [112, 113].

Probiotics can also stimulate growth of certain microorganisms of the gut microbiota. VSL#3 is a probiotic mixture that contains eight different strains of bacteria and it was evaluated against different diseases, including IBD models. It was reported that the improvement of colitis in a DSS-induced murine model by VSL#3 supplementation (i.e. significant reduction in disease severity score) was associated with modifications in ileal microbiota composition. The ileal microbiota of inflamed animals treated with VSL#3 was characterized by enrichment of *Enterobacteriaceae* compared to the colitis control mice [114]. This same probiotic mixture was also evaluated previously in a TNBS induced model of colitis in rats. Microbial composition was analyzed by terminal restriction fragment length polymorphism (T-RFLP) of the bacterial 16 S rRNA gene. The reduction of colitis severity in VSL#3-fed rats was associated to alteration in the composition and diversity of the intestinal microbiota [115]. VSL#3 was also assayed in a colitis-associated cancer model in rats and it was shown that animals treated with the probiotic mixture had significantly less intestinal damage (without developing carcinomas or high-grade dysplasia) than the vehicle treated-controls; and this correlated with decreased richness and diversity of the mucosally-adherent microbiota [116]. *Lactobacillus reuteri* was evaluated in IL-10 knock-out (KO) mice and its administration prevented colitis associated to this model by increasing the number of lactobacilli in the gastrointestinal tract [117]. *L. salivarius* UCC118 was another LAB evaluated and using a placebo-controlled trial it was reported that its oral administration reduced mucosal inflammatory activity and prevalence of colon cancer in IL-10 KO mice by modifying the intestinal microbiota where a significant decrease of *C. perfringens*, coliforms, and enterococcus groups were observed in the probiotic administered group [118]. In addition to the studies with specific LAB strains and mixtures of LAB, fermented products containing these microorganisms have also been analyzed against IBD using animal models. The oral administration of yoghurt, made with potential probiotic strains, decreased the inflammation in a TNBS-induced mouse model by modulating the large intestine microbiota of the mice, with increase of the bifidobacterial population. This effect was accompanied by a regulatory and anti-inflammatory response in the intestine, compared with the inflamed control animals [119]. The same yoghurt maintained the remission period in a model of recurrent inflammation through the modulation of some intestinal bacteria populations and the gut immune response [120].

The translation of probiotic to be used for IBD patients remains uncertain [121]. It was shown that specific probiotics promote favorable intestinal colonization, and also that some fermented products have anti-inflammatory properties, and immunomodulatory and metabolic effects in animal. However, when evaluated in clinical trials, the effects are variable, preliminary, or limited in magnitude. Similarly, the role of probiotic for the beneficial management of the endogenous intestinal microbiota appeared to be a promising strategy due to their effectiveness in animal models; however, research in humans has been scarce as there are only few reports in the past three years where the fecal microbial composition of the patients was evaluated. In this sense, it has been shown that the supplementation of the probiotic Ecologic 825 (Winclove, Amsterdam, the Netherlands) to patients with UC and severe pouchitis restored the mucosal barrier, and this effect was correlated with the bacterial diversity of mucosal pouchitis microbiota [122].

12. Conclusions

The dysbiosis of the intestinal microbiota appears as one of the most important contributing factors in the development of IBD. The shared distinct microbial community [31] and immune abnormalities [36] observed in both CD patients and their healthy siblings, as opposed to unrelated healthy controls, strongly suggests a potential genetic or familial influence on dysbiosis mediated by the interplay between immune system and microbiota. This implies that genetic risk factors leading to dysbiosis could be a precondition for individuals affected by CD, emphasizing the need for further research to elucidate the intricate relationship between genetics, microbiota, immunity, and the development of CD.

In the last years certain bacterial species were analyzed as either being beneficial or deleterious markers of patients suffering IBD and also in healthy people that have a predisposition to develop intestinal inflammation. *F. prausnitzii* aroused the interest of different groups that investigate the IBD (causes and possible treatments) since certain studies showed its potential as an IBD treatment.

Considering the importance of intestinal dysbiosis in IBD, FMT is a promising therapy for patients that suffer these pathologies; however, more controlled trials of FMT in specific disorders are needed before these can be accepted and applied clinically. The possibility to use specific fecal microorganisms in the FMT appears to be an interesting and more standardized alternative. A compositional balance between bacteria that increase (e.g., *Escherichia*) and decrease (e.g., *Lactobacillus*) the production of mucosal anti-α-Gal IgM and/or IgG and/or IgA may contribute to control IBD severity. Enrichment of microbiota with *Lactobacillus* bacteria may reduce mucosal anti-α-Gal antibodies with consequences for amelioration of IBD symptoms.

In conclusion, the administration of probiotics represents another possibility to stabilize and improve the balance of the intestinal microbiota that is altered in IBD, and this effect is associated with modulation of the intestinal immune response (induction of the anti-inflammatory response) in the inflamed host, at least in preclinical models. However, as in the case of TFM, not enough human clinical trials have been published in which the application of probiotics in IBD patients has been tested. These trials are essential before the medical community can accept the addition of probiotics as supplements for IBD patients.

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Author's contributions

A.C-C and L.G.B-H conceived and designed the study. A.C-C and L.G.B-H performed data analysis and wrote the original draft preparation. A.C-C and L.G.B-H reviewed and editing the final version. All authors have read and agreed to the published version of the manuscript.

Disclosure of interest

A.C-C and L.G.B-H are co-founders of the startup microXpace, which aims to exploit the potential of natural alpha-gal antibodies to fight infectious diseases in human and animal health.

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