Mechanisms of Immunomodulation by Probiotics in Inflammatory Bowel Disease

by

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Abstract

Inflammatory bowel disease (IBD) is an intestinal inflammatory disease with a poorly understood multifactorial pathogenesis. The two main types of IBD, Crohn's disease (CD) and ulcerative colitis (UC), are distinct disease forms with differences in genetic risk factors, involved immune cells, and affected parts of the gastrointestinal (GI) tract. Additionally, environmental factors and the intestinal microbiome also contribute to CD and UC pathogenesis. CD and UC are both characterized by chronic, relapsing inflammation of the GI tract. Currently, treatment consists of untargeted immunomodulatory therapies, targeted biologic therapies, and surgery. Despite the variety of treatment options available, curative treatment of IBD is not (yet) possible. Because the intestinal microbiome and immunological factors contribute to IBD pathogenesis, probiotics are being investigated for their therapeutic potential. Probiotics are live microorganisms that, upon administration, can provide a health benefit to the host. One of the mechanisms of action of probiotics is immunomodulation. In this literature review, the mechanisms of immunomodulation by probiotics in IBD patients and disease models are discussed. The immunomodulatory effects of probiotics on immune cells involved in IBD pathogenesis are reviewed individually, which includes the effects on intestinal epithelial cells (IECs), neutrophils, macrophages, dendritic cells (DCs), T cells, and B cells. It is discussed that probiotics can affect cytokine production, cell signaling, gene expression, cell differentiation, immune cell infiltration, number of pro-inflammatory and immunoregulatory cells, and/or the production of immunoglobulins in IBD patients and disease models. Furthermore, recommendations for future studies on probiotics in IBD treatment are discussed.

Plain Language Summary

Inflammatory bowel disease (IBD) is an intestinal inflammatory disease with a poorly understood pathogenesis. The two major types of IBD, Crohn's disease (CD) and ulcerative colitis (UC), are distinct disease forms with differences in genetic risk factors, involved immune cells, and affected parts of the gastrointestinal (GI) tract. The precise disease mechanisms are unknown, but it has been noted that genetic, immunological and environmental factors, and intestinal microbiome are all involved in CD and in UC pathogenesis. In IBD patients, these factors contribute to chronic, relapsing inflammation of the GI tract, a defective and leaky intestinal barrier, an altered intestinal microbiome, and defects in the immune system. Currently, there is a variety of treatment options available, which includes untargeted and targeted therapies that suppress the immune system, and surgery. Curative treatment of IBD is, however not (yet) possible. Because the intestinal microbiome and immunological factors are involved in IBD pathogenesis, numerous studies have investigated the therapeutic potential of probiotics. Probiotics are live microorganisms that, upon administration, can provide a health benefit to the host. In general, probiotics have four different mechanisms of action: antimicrobial activity, interaction with the host microbiome, enhancement of the gut barrier function, and modulation of the immune system (immunomodulation). Every probiotic strain has one or multiple mechanisms of action to potentially provide a health benefit to the consumer. In studies assessing the therapeutic potential of probiotics in IBD treatment, mixed results were obtained. Several clinical studies have shown positive effects of the administration of probiotics on remission in IBD patients. In contrast, numerous others did not show positive effects of probiotics administration. These contrasting results in IBD patients are likely caused by

human heterogeneity, differences in used probiotic strain(s), differences in factors underlying CD or UC, and quality of the clinical studies. In this literature review, immunomodulation by probiotics (one the four probiotics mechanisms of action) in IBD patients and IBD disease models is discussed, which contributes to the better understanding of the therapeutic potential of probiotics in the treatment of IBD. The immunomodulatory effects of probiotics on different immune cells involved in IBD pathogenesis are reviewed individually, which includes the effects on intestinal epithelial cells (IECs), neutrophils, macrophages, dendritic cells (DCs), T cells, and B cells. In this review, it is discussed that the administration of probiotics in IBD patients and IBD disease models can affect the production of pro-inflammatory and immunoregulatory proteins, gut barrier function, gene expression, immune cell infiltration into the gut and associated tissues, number of pro-inflammatory and immunoregulatory immune cells, and/or cell signaling. These probiotics-induced immune system alterations could potentially help IBD treatment. Besides, recommendations for future studies on probiotics in IBD treatment are discussed. This includes the recommendation that future studies on probiotics should focus more on patient-, disease-, and target-oriented research instead of only determining clinical outcomes in patients. Additionally, the use of new probiotic strains and techniques could also contribute to better understanding of the therapeutic potential of probiotics in the treatment of IBD and other diseases.

Introduction

Inflammatory bowel disease (IBD), which is comprised of Crohn's disease (CD), ulcerative colitis (UC), and IBD unclassified (IBDU), is an intestinal inflammatory disease with a poorly understood multifactorial pathogenesis (1–3). CD and UC, the two major types of IBD, are distinct disease forms with differences in genetic risk factors, involved immune cells, and affected parts of the gastrointestinal (GI) tract (3). Despite the differences, it is known that genetic, immunological and environmental factors, and the intestinal microbiome all contribute to CD and to UC pathogenesis, however the precise disease mechanisms are unknown (4,5).

CD is characterized by chronic, relapsing transmural inflammation (affecting all layers of the intestinal wall) and by patchy intestinal lesions that are scattered along the GI tract (4,5). In CD patients, the transmural inflammation contributes to intestinal fibrosis, strictures, and perforations, which causes abdominal pain, obstruction, and non-bloody diarrhea (4,5). In contrast, UC is characterized by chronic, relapsing superficial inflammation (affecting the mucosal layer) and by continuous intestinal lesions that are only present in the colon (4,5). The superficial inflammation causes intestinal perforations, ulcers, abdominal pain, and bloody diarrhea in UC patients (4,5). Furthermore, extraintestinal manifestations, which includes musculoskeletal and dermatological complications, also occur in CD and UC patients (4–6). Additionally, IBD patients have an increased risk of developing different types of gastrointestinal cancer (4,5).

The onset and progression of CD and UC is mediated by genetic, immunological, and environmental factors, and by the intestinal microbiome, however the mechanisms are not completely understood (4,5). Examples of environmental factors that increase the risk of CD and/or UC development include smoking (CD), antibiotics exposure (CD and UC), use of oral contraceptives (CD and UC), urban living (CD and UC), and certain dietary products (e.g. sucrose consumption increases the risk of UC and CD) (4,7). There are

also environmental factors that decrease the risk of developing CD and/or UC, which includes breastfeeding (CD and UC), physical activity (CD), presence of gastric *Helicobacter pylori* (CD and UC), and certain dietary products (e.g. tea consumption decreases UC risk) (7). Besides, genome-wide association studies have identified more than 350 IBD risk loci, and many of these are associated with immune cells and intestinal barrier function (8–11). Most of the IBD risk loci only increase the risk of CD or UC development, whereas some other loci are shared between CD and UC (10). For example, genetic variants of *IL23R* (encoding the IL-23 receptor, which is involved in a pro-inflammatory signaling pathway) are associated with an increased risk of CD and UC development, and genetic variants in *NOD2* (encoding for a intracellular pattern recognition receptor) are only associated with an increased risk of CD development (4). The presence of one or multiple IBD risk loci is not enough for the development of CD and UC, as studies have shown that concordance rates in monozygotic twins are ~50% for CD and ~15% for UC, which indicates that genetics are more important in the development of CD, and that other factors also contribute to CD and UC pathogenesis (4–6). Furthermore, it has been determined that IBD risk loci only explain 13.1% (CD) and 8.2% (UC) of the variance in disease liability, which indicates that other factors, such as environmental risk factors, contribute more to the development of CD and UC (8,12).

The pathogenesis of CD and of UC are not completely understood (4,5). Nevertheless, it is has been established that CD and UC are chronic, relapsing inflammatory diseases of the GI tract, and that both are characterized by an impaired intestinal barrier, dysregulated immune system, and altered microbiome (4,5,13). In brief, early in the pathogenesis of CD and UC, an impaired intestinal barrier can be observed (4,5,12). The intestinal barrier, which includes the mucus layer and intestinal epithelium, provides a physical and chemical barrier separating the intestinal lumen from the host tissue, thereby acting as a first line of defense (14–16). Impairment of the intestinal barrier can lead to invasion of microbiota into the host tissue, which can cause intestinal inflammation (4,5). Furthermore, the impairment of the intestinal barrier can also directly affect the intestinal immune system through altered signaling and altered production of (immune) proteins (*e.g.* production of the chemokine CCL20) (17,18). However, it is not known whether a defective intestinal barrier is a cause or consequence of inflammation in IBD (15). Environmental and genetic factors can contribute to the impairment of the intestinal barrier (4,5). For example, smoking and alcohol consumption can increase intestinal permeability, which potentially contributes to impairment of the intestinal barrier (12). Additionally, IBD risk loci are associated with the intestinal barrier, which includes genes encoding tight-junctions proteins (*e.g. Cadherin 1, TJP1*) and proteins involved in the immune function of intestinal epithelial cells (IECs) (*e.g. NOD2*) (4,5).

Besides the impaired intestinal barrier, dysregulation of the innate and adaptive immune system is also observed in CD and UC (4,5,13). It has been noted that in CD and UC, there is an increase in number of pro-inflammatory cytokines and (pro-inflammatory) innate immune cells (4,5,19). This includes an increase in number of neutrophils, pro-inflammatory macrophages, and pro-inflammatory DCs (4,5,19). The increase in number of innate immune cells in IBD leads to a more pro-inflammatory state in the GI tract, which contributes to IBD pathogenesis (4,5,20). To illustrate this, neutrophil infiltration into the intestinal barrier is one of the first signs of intestinal inflammation, and infiltrated neutrophils are observed throughout the course of active IBD (4,5,20). The accumulation of neutrophils leads to increased production of pro-inflammatory cytokines, which subsequently results in the recruitment and pro-

inflammatory polarization of other immune cells (4,5,20). Additionally, the adaptive immune system is also dysregulated in the CD and UC (4,5,20). In the inflamed GI tract of CD patients, an increase in number of pro-inflammatory T helper 1 (Th1) cells and Th17 cells is observed (16,21–23). In contrast, increased levels of the pro-inflammatory Th2 cells, Th9 cells and Th17 cells are observed in the inflamed colon of UC patients (16,20–23). This increase in number of pro-inflammatory T helper cells is likely mediated by the increase in pro-inflammatory cytokines levels, which are produced by the increased number of (pro-inflammatory) innate immune cells in CD and UC (4). The increased number of T helper cells potentially contributes to IBD pathogenesis by producing pro-inflammatory cytokines, thereby affecting other immune cells (21). Furthermore, in the inflamed mucosa of IBD patients, there is an increase in number of regulatory T (Treg) cells (20,24,25). This could indicate that Treg cells are functionally deficient in IBD patients, however this is not known (16). Additionally, a change in the ratio of Treg cells and pro-inflammatory T cells could also contribute to IBD pathogenesis (16,26). Furthermore, effector $CD8^+$ T cells are likely also involved in IBD pathogenesis (27,28). Besides, the role of B cells in CD and UC pathogenesis is less studied, however in inflamed IBD tissue there is a IgG predominance, whereas IgA predominance is observed in healthy tissue (16). IgG can contribute to IBD pathogenesis by activating immune cells via the interaction with Fc-gamma receptors (29). Additionally, environmental and genetic risk factors can contribute to the dysregulated immune system in IBD (4,5). For example, certain variants in IBD risk genes (*e.g. IL23A* and *IL12B*, encoding the heterodimer IL-23, which is a pro-inflammatory cytokine) are associated with the stabilization and promotion of Th17 cell differentiation (11). In addition, smoking, which increases the risk of CD and UC, is associated with immunosuppression (7).

CD and UC are also both characterized by an altered microbiome (4,5,13). The altered microbiome likely contributes to CD and UC pathogenesis, however the precise mechanisms are unknown (4,5,13). One of the proposed mechanisms is that the loss of commensals in the microbiome benefits the colonization of pathobionts in the GI tract, which can cause intestinal inflammation (30). Furthermore, bacteria from the microbiome could activate the intestinal immune system after impairment of the intestinal barrier, which can also contribute to intestinal inflammation (31). Moreover, the microbiome is also able to regulate the intestinal immune system, and an altered microbiome could lead to a dysregulated immune system (31). Additionally, alterations in the intestinal microbiome can also result in a changed intestinal metabolome, which is observed in IBD patients, and this altered intestinal metabolome can contribute to IBD pathogenesis (32). To illustrate this, short-chain fatty acids, which are able to regulate the number of intestinal Treg cells, show reduced levels in IBD patients, and a subsequent reduction in Treg cells can potentially contribute to the pathogenesis of CD and UC (32). Besides, the composition of the altered microbiome in CD and UC patients has been investigated in numerous studies (33–38). In brief, a decrease in microbiome diversity, which is called dysbiosis, is observed in CD and in UC patients (5,6,37). The altered intestinal microbiome of CD and UC patients show similarities, however certain alterations in the microbiome are only observed in CD or only in UC patients (37). For example, the abundances of some species from the *Enterobacteriaceae* family (*e.g. Escherichia fergusonii* and *Shigella flexneri*) is increased in CD patients only (37). *Escherichia* and *Shigella* species are known to invade the intestinal barrier, cause ulceration of the colon, and cause bloody diarrhea (37). In contrast, in both CD and UC patients, the abundance of the phylum *Firmicutes* is reduced (6,19,37). A reduction in *Firmicutes* abundance is also associated with other diseases, including asthma and type 2 diabetes mellitus (39). Additionally, the

abundance of *Faecalibacterium prausnitzii* is also reduced in CD and UC patients (6,19,37). *F. prausnitzii*, which is one of the most abundant bacterial species in the healthy microbiome, is involved in promoting gut health through the production of short-chain fatty-acids (6,19,40). In addition, the abundance of *Bacteroides* is increased in CD and UC patients, and these commensal bacteria are known to be opportunistic pathogens (37). The increase of specific strains of *Bacteroides* (e.g. *Bacteroides fragilis* and *Bacteroides vulgatus*) are linked to the genetic variations in *NOD2*, a CD risk loci (37). Despite the identified alterations in the microbiome, a particular microbiome composition or marker microorganism that is specific for CD or for UC has not (yet) been identified (4,5). Besides, the composition of the microbiome is associated with the impaired intestinal barrier and dysregulated immune system in CD and UC patients (4,5,30). However, it is not known whether the altered microbiome causes the impaired intestinal barrier and/or dysregulation of the immune system, or whether the observed dysbiosis of the intestinal microbiome is a consequence of the dysregulated immune system and/or impaired intestinal barrier (20,30). Additionally, the identified IBD environmental risk factors can also influence the microbiome composition, which includes antibiotics exposure, drug use, and diet (*e.g.* low-fiber diet can result in microbiome dysbiosis (20,30). IBD risk loci can also contribute to an altered microbiome (31). For example, the genetic variants in *NOD2*, a pattern recognition receptor, can affect the production of anti-microbial peptides, which subsequently can alter the composition of the intestinal microbiome (31).

Currently, there is variety of treatment options available for CD and UC, however complete treatment of IBD is not (yet) possible, and management of disease is therefore based on individual disease characteristics (4,5). The treatment options for CD and UC can be divided into untargeted therapies, targeted biologic therapies, and surgery (4,5,16). Untargeted therapies have been used for many decades in the management of CD and UC, and include corticosteroids, 5-aminosalicylicates (mesalazine), and immunomodulators (4,5,16). Corticosteroids, which are broadly active anti-inflammatory and immunosuppressive drugs, are used in CD and UC to induce remission in mildly, moderately and severely active CD and UC (4,5,41,42). Corticosteroids are not used to maintain remission as adverse events and disease remission are observed after long-term use (4,5,41,42). 5-aminosalicylicates, which have an unknown mechanisms of action, are given to mildly-to-moderately active CD and UC patients to induce and/or maintain remission, and long-term usage is common (4,5,41,42). Broadly active immunomodulators are used in the maintenance of mildly-to-moderately active CD and UC (4,5,41,42). These untargeted therapies can be used in combination or as monotherapies (4,5,41,42). For the treatment of moderate-to-severe CD and UC, targeted biologic therapies are commonly used (4,5,41,42). Several biologic therapies for the treatment of CD and UC are available, and their mechanisms of action include: neutralization of pro-inflammatory cytokines (*e.g.* anti-TNF antibodies and anti-IL-12 antibodies), blocking of pro-inflammatory signaling pathways (*e.g.* JAK inhibitors), and modulation of immune cell trafficking (*e.g.* anti–α4β7 integrin antibodies) (16). These biologic therapies specifically target immune cells or immune cell products that are involved in CD and/or UC pathogenesis (4,5). Biologic therapies are used to induce and/or maintain remission in moderate-to-severe CD and UC, and have been very successful (4,5,41,42). Furthermore, biologic therapies are used in combination with untargeted therapies to increase effectiveness (4,5). However, despite the success of biologic therapies in the treatment of CD and UC, 30% of patients do not have an initial response to biologic therapies, and up to 50% of patients lose responsiveness to the biologic therapies over time (16,43). Additionally, CD and UC patients can also

experience side effects and drug intolerance from biologic therapies (44,45). Furthermore, biologic therapies are way more expensive than untargeted therapies (8 times more expensive than 5-aminosalicylicates and 36 times more expensive than immunomodulators) (46). Lastly, surgery is performed in CD and UC patients in case of severe active disease and/or medically refractory disease (6,19). In total, 50-80% of CD patients and 10-30% of UC patients undergo surgery during their lifetime (47). Nevertheless, the number of CD and UC patients requiring surgery is decreasing, which is contributable to the use of biologic therapies in treatment CD and UC treatment (47,48). Because complete treatment of IBD is not (yet) possible, new treatment options are being investigated, which includes the development of new biologic therapies (49). Additionally, because the microbiome and intestinal immune system play a role in IBD pathogenesis, therapies that target and/or use the microbiome or specific microorganisms are tested for their therapeutic potential in the treatment of CD and/UC, with mixed success (50,51). This includes fecal microbiota transplantation (FMT) and probiotics (50,51).

Probiotics, which are studied for their therapeutic potential in the treatment of CD and/or UC, are “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (52). The Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) established this consensus definition in 2001, which was grammatically corrected in 2014 (52). Probiotics can be consumed and/or are administered in different forms, which includes probiotic drugs, fresh fermentation products, and probiotic dietary supplements (52). Research on probiotics has been performed for over 100 years (53). One of the first scientific reports on probiotic bacteria dates back to 1907 (53,54). Elie Metchnikoff described in his report a correlation between the consumption of lactic microbes and enhanced longevity (53,54). Since then, numerous probiotics have been identified. The most commonly used probiotic strains belong to the *Bifidobacterium, Lactobacillus* and *Saccharomyces* genera (55). Additionally, microbial strains that belong to the *Bacillus, Escherichia, Propionibacterium* and *Streptococcus* genera are also used as probiotics (55). Besides, probiotic strains isolated from healthy human individuals are used as ‘next generation’ probiotics (55). Furthermore, genome engineering is used to enhance probiotic efficacy of known probiotics, and to identify new probiotic strains (56–58). The different probiotic strains are experimentally tested for their therapeutic effects in different diseases and disease models, including in IBD, with mixed success (2,51,53). Some clinical studies have shown a positive effect of the administration of a single probiotic strain or a mixture of probiotics on remission in IBD patients, especially in UC patients (2,59). However, in other clinical studies no positive effects of the probiotics administration on IBD symptoms were observed (2,59). Contrasting results were also obtained in clinical studies investigating the potential of probiotics in the treatment of other diseases, including in different forms of diarrhea and respiratory infections (51).

In general, probiotics have four different mechanisms of action: 1) antimicrobial activity, 2) interaction with the microbiome, 3) enhancement of barrier function, and 4) immunomodulation (51,60,61). These mechanisms of action do not exist in every probiotic strain, and the mechanisms are not always shared between probiotic strains from the same genus (62). Besides, the mechanisms of action of a specific probiotic strain will also depend on the context (62). For example, some probiotic strains require the presence of other strains or environmental factors to execute their mechanisms of action (62).

Nevertheless, every probiotic strain requires a mechanism of action to provide a health benefit to the consumer. 1) Antimicrobial activity or protection against pathogens by probiotics has been shown in cell culture and in animal models (51,60,63). Probiotics are able to antagonize pathogenic bacteria by inhibiting bacterial adherence, the production of a physiologically restrictive gut (e.g. lower luminal pH), and the production of antimicrobial molecules (60,64). 2) Interaction with the microbiome is another proposed mechanism of action of probiotics (51). First of all, probiotics can prevent the colonization of pathogenic bacteria and enhance the colonization of beneficial intestinal bacteria by competitive exclusion and nutritional competition (61,65). However, it is also possible that probiotics can inhibit beneficial intestinal bacteria (66). Compared to spontaneous recovery after antibiotics-treatment, it was observed that the administration of a mixture of probiotics delayed the reconstitution of the microbiome and transcriptome in mice and humans (66). In contrast, the transfer of autologous fecal microbiome resulted in faster reconstitution of the microbiome and transcriptome (66). Besides, probiotics can also regulate the intestinal microbiome through metabolite secretion (61). Probiotics can increase levels of short-chain fatty acids (e.g. butyrate) in the gut, either directly or via cross-feeding of other bacteria in the microbiome, leading to a beneficial environment in the GI tract (62). Moreover, probiotics can also modulate the intestinal microbiome (61). However, this mechanism is controversial, as it has been shown that administration of probiotics in healthy individuals does, in most cases, not result in an altered microbiome (67,68). Furthermore, most studies investigating the effect of probiotics on the composition of the microbiome used fecal stool samples, whereas in recent years it has been shown that these fecal samples are not representative for the gut microbiome (51,68,69). 3) Probiotics can improve the gut barrier function through different mechanisms (70,71). Firstly, probiotics can enhance the epithelial barrier function via signaling in intestinal epithelial cells (IECs) (70,71). The interaction between probiotics and pattern recognition receptors (PRRs) on the cell surface of IECs can result in the activation of signaling pathways involved in the suppression of apoptosis, proliferation of IECs, secretion of cytokines, and upregulation of tight junction proteins (61,70,71). Besides, probiotics can also enhance the epithelial barrier function by improving the intestinal chemical barrier (71). It has been shown that probiotics can increase the expression of mucins in the gut (51,61,71). 4) Immunomodulation, the last mechanism of action, will be discussed in more detail in the main part of this literature review, with a focus on the mechanisms of immunomodulation by probiotics in IBD patients and disease models.

Several different experimental models are used to investigate the mechanisms of action of probiotics, with each model having its advantages and limitations (71). First of all, epithelial cell lines such as Caco-2, HT-29, IPEC-1/IPEC-J2 and T84 are commonly used in probiotic research, with the major advantage being ease of use (2,71). These cell lines are used to study the effect of probiotics on the epithelial barrier function, tight junctions, and signaling (71). Furthermore, specific interactions between structural components of probiotics or secreted molecules by probiotics with the host can be studied in detail *in vitro* with these immortalized cell lines (72). Major limitations of cell lines for the study of probiotics include the many non-physiological characteristics, the influence of culture medium, the lack of the microbiome, limited interaction with the host, and difficulty to mimic different diseases (2,71). Studies improving the quality of *in vitro* models for the study of probiotics are ongoing (2). These newly developed *in vitro* models include organoids, gut-on-a-chip, primary intestinal cells, and coculture models (2,73,74). Besides, animal models are also used to study the mechanisms of action of probiotics (2). Especially mouse

model are frequently used (2). Among other factors, this is because mouse models are relatively cheap and they have great experimental flexibility (2). Furthermore, there are numerous different mouse models available, including different healthy and disease-mimicking models (75). The major limitation of all mouse models for the study of probiotics is that the mouse gut is not representative for the human gut (2). This is because there are structural differences (e.g. the lack of mucosal folds in mice) and differences in the microbiome (2). The most commonly used mouse models include healthy mice and germ-free mice for the general study of probiotics (60,75). To study the potential of probiotics in IBD treatment, mainly dextran sodium sulfate (DSS)-, and trinitrobenzene sulfonic acid (TNBS)-induced colitis mice are used, with DSS-induced colitis mice being used more frequently (60,75). DSS is a sulfated polysaccharide that induces colitis in the gut of mice by affecting the epithelial barrier integrity, resulting in inflammation in the gut (75). One of the advantages of using DSS to induce colitis in mice is that different dosages and concentrations can be used to induce acute, chronic, or relapsing models of IBD (75). In contrast, the major limitation is that the adaptive immune system is not required for the induction of DSS-induced colitis, whereas in humans, there is an important role of T and B cells in the pathogenesis of CD and UC (75). Furthermore, the induction of colitis in mice by DSS or another chemical can also affect the microbiome in the gut (76). Lastly, humans are also used to study probiotics (72). In humans, transcriptomics, proteomics, metabolomics, and metagenomics are commonly used to study the effect of probiotics on a systemic or organ level (77). Therefore, the outcomes of probiotics studies in humans tend to be more clinical, and focus less on understanding the mechanisms of action of probiotics (77,78). Besides, *ex vivo* studies investigating the effects of probiotics on isolated immune cells are also performed (79–81). New techniques to study the human GI tract are being developed (82). These techniques can potentially be used in the future to study the mechanisms of action of probiotics in humans *in vivo*.

In this literature review, the research question, What are the mechanisms of immunomodulation by probiotics in IBD patients and disease models? is investigated. Immunomodulation by probiotics, one of the mechanisms of action of probiotics, holds the potential to contribute to the treatment of different diseases. By discussing the mechanisms of immunomodulation by probiotics in IBD patients and disease models, the therapeutic potential of probiotics in the treatment of CD and/or UC is explored. The immunomodulatory effects of probiotics on the different immune cells involved in IBD pathogenesis are reviewed individually, which includes the immunomodulatory effects on IECs, neutrophils, macrophages, DCs, T cells, and B cells. In this review, it is discussed that probiotics can affect cytokine production, cell signaling, gene expression, cell differentiation, immune cell infiltration, number of pro-inflammatory and immunoregulatory cells, and/or the production of immunoglobulins in IBD patients and disease models. To illustrate this, articles are highlighted that studied the mechanisms of immunomodulation by probiotics in detail. Furthermore, recommendations for future studies on probiotics in IBD treatment are discussed.

Mechanisms of Immunomodulation of IECs by Probiotics in IBD

The intestinal epithelium, which is comprised of a single layer of IECs intercalated with immune cells, provides a physical and chemical barrier separating the intestinal lumen from the host tissue, thereby acting as a first line of defense (14–16). The cellular and architectural structure of the single layer intestinal epithelium varies along the gut (14). There are different subtypes of IECs, with each cell having a different

function (14). This includes enterocytes, which form the physical barrier, and the mucus-secreting goblet cells (14). In IBD patients, it has been noted that early in the pathogenesis, a defect in the epithelial barrier can be observed (**Fig. 1a**) (5). The loss of barrier function can lead to the failure of immune tolerance and the infiltration of microbiota, resulting in the activation of immune cells, and production of pro-inflammatory cytokines and chemokines, leading to (enhanced) inflammation (5). However, it is not known whether a defective epithelial barrier is a cause or consequence of inflammation in IBD (15). The breakdown of the epithelial barrier could also lead to dysbiosis (loss of microbiome diversity), which is observed in CD and UC patients, and this potentially contributes to IBD pathogenesis (70). Besides, IECs can also contribute to CD and UC pathogenesis through some of the IBD risk loci that have been identified (4,5,8,10). These include genes encoding proteins involved in epithelial polarity (*e.g. TTC7A*), proteins involved in tight-junctions (*e.g. MAGI2, MAGI3,* and *CDH1*), and proteins involved in the mucosal immune response, such as NOD2 (a intracellular pattern recognition receptor) (3,11,83). Additionally, IECs can contribute to IBD pathogenesis through antigen presentation (4,14,84). By acting as antigen presenting cells, IECs can affect other immune cells that are involved in IBD pathogenesis (4,14,84).

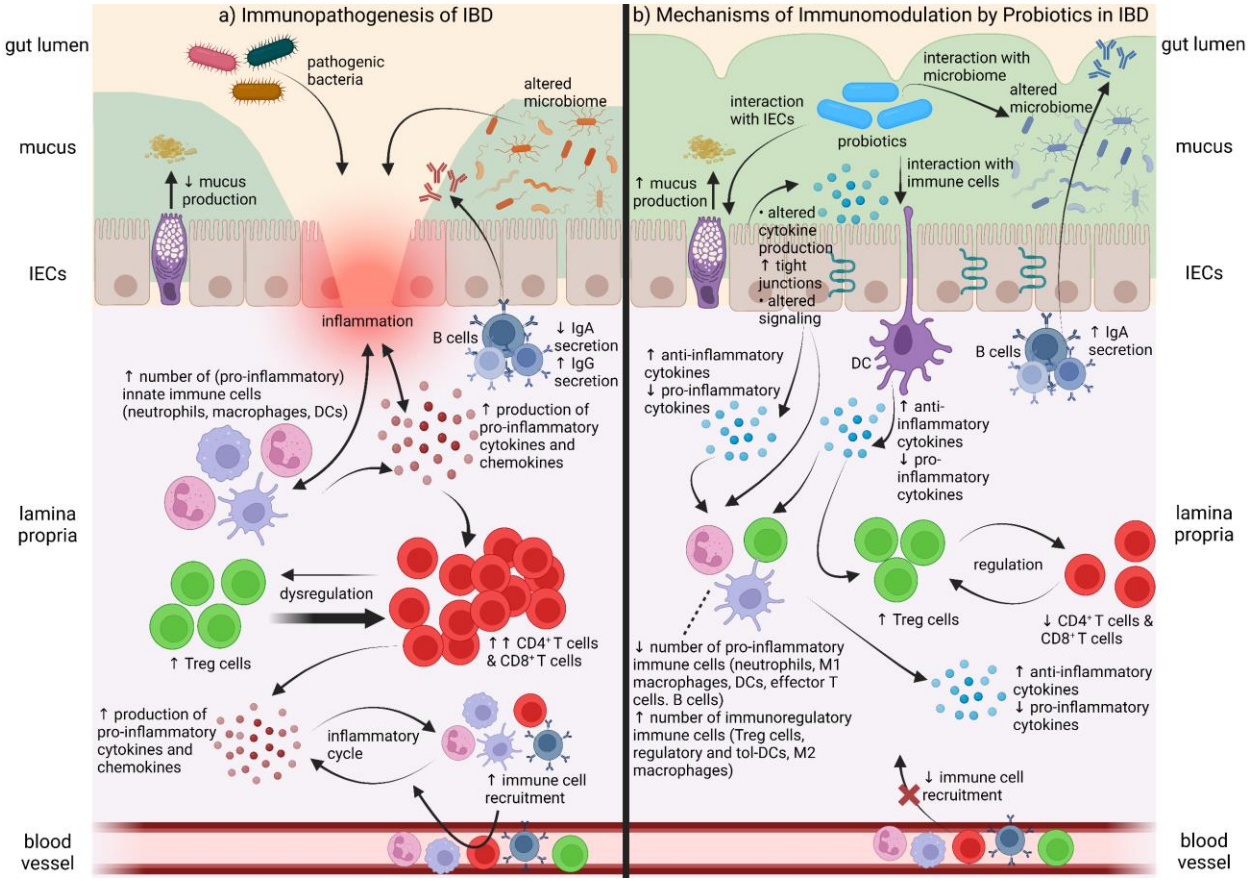

Fig. 1. Overview of immunopathogenesis of IBD, and of mechanisms of immunomodulation by probiotics in IBD. **a)** Numerous immune system alterations are observed in IBD patients. This includes the breakdown of the intestinal barrier, altered microbiome, increase in pro-inflammatory cytokines and decrease in anti-inflammatory cytokines, increased infiltration and proliferation of pro-inflammatory immune cells, increased pro-inflammatory cell-polarization, disbalance in ratio of pro-inflammatory and immunoregulatory cells, IgG predominance in the gut, and immune cell-related IBD risk loci. However, the precise mechanisms of immunopathogenesis of IBD are unknown.

b) The administration of probiotics can have immunomodulatory effects on IECs, neutrophils, macrophages, DCs, T cells, and/or B cells in IBD patients and disease models. It has been observed that probiotics can affect cytokine production, cell signaling, gene expression, cell differentiation, immune cell infiltration, number of pro-inflammatory and immunoregulatory cells, and/or the production of immunoglobulins. Some of these probiotic-induced alterations are likely connected to each other. However, the precise mechanisms of immunomodulation by probiotics in IBD are unknown. Figure inspired by Abraham and Cho, 2009 (85), and by Liu *et al*., 2020 (70). Created with BioRender.com.

Because IECs play a role in the pathogenesis of IBD, numerous studies have investigated the potential of probiotics to target IECs in IBD patients and disease models, with the goal to enhance the intestinal epithelial barrier and/or improve (immune) functions of IECs. To study this, mainly colitis-induced mouse models are used, despite the earlier discussed limitations of these animal models. This is because, in immortalized cell lines it is difficult to mimic the intestinal epithelium with the right cellular composition, microbiome and chemical barrier, and IBD pathogenesis (86). Furthermore, IECs are also difficult to study in humans *in vivo*. Overall, it was observed that the administration of probiotics in colitis-induced mouse models had an effect on cytokine and chemokine production, signaling, tight junctions and/or mucus production in/by IECs (**Fig. 1b**). In most studies, it has been observed that probiotic strains or a mixture of probiotics could reduce the production of pro-inflammatory cytokines and increase anti-inflammatory cytokine production (**Fig. 1b**) (76,87–104). Cytokine and chemokine production in the GI tract is not exclusively performed by IECs, and the observed effects of administration of probiotics on cytokine and chemokine production are therefore not only contributable to an altered production in IECs. Nevertheless, in a DSS-induced colitis model it was shown that the administration of *Lactobacillus jensenii* TL2937 reduced the production of the proinflammatory cytokines CXCL1, IL-1, IL-15, IL-17, MCP-1 and TNF-α and increased the production of IL-10 and IL-27, which are immunoregulatory cytokines, in the colon (89). This resulted in lower disease activity and alterations in the colon (89). Furthermore, in the same study it was observed that the addition of *L. jensenii* TL2937 to DSS-treated porcine intestinal epithelial cells or DSS-treated Caco-2 cells also reduced the production of pro-inflammatory cytokines and chemokines (89). The proposed mechanism involves reduced levels of phosphorylated JNK, which is a kinase involved in apoptosis, tight junctions, and the production of pro-inflammatory cytokines (89). In another study it was noted that the probiotic *Lactobacillus casei* strain Shirota increased the expression of the anti-inflammatory cytokine *IL-10* and reduced the expression of the pro-inflammatory *IFN-γ* and *iNOS* (92). In the same study it was observed that signaling in IECs of colitis-induced mouse models can also be affected by the administration of probiotics (92). It was shown that the NF-κB signaling pathway was activated in DSS-induced colitis mice, and that the addition of the probiotic *L. casei* strain Shirota downregulated the NF-κB pathway, leading to a reduction in active NF-κB in the nuclei of colon cells (92). The NF-κB pathway is involved in the immune response, and, among other functions, regulates the expression of a variety of proinflammatory cytokines and chemokines (105). Downregulation of the NF-κB signaling pathway could therefore contribute to the anti-inflammatory effects of probiotics in the colon of DSS-induced colitis mice. Altered signaling in IECs after administration of probiotics in IBD disease models has also been observed in other studies (**Fig. 1b**) (87–89,92,94,96,97,101,102,104,106–109). This includes numerous studies in which increased expression and production of tight junction proteins was observed (**Fig. 1b**) (76,91,92,95–98,100,102,104,109–112). For example, the administration of the probiotic *Bacteroides uniformis*

JCM5828 resulted in increased gene expression and protein levels of Claudin-1, Occludin, and ZO-1 in DSS-induced colitis mice, which was associated with alleviation of colitis (104). Another study showed that the administration of *Lactobacillus reuteri* resulted in upregulation of the tight junction protein encoding genes *Tjp1* and *Ocln* in DSS-induced colitis mice (76). In the same study it was noted that the treatment of *L. reuteri* resulted in normalized mucus morphology in DSS-induced colitis mice (76). Other studies have shown that the administration of probiotics in DSS-induced colitis mice can result in increased expression and production of mucus (**Fig. 1b**) (92,96,98,100,102,104). For example, in DSS-induced colitis mice it was shown that the administration of *Lactobacillus plantarum* KLDS 1.0386 and tryptophan resulted in increased expression of *Muc1* and *Muc2*, improving the epithelial barrier function (102). Besides, in another study it was shown that the administration of probiotic strains *Bifidobacterium breve* M1 and *B. breve* M2 each protected DSS-induced colitis mice from the destruction of goblet cells and mucus layer (98). In conclusion, studies investigating the mechanisms of action of probiotics in IBD disease models and patients showed that probiotics can affect cytokine and chemokine production, signaling, tight junctions and/or mucus production in IECs (**Fig. 1b**). However, the exact mechanisms are unknown, and the causes and consequences of the observed alterations are not clear. Furthermore, since most of these results are obtained from *in vitro* studies, *ex vivo* studies and animal models, it is not known how probiotics potentially can influence IECs and the epithelial barrier in IBD patients.

Mechanisms of Immunomodulation of Neutrophils by Probiotics in IBD

Neutrophils, which are the most abundant immune cells in the blood, are innate immune cells with antimicrobial activities (10,113). One of the hallmarks of active IBD is the infiltration of neutrophils into the intestinal barrier (**Fig. 1a**) (10). Additionally, fecal biomarkers for IBD disease activity include neutrophil proteins (10). Despite the potential role of neutrophils in IBD pathogenesis, research on neutrophils is limited, and IBD research has focused more on other immune cells (10). It has been suggested that neutrophils have a dual function in IBD pathogenesis, as they can have both protective and harmful effects (10). Neutrophils are responsible for the maintenance of the epithelial barrier, host defense and resolving inflammation, but they can also cause tissue injury and chronic inflammation (10). Besides, the interaction between neutrophils and the microbiome is likely also important for IBD pathogenesis, as neutrophils are able to modulate the microbiome composition through phagocytosis, reactive oxygen species production, production of anti-microbial peptides, and the production of pro-inflammatory cytokines (10). Additionally, many of the IBD risk loci are associated with neutrophils and their anti-microbial activity (10). This includes IBD risk loci involved in reactive oxygen species production (*e.g. CYBA, NCF4*), neutrophil autophagy (*e.g. PTAFR*), and neutrophil recruitment and migration *(e.g. LSP1* and *MMP9*) (10).

Despite the suggested roles of neutrophils in IBD pathogenesis, research on the effect of the administration of probiotics on neutrophils in IBD patients and disease models is limited. Studies that looked at the effect of the administration of probiotics on neutrophils in IBD patients and disease models all looked at neutrophil infiltration and/or recruitment (**Fig. 1b**) (76,91,94,99,100,114–121). For this, most studies looked at the levels of myeloperoxidase, which is commonly used as a marker for neutrophil infiltration (99). For example, the administration of the probiotic *Escherichia coli* strain Nissle 1917

reduced the number of neutrophils in the colon of DSS-induced colitis mice (118). In the same study, reduced levels of the neutrophil-recruiting chemokine CXCL1/KC were observed in the colon (118). In another study it was noted that the administration of *Bacillus smithii* XY1 reduced neutrophil mobilization in a DSS-induced colitis zebrafish model as shown by live imaging, and the effect was comparable to treatment with prednisolone (a corticosteroid), which is a commercial drug for IBD treatment (116). This effect on neutrophil mobilization was not observed when *Lactobacillus rhamnosus* GG, another probiotic strain, was administered (116). Besides, in a non-IBD cell culture model, consisting of murine bone-marrow derived neutrophils or differentiated HL-60 cells stimulated with PMA or *S. aureus* to induce neutrophil extracellular trap (NET) formation, it was observed that the administration of *L. rhamnosus* GG inhibited NET formation, reduced the production of reactive oxygen species by neutrophils, and dampened the phagocytic capacity (122). NETs are present in the inflamed tissue and may contribute to IBD pathogenesis (122,123). Thus, studies investigating the effect of administration of probiotics in IBD disease models and patients have shown that probiotics can affect neutrophil infiltration and recruitment (**Fig. 1b**). However, the number of studies is limited, and the mechanisms are unknown. Since it has been shown that neutrophils play a role in IBD pathogenesis, more research on the therapeutic potential of probiotics on neutrophils in IBD disease models and patients is required.

Mechanisms of Immunomodulation of Macrophages by Probiotics in IBD

Macrophages can be found along the GI tract, and they are involved in intestinal homeostasis (124,125). Dysregulation of macrophages is commonly observed in inflammatory diseases, including in IBD (124). Macrophages are known to adapt to their tissue of residence, and large heterogeneity is therefore observed among macrophages, which includes the presence of different kinds of macrophages in the GI tract (124,125). In healthy situations, macrophages are involved in phagocytosis of bacteria, clearing of apoptotic cells, production of immunoregulatory and pro-inflammatory cytokines to maintain gut homeostasis, interaction with T cells, and interaction with the nervous system (124). On the contrary, macrophages are also involved in IBD pathogenesis (**Fig. 1a**) (124). Macrophages contribute to IBD pathogenesis through different mechanisms, which includes differentiation into pro-inflammatory macrophages, production of pro-inflammatory cytokines, and an increased response against commensal bacteria (124). Furthermore, it has been noted that certain IBD risk loci are related to macrophage function, which includes the earlier discussed *NOD2* mutations that are also involved the function of other (immune) cells (*e.g.* IECs and neutrophils) (10,124). For macrophages specifically, it has been shown that *NOD2* mutations can lead to aberrant macrophage function, as *NOD2* variants result in increased activation and polarization of pro-inflammatory macrophages (124,126). Additionally, clinical remission in IBD post-treatment has been associated with a reduction in activity of pro-inflammatory macrophages and reduced recruitment of macrophages to the gut (124).

Since macrophages are involved in IBD pathogenesis, numerous studies have investigated the therapeutic potential of probiotics to target macrophages in IBD patients and disease models. Again, to study this, mainly colitis-induced mouse models were used. In general, it was observed that the administration of probiotics had an effect on macrophage polarization, cytokine production, signaling, and/or infiltration (**Fig. 1b**). First of all, most studies investigating the therapeutic potential of probiotics looked at the effect

of probiotics on macrophage polarization (**Fig. 1b**) (127–133). Classically, there are two types of macrophages, which are the pro-inflammatory and bactericidal M1 macrophages, and the M2 macrophages, which are associated with tissue repair and immunosuppression (124,134). It was shown that TNBS-induction of colitis increased the expression of M1 macrophage markers (arginase-2, IL-1ß, and TNF-α) in mice, whereas the expression of M2 markers (arginase-1, IL-10, and CD206) was reduced (135). In the same study it was noted that treatment with *Lactococcus lactis* EJ-1 suppressed the expression of the same M1 macrophage markers and restored the expression of the same M2 macrophage markers (135). The effect of *L. lactis* EJ-1 treatment on macrophages polarization was similar to the effect of treatment with sulfasalazine (a drug that is metabolized into 5-aminosalicylic acid in the colon), a commonly used medicine in IBD treatment (135). The observed differences in macrophage polarization after administration of probiotics could potentially be mediated by probiotics-induced alterations in signaling pathways (130). However, since it is difficult to correlate probiotics-induced alterations in signaling pathway to macrophage polarization in IBD disease models, no study has yet shown a direct association between them. In general, pathways associated with macrophage polarization include NF-κB, PI3K/Akt, MAPK, AP-1, and STAT (130). Several studies have shown that certain probiotic strains or a mixture of probiotics could alter signaling and influence macrophage polarization, and these changes could potentially be related to each other (**Fig. 1b**) (127,130,132,133,135–137). For example, in one study it was shown that a purified protein (GroEL) from *L. reuteri* was able to inhibit M1 macrophage markers (HLA-DR) and promote M2 macrophage polarization (CD206) *ex vivo*, and at the same time affect the TLR4 non-canonical pathway, which is involved in the production of the anti-inflammatory cytokine IL-10 (127). Besides, another observed effect of administration of probiotics on macrophages in IBD disease models is a reduction in macrophage infiltration (128,136,138). In one study it was shown that the probiotic mixture VSL#3, which is a popular commercially available probiotics mixture consisting of *B. breve, Bifidobacterium infantis, Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus delbrueckii, Lactobacillus paracasei, L. plantarum,* and *Streptococcus salivarius*, reduced the number of infiltrated macrophages in the mucosa and submucosa in the active colitis model, but not in the chronic colitis model (128,139). Lastly, cytokine production by macrophages could also be affected by the treatment with probiotics (**Fig. 1b**) (127–129,132,133,140,141). However, again this is difficult to prove in IBD disease models, as macrophages are not the only cells producing cytokines. In one study, peritoneal macrophages were isolated from healthy mice, before they were stimulated with LPS and treated with probiotics to determine the effect of probiotics on cytokine production by macrophages i*n vitro* (132). It was shown that *L. plantarum* CLP-0611 inhibited the expression of pro-inflammatory cytokines (TNF-α, IL-1ß, and IL-6) and activation of the pro-inflammatory signaling pathways NF-κB and AP1 (132). In the same study it was determined that treatment of TNBS-induced colitis mice with *L. plantarum* CLP-0611 resulted in inhibition of TNBS-induced M1 macrophage markers (arginase-2, IL-1ß, and TNF-α) expression and increased expression of a M2 macrophage markers (arginase-1, IL-10, and CD206) (132). Thus, studies investigating the therapeutic potential of probiotics in IBD disease models and patients showed that probiotics can affect macrophage polarization and infiltration, which could lead to reduced inflammation (**Fig. 1b**). In addition, studies have also suggested that probiotics could affect macrophage signaling and cytokines production (**Fig. 1b**). Further research is required to investigate the potential of probiotics to target macrophages in IBD patients *in vivo*.

Mechanisms of Immunomodulation of DCs by Probiotics in IBD

DCs are APCs that connect the innate immune system with the adaptive immune system (142). DCs regulate immunity or immune tolerance by cytokine production, cell-cell interactions, and the presentation of sampled antigens to T cells (143). Through the presentation of antigens, DCs are able to initiate naïve T cell differentiation towards inflammatory or regulatory phenotypes (144,145). There are different types of DCs with distinct features and functions (142,143,146). In the gut, DCs sample antigens from the intestinal lumen, and subsequently present these antigens to naïve T cells (144). Thereby, DCs are able to regulate T cell polarization in the gut, and mediate the balance between immune tolerance and immunity (144). Besides, the exact mechanisms are unknown, but it seems that DCs play an role in IBD pathogenesis (**Fig. 1a**) (145–147). Regulation of T cells by DCs is one of the proposed mechanisms of how DCs can contribute to IBD pathogenesis (148). Moreover, in some IBD patients it has been noted that the mucosa was enriched with (activated) DCs (146,149–151). Furthermore, in UC patients a reduction in $CD83^+$ DCs was observed, and the loss of $CD83^+$ DCs led to increased inflammation in a colitis mice model in a separate study (145,147). Moreover, it has been observed that the number of regulatory DCs in UC patients is reduced (152). Additionally, some of the IBD risk loci are associated with the function of DCs (10,153). For example, IBD risk loci have been identified that are associated with microbiota sensing (*e.g. CARD9*), and chemokine receptors (*e.g. CCR7*) (10,154).

Studies investigating the therapeutic potential of probiotics to target DCs in IBD patients and disease models either looked at DCs in colitis-induced mice models or studied the effects of probiotics on DCs *ex vivo*. In some of these *ex vivo* studies, probiotics-stimulated DCs were transferred into colitis-induced mouse models. First of all, a few studies looked at the effect of the administration of probiotics on the number of pro-inflammatory and/or immunoregulatory DCs in colitis-induced mice (**Fig. 1b**) (76,120,155–157). For example, the administration of *L. reuteri* to DSS-induced colitis mice reduced the number of pro-inflammatory $CD11b^+CD11^+$ DCs in mesenteric lymph nodes (76). In the same study it was shown that the induction of colitis by DSS treatment increased the number of bacterial antigens in mesenteric lymph nodes, whereas the administration of *L. reuteri* resulted in similar bacterial loads in the mesenteric lymph nodes to the control group (76). This is likely the result from altered DC functioning, as DCs are responsible for the transfer of bacterial antigens from the intestine to the mesenteric lymph nodes (76). Besides, in two other studies it was shown that the administration of probiotics in DSS-induced colitis mice could increase the number of immunoregulatory $CD103^+$ DCs (156,157). In the first study, it was noted that administration of the probiotic *L. plantarum* 22A-3 increased the number of $CD103^+$ DCs in the ileum of DSS-induced colitis mice (156). In the other study it was shown that the administration of *Enterobacter ludwigii*, which was isolated from the feces of mice treated with metronidazole (an antibiotic), resulted in increased numbers of $CD103^+$ DCs in the mesenteric lymph nodes and colonic lamina propria in DSS-induced colitis mice (157). In this study, the proposed mechanism for the increase in immunoregulatory activity and number of $CD103^+$ DCs was that choline, a metabolite produced by *E. ludwigii*, could bind to α7nAChR on the cell surface of $CD103^+$ DCs, which increased the expression of immunoregulatory genes, increasing the immunomodulatory activity of $CD103^+$ DCs (157). Besides, in both studies an increase in the number of Treg cells was observed, and this is likely related to the immunoregulatory function of $CD103^+$ DCs (156,157). Other studies investigating the therapeutic potential of probiotics to target DCs in IBD

patients and disease models, performed *ex vivo* studies of DCs, in which the expression of co-stimulatory molecules, DC markers and/or receptors, and cytokine production was investigated (80,81,158–161). For example, DCs from UC patients treated with the probiotic mixture VSL#3, corticosteroids, or placebo were analyzed for the expression of cytokines and activation markers (160). It was shown that VSL#3 treatment increased the production of the anti-inflammatory cytokine IL-10, and in addition a decrease in the production of IL-12p40 was observed (160). IL-12p40 is a subunit of the T cell stimulating cytokine IL-12 (160). Treatment with corticosteroids also resulted in increased production of IL-10 and reduced production of IL-12p40 (160). Besides, other studies have investigated the therapeutic potential of probiotics to induce tolerogenic DCs (tol-DCs) *ex vivo* (80,120,161). For example, in one study, mouse-derived DCs were incubated with different probiotic strains (120). It was noted that the probiotic strains *L. rhamnosus* Lr32 and *Lactobacillus salivarius* Ls33 were able to induce tol-DCs (120). When these probiotics-treated tol-DCs were intra-peritoneal administered to TNBS-induced colitis mice, alleviation of colitis and a reduction in inflammation scores were observed (120). The observed effects of probiotics-induced tol-DCs were similar to treatment with the anti-inflammatory drug prednisone (a corticosteroid) (120). Furthermore, the administration of the tol-DCs reduced the expression of pro-inflammatory cytokines (*e.g.* TNF-α, IL-6, and IL-1ß) (120). Moreover, an increase in number of Treg cells was observed (120). In conclusion, the administration of probiotics can affect the number of pro-inflammatory and immunoregulatory DCs in IBD disease models and patients (**Fig. 1b**). Additionally, the induction of tol-DCs by probiotics could potentially help IBD treatment in the future (148). Further research is needed to test the therapeutic potential of probiotics that target DCs in the treatment of IBD.

Mechanisms of Immunomodulation of T cells by Probiotics in IBD

Naïve T cells are activated after interaction with antigen-presenting DCs (162). In the gut, this process takes place in the gut-associated lymphoid tissue (GALT) and mesenteric lymph nodes (16). After activation, naïve T cell undergo proliferation and differentiation into $CD4^+$ or $CD8^+$ effector, memory, or regulatory T cells, with each T cell type having a distinct function (16,162). For example, intestinal Treg cells regulate T cells and B cells, thereby controlling the responses against pathogenic bacteria, commensal bacteria from the microbiome, and food antigens in the gut (163). T cells, in addition to other immune cells, are also involved in the IBD pathogenesis, however the exact mechanisms are not known (**Fig. 1a**) (21,24). It has been noted that in the inflamed intestine of CD patients, an increase in T helper 1 (Th1) cells and Th17 cells are observed (16,21–23). In contrast, increased levels of Th2 cells, Th9 cells and Th17 cells are observed in the inflamed colon of UC patients (16,20–23). The produced cytokines by all of these $CD4^+$ Th cells potentially contribute to IBD pathogenesis (21). Furthermore, in the inflamed mucosa of IBD patients, an increase in number of Treg cells has been shown (20,24,25). This could mean that these Treg cells are functionally deficient in the intestine of IBD patients, however this is unknown (16). Additionally, a relative increase in more pro-inflammatory Treg cells could also contribute to IBD pathogenesis, as well as a change in ratio of Treg cells and pro-inflammatory T cells (16,26). Moreover, it is not known whether the increase in Treg cells is a cause or consequence of inflammation in IBD (16). Memory T cells population may also contribute to IBD pathogenesis, especially to the chronicity of IBD (16). Furthermore, effector $CD8^+$ T cells are likely also involved in IBD pathogenesis (27,28). Additionally,

some of the IBD risk loci are T cells, including *RORC*, which encodes for the Th17 cell transcription factor RORγt, and *IL23A* and *IL12B*, which encode the heterodimer IL-23 (induces RORγt expression) (11).

Numerous studies have investigated the potential of targeting different types of T cells in IBD patients and disease models, including studies that used probiotics, with mixed success (164). The notion that bacteria can influence T cell proliferation and differentiation has been shown in non-IBD and IBD studies. For example, it has been shown that short-chain fatty acids, which are produced by commensal bacteria (*e.g. F. prausnitzii*), can boost the number of Treg cells (40,165). In another study, it was shown that the transfer of the microbiome of IBD patients into germ-free mice resulted in a more pro-inflammatory T cell population in the mice (166) Furthermore, it was shown that the administration of *B. infantis* EVC001 to breastfed infants beneficially altered T cell polarization (Th1-skewing) (167). Studies that investigated the therapeutic potential of probiotics to target T cells in IBD patients and disease models mainly looked at the number and/or ratio of different types of T cells, including Treg cells and Th17 cells (**Fig. 1b**) (76,99,104,120,121,129,155–157,168–189). In addition, some studies looked at T cell infiltration and/or T cell apoptosis (190–192). One of the earlier studies investigating the effect of the administration of probiotics on T cells showed that treatment with the probiotic mixture VSL#3 increased the number of $CD4^+$ Treg cells in TNBS-induced colitis mice (168). The transfer of these $CD4^+$ Treg cells to other TNBS-induced colitis mice prevented the development of colitis (168). In another study it was shown that the administration of cell surface ß-glucan/galactan (CSGG) polysaccharides obtained from the probiotic *Bifidobacterium bifidum* could prevent colitis formation in mice, reduce the number of pro-inflammatory cytokine-producing effector T cells, and increase the number of immunomodulatory Treg cells (177). These immunomodulatory Treg cells had a diverse specificity to commensal bacteria, *B. bifidum*, and food antigens (177). The suggested mechanism of action involved the conversion of standard DCs into regulatory DCs by CSGG polysaccharides, which was mediated by TLR2 signaling (177). These regulatory DCs subsequently produce anti-inflammatory cytokines, thereby regulating the number of Treg cells (177). The ratio of pro-inflammatory T cells and Treg cells in IBD patients can also be affected by inhibiting Th17 proliferation and differentiation (104,171,173,178,180,185). For example, it was shown that the administration of *B. uniformis* JCM5828 inhibited Th17 differentiation in DSS-induced colitis mice (104). The proposed mechanism is that *B. uniformis* JCM5828 increased the production of bile acids in the gut, which resulted in inhibition of Th17 cell differentiation (104). Administration of probiotics in IBD disease models can also affect T cell infiltration (190). It was observed that the administration of *L. paracasei* to a colitis mouse model reduced intestinal inflammation and reduced the number of infiltrated T cells (190). This reduction in infiltrated T cells was associated with reduced levels of IP-10, which is a pro-inflammatory chemokine (190). In the article it is shown that lactocepin, a protein from *L. paracasei*, was able to degrade IP-10, and as a consequence reduced intestinal inflammation in the colitis mouse model was observed (190). In conclusion, the administration of probiotics to IBD disease models and patients can influence T cell infiltration and/or the ratio of pro-inflammatory T cells and Treg cells, which potentially could help IBD patients (**Fig. 1b**). Further research is needed to study the therapeutic potential of probiotics on T cells in IBD patients *in vivo*.

Mechanisms of Immunomodulation of B cells by Probiotics in IBD

Along the gut, B cells can be found in the GALT and in the gut lamina propria (193). In the GALT, antigens sampled from the gut lumen are presented to B cells, either T-cell dependent or independent, which leads to immune induction and differentiation (193). After immune induction, B cells are transported via the blood into the gut lamina propria (193). Here, B cells undergo terminal differentiation into plasma cells (193). These plasma cells mainly produce IgA antibodies, which are then secreted into the gut lumen (193,194). The secreted IgA antibodies bind to antigens in the gut lumen, and thereby IgA antibodies can mediate the immune response, clearing of antigens, and regulation of the microbiome (193,195). In IBD patients it has been observed that the immune cell composition of the GALT and the gut lamina propria is altered (193,196). Furthermore, in the inflamed mucosa of IBD patients, there is a predominance of IgG antibodies, which is in contrast to the IgA predominance observed in the healthy gut (**Fig. 1a**) (16,193). The reduction in IgA antibodies, the dominance of IgG antibodies in the gut, or both could contribute to IBD pathogenesis (16). In mice, it has been noted that a decrease in IgA can lead to a reduction in microbiome diversity, and can result in intestinal inflammation (16,197). Besides, in another study it was shown that the induction of IgG antibodies against commensal bacteria resulted in intestinal inflammation (16,198). The proposed mechanism of how IgG predominance in the gut could lead to intestinal inflammation includes the infiltration of neutrophils, activation of macrophages, activation of the complement system, and increased production of pro-inflammatory cytokines (16,193,198). This is likely mediated by the interaction between IgG and the Fc-gamma receptors on the cell surface of neutrophils and macrophages (29). Additionally, several IBD risk loci are associated with B cell function, including a *FCGR2A* variant (encoding an antibody receptor), which changes the binding affinity for IgG (10,198,199). Besides, therapeutic studies targeting B cells in IBD patients are limited and have been unsuccessful (200). For example, treatment with rituximab, an anti-CD20 B cell-depleting antibody, did not result in remission of UC (16,200,201). Moreover, it has been noted that rituximab can even induce CD (200,202).

The number of studies investigating the potential of probiotics to target B cells in IBD disease is small (172,189,203,204). All studies at the minimum looked at the effect of administration of probiotics on IgA antibody production (**Fig. 1b**). In one of the studies, it was shown that the levels of secretory IgA were increased in the feces of DSS-induced colitis mice, and that the administration of *L. lactis* NCDO 2118 did not affect secretory IgA production (172). In contrast, in two other studies it was shown that IgA production was altered by administration of probiotics (189,203). The administration of *Coprococcus eutactus* resulted in increased IgA levels in the feces, colon tissue, and serum of DSS-induced colitis mice compared to untreated DSS-induced colitis mice (203). Furthermore, the number of IgA-producing plasma cells in the colon was increased after administration of *C. eutactus* (203). In contrast, in the other study it was shown that DSS treatment resulted in increased levels of IgA, IgG, and IgM in the gut mucosa, and that the administration of the probiotic mixture VSL#3 reduced the levels of these immunoglobulins (189). Besides, only one study extensively investigated the effect of probiotics administration on B cells in a IBD disease model (204). It was shown that DSS treatment resulted in reduced numbers of B cells in the GALT, smaller GALT size, increased accumulation of B cells in the mesenteric lymph nodes, increased infiltration of IgA^+ plasma cells into the gut, and the level of IgA antibodies bound per bacteria was increased (204). The administration of *L. reuteri* prevented gut disruption, and reduced colitis symptoms (204).

Furthermore, treatment with *L. reuteri* preserved the number of B cells in the GALT and GALT size, reduced IgA^+ plasma cell infiltration, reduced B cell accumulation in the mesenteric lymph nodes, and the level of IgA antibodies bound per bacteria was comparable to control mice (204). The suggested mechanism of action is that treatment with *L. reuteri* maintains the functions of GALT by interacting with immune cells in the GALT, and as a result, B cell functions and intestinal IgA production are controlled, leading to the prevention of intestinal inflammation (204). Thus, the administration of probiotics in IBD disease models can positively affect B cell function (**Fig. 1b**). However, the number of studies is limited, and the mechanisms are unknown. Further research is required to determine the mechanisms of action and the therapeutic potential of probiotics on B cells in IBD disease models and patients.

Conclusion

Overall, numerous studies have shown that probiotics can have immunomodulatory effects on IECs, neutrophils, macrophages, DCs, T cells, and/or B cells in IBD patients and disease models (**Fig. 1b**). It has been observed that the administration of probiotics can affect cytokine production, cell signaling, gene expression, cell differentiation, immune cell infiltration, number of pro-inflammatory and immunoregulatory cells, and/or the production of immunoglobulins (**Fig. 1b**). Some of these probiotic-induced alterations are likely connected to each other. However, the precise molecular mechanisms of immunomodulation are unknown for most of the studied probiotics in IBD patients and disease models.

The majority of the studies discussed in this literature review solely looked at the (clinical) outcomes of the administration of probiotics on immune cells, and did not investigate the molecular mechanisms of immunomodulation by probiotics. The discussed (clinical) outcomes of these studies include clinical symptoms, the production of immune cell-related products (e.g. cytokine production), and the presence or number of immune cells. Despite not discussing the molecular mechanisms, these studies can still contribute to the understanding of the immunomodulatory effects of probiotics. This is because the (clinical) outcomes can demonstrate which immune cells are affected by probiotics. Moreover, the observed (clinical) outcomes on immune cells could help direct research on the molecular mechanisms of immunomodulation by probiotics. Accordingly, investigating the (clinical) outcomes of the administration of probiotics in humans *in vivo* before studying the molecular mechanisms of immunomodulation by probiotics in IBD animal models and in *in vitro* studies, is likely the most effective and fastest way to identify beneficial immunomodulatory probiotics (51).

In contrast to the studies that looked at (clinical) outcomes, there are a few studies that have extensively investigated the molecular mechanisms of immunomodulation by probiotics, and how the observed effects on the different immune cells are related. Most of these studies are highlighted in this literature review. The studies investigating the molecular mechanisms of immunomodulation by probiotics in detail, commonly used a combination of *in vitro* and *in vivo* experiments. In these studies, it was shown that specific components or secreted molecules from probiotics were able to interact with a specific part of the immune system of the recipient. For example, choline secreted by *E. Ludwigii* was able to interact with α7nAChR on the cell surface of DCs, which resulted in the production of immunoregulatory molecules by the DCs, leading to Treg cell-mediated immune tolerance (157). Besides, in another study it was shown that *B. bifidum*—derived CSGG interacted with TLR2 of DCs, which resulted in the differentiation of DCs

into regulatory DCs (177). The increase in regulatory DCs led to an increased production of the inhibitory cytokines TGF-ß1 and IL-10, and subsequent induction of Treg cells (177). These two articles are good examples of studies that have investigated the molecular mechanisms of immunomodulation by probiotics in detail. More of these in-depth studies are needed to better understand the molecular mechanisms of immunomodulation by probiotics.

The need of in-depth studies on the molecular mechanisms of immunomodulation by probiotics is confirmed by the notion that probiotics have shown therapeutic potential *in vitro*, in animal disease models, and in some human *in vivo* studies, but not consistently in (large) studies investigating the potential of probiotics in the treatment of IBD patients. Several clinical studies have shown positive effects of the administration of probiotics on remission in IBD patients (2). However, numerous others did not show positive effects of probiotics administration (2). Therefore, future studies should, besides studying the molecular mechanisms of action of probiotics in detail, also investigate why certain health benefits of probiotics that are observed in *in vitro* studies and animal model studies are not translatable to IBD patients. Possible explanations for the lack of translatability and the contrasting results in clinical studies include human heterogeneity, disease heterogeneity, differences in used probiotic strain(s), differences in factors underlying CD or UC, and quality of the clinical studies (2,51). However, research is needed to investigate and establish this.

Besides, in future studies researchers should investigate the immunomodulatory effects of probiotics on all of the immune cells involved in IBD pathogenesis, and not focus on only a fraction of the immune cells. In the majority of studies included in this literature review, the immunomodulatory effects of administration of probiotics were only determined for some of the immune cells involved in IBD pathogenesis. As a consequence, the complete therapeutic potential of most probiotics remains unknown. In addition, investigating the immunomodulatory effect of a probiotic strain or mixture of probiotics for all immune cells involved in IBD pathogenesis may help contribute to the understanding of IBD pathogenesis and the role of specific immune cells in the pathogenesis of IBD.

Additionally, studies investigating the therapeutic potential of probiotics in (IBD) patients should focus more on patient-, disease-, and target-oriented research instead of only determining the clinical outcomes (51). In these future studies, specific probiotic strains or mixtures of probiotics should be devised beforehand instead of administering ‘random’ probiotics in the hope of obtaining beneficial health effects (51). The design of the specific probiotic strains or specific mixtures of probiotics should be based on the molecular target, disease of the recipient, and patient characteristics (51,68,77). Relevant patient characteristics include microbiota composition, host genetics and immune composition (51,68,77). Moreover, determining these patient characteristics before treatment can also help prevent probiotic colonization resistance, as it has been shown that probiotic colonization can be predicted based on patient characteristics (68). In addition to studying the effects of probiotics administration on clinical symptoms, also the effects on cytokine production, different immune cells, metabolites, and the microbiome should be investigated in future (large) clinical studies in humans, in order to completely understand the (immunomodulatory) effect of probiotics in (IBD) patients (51).

With the development of new techniques, the quality of studies investigating the therapeutic potential of probiotics will likely increase (77). One of the new techniques that could contribute to better understanding of the therapeutic potential of probiotics in IBD treatment, is a sampling capsule that collects luminal contents at different locations in the gut of humans *in vivo* (82,205). The obtained luminal liquid can be used for multi-omics analyses (82,205). With this sampling capsule, researchers were able to investigate the microbiome composition, host proteome, bile acids, and the metabolome in time and space, in humans (82,205). In addition to using new techniques, studies should also consider investigating the therapeutic potential of new probiotics (51). This includes the engineering or editing of existing probiotics to increase effectivity, or the identification of new microorganisms that can be used as probiotics (51,56,58,206). Furthermore, short-term and long-term safety of probiotics should also be investigated in more detail (51,207). This is especially important for the administration of probiotics in vulnerable target populations, which includes IBD patients (207). Additionally, treatment with probiotics can enhance antibiotic-resistance in the gut, and this should also be considered in future studies (69). Besides, similar to other medical treatments, medical regulation of probiotics is required to ensure effectiveness and safety of probiotics (51,55).

Altogether, the therapeutic potential of probiotics in the treatment of IBD is limited at the moment. Up to now, clinical studies have not shown clear and consistent, positive effects of the administration of probiotics in the treatment of IBD. Nevertheless, as discussed in this literature review, probiotics can have immunomodulatory effects in IBD disease models and patients. The immunomodulatory effects of specific probiotics may contribute to the treatment of CD and/or UC in the future, however more research is needed. The molecular mechanisms of immunomodulation by probiotics in IBD treatment should be investigated in-depth in future studies.

References

1. Li C, Peng K, Xiao S, Long Y, Yu Q. The role of Lactobacillus in inflammatory bowel disease: from actualities to prospects. Cell Death Discov. 2023 Sep 29;9(1):1–12.

2. Jensen BAH, Heyndrickx M, Jonkers D, Mackie A, Millet S, Naghibi M, et al. Small intestine vs. colon ecology and physiology: Why it matters in probiotic administration. Cell Rep Med. 2023 Sep;4(9):101190.

3. Caruso R, Lo BC, Núñez G. Host–microbiota interactions in inflammatory bowel disease. Nat Rev Immunol. 2020 Jul;20(7):411–26.

4. Roda G, Chien Ng S, Kotze PG, Argollo M, Panaccione R, Spinelli A, et al. Crohn's disease. Nat Rev Dis Primer. 2020 Apr 2;6(1):1–19.

5. Kobayashi T, Siegmund B, Le Berre C, Wei SC, Ferrante M, Shen B, et al. Ulcerative colitis. Nat Rev Dis Primer. 2020 Sep 10;6(1):1–20.

6. Dolinger M, Torres J, Vermeire S. Crohn's disease. The Lancet. 2024 Mar;403(10432):1177–91.

7. Piovani D, Danese S, Peyrin-Biroulet L, Nikolopoulos GK, Lytras T, Bonovas S. Environmental Risk Factors for Inflammatory Bowel Diseases: An Umbrella Review of Meta-analyses. Gastroenterology. 2019 Sep;157(3):647-659.e4.

8. Liu JZ, Van Sommeren S, Huang H, Ng SC, Alberts R, Takahashi A, et al. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. Nat Genet. 2015 Sep;47(9):979–86.

9. De Lange KM, Moutsianas L, Lee JC, Lamb CA, Luo Y, Kennedy NA, et al. Genome-wide association study implicates immune activation of multiple integrin genes in inflammatory bowel disease. Nat Genet. 2017 Feb;49(2):256–61.

10. Danne C, Skerniskyte J, Marteyn B, Sokol H. Neutrophils: from IBD to the gut microbiota. Nat Rev Gastroenterol Hepatol. 2024 Mar;21(3):184–97.

11. Graham DB, Xavier RJ. Pathway paradigms revealed from the genetics of inflammatory bowel disease. Nature. 2020 Feb 27;578(7796):527–39.

12. Rudbaek JJ, Agrawal M, Torres J, Mehandru S, Colombel JF, Jess T. Deciphering the different phases of preclinical inflammatory bowel disease. Nat Rev Gastroenterol Hepatol. 2024 Feb;21(2):86–100.

13. DeGruttola AK, Low D, Mizoguchi A, Mizoguchi E. Current Understanding of Dysbiosis in Disease in Human and Animal Models: Inflamm Bowel Dis. 2016 May;22(5):1137–50.

14. Allaire JM, Crowley SM, Law HT, Chang SY, Ko HJ, Vallance BA. The Intestinal Epithelium: Central Coordinator of Mucosal Immunity. Trends Immunol. 2018 Sep;39(9):677–96.

15. Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nat Rev Immunol. 2014 Mar;14(3):141–53.

16. Chang JT. Pathophysiology of Inflammatory Bowel Diseases. Longo DL, editor. N Engl J Med. 2020 Dec 31;383(27):2652–64.

17. Thoo L, Noti M, Krebs P. Keep calm: the intestinal barrier at the interface of peace and war. Cell Death Dis. 2019 Nov 7;10(11):849.

18. Martini E, Krug SM, Siegmund B, Neurath MF, Becker C. Mend Your Fences: The Epithelial Barrier and its Relationship With Mucosal Immunity in Inflammatory Bowel Disease. Cell Mol Gastroenterol Hepatol. 2017 Jul;4(1):33–46.

19. Le Berre C, Honap S, Peyrin-Biroulet L. Ulcerative colitis. The Lancet. 2023 Aug;402(10401):571–84.

20. De Souza HSP, Fiocchi C. Immunopathogenesis of IBD: current state of the art. Nat Rev Gastroenterol Hepatol. 2016 Jan;13(1):13–27.

21. Geremia A, Biancheri P, Allan P, Corazza GR, Di Sabatino A. Innate and adaptive immunity in inflammatory bowel disease. Autoimmun Rev. 2014 Jan;13(1):3–10.

22. Maynard CL, Weaver CT. Intestinal Effector T Cells in Health and Disease. Immunity. 2009 Sep;31(3):389–400.

23. Tindemans I, Joosse ME, Samsom JN. Dissecting the Heterogeneity in T-Cell Mediated Inflammation in IBD. Cells. 2020 Jan 2;9(1):110.

24. Clough JN, Omer OS, Tasker S, Lord GM, Irving PM. Regulatory T-cell therapy in Crohn's disease: challenges and advances. Gut. 2020 May;69(5):942–52.

25. Smids C, Horjus Talabur Horje CS, Drylewicz J, Roosenboom B, Groenen MJM, Van Koolwijk E, et al. Intestinal T Cell Profiling in Inflammatory Bowel Disease: Linking T Cell Subsets to Disease Activity and Disease Course. J Crohns Colitis. 2018 Mar 28;12(4):465–75.

26. Hovhannisyan Z, Treatman J, Littman DR, Mayer L. Characterization of Interleukin-17–Producing Regulatory T Cells in Inflamed Intestinal Mucosa From Patients With Inflammatory Bowel Diseases. Gastroenterology. 2011 Mar;140(3):957–65.

27. Casalegno Garduño R, Däbritz J. New Insights on CD8+ T Cells in Inflammatory Bowel Disease and Therapeutic Approaches. Front Immunol. 2021 Oct 11;12:738762.

28. Corridoni D, Antanaviciute A, Gupta T, Fawkner-Corbett D, Aulicino A, Jagielowicz M, et al. Single-cell atlas of colonic CD8+ T cells in ulcerative colitis. Nat Med. 2020 Sep;26(9):1480–90.

29. Canales-Herrerias P, Uzzan M, Seki A, Czepielewski RS, Verstockt B, Livanos AE, et al. Gut-associated lymphoid tissue attrition associates with response to anti-α4β7 therapy in ulcerative colitis. Sci Immunol. 2024 Apr 19;9(94):eadg7549.

30. Shan Y, Lee M, Chang EB. The Gut Microbiome and Inflammatory Bowel Diseases. Annu Rev Med. 2022 Jan 27;73(1):455–68.

31. Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. Cell Res. 2020 Jun;30(6):492–506.

32. Lavelle A, Sokol H. Gut microbiota-derived metabolites as key actors in inflammatory bowel disease. Nat Rev Gastroenterol Hepatol. 2020 Apr;17(4):223–37.

33. Gevers D, Kugathasan S, Denson LA, Vázquez-Baeza Y, Van Treuren W, Ren B, et al. The Treatment-Naive Microbiome in New-Onset Crohn's Disease. Cell Host Microbe. 2014 Mar;15(3):382–92.

34. Lewis JD, Chen EZ, Baldassano RN, Otley AR, Griffiths AM, Lee D, et al. Inflammation, Antibiotics, and Diet as Environmental Stressors of the Gut Microbiome in Pediatric Crohn's Disease. Cell Host Microbe. 2015 Oct;18(4):489–500.

35. Ning L, Zhou YL, Sun H, Zhang Y, Shen C, Wang Z, et al. Microbiome and metabolome features in inflammatory bowel disease via multi-omics integration analyses across cohorts. Nat Commun. 2023 Nov 6;14(1):7135.

36. Franzosa EA, Sirota-Madi A, Avila-Pacheco J, Fornelos N, Haiser HJ, Reinker S, et al. Gut microbiome structure and metabolic activity in inflammatory bowel disease. Nat Microbiol. 2018 Dec 10;4(2):293–305.

37. Vich Vila A, Imhann F, Collij V, Jankipersadsing SA, Gurry T, Mujagic Z, et al. Gut microbiota composition and functional changes in inflammatory bowel disease and irritable bowel syndrome. Sci Transl Med. 2018 Dec 19;10(472):eaap8914.

38. Lloyd-Price J, Arze C, Ananthakrishnan AN, Schirmer M, Avila-Pacheco J, Poon TW, et al. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. Nature. 2019 May;569(7758):655–62.

39. Hou K, Wu ZX, Chen XY, Wang JQ, Zhang D, Xiao C, et al. Microbiota in health and diseases. Signal Transduct Target Ther. 2022 Apr 23;7(1):135.

40. Lopez-Siles M, Duncan SH, Garcia-Gil LJ, Martinez-Medina M. *Faecalibacterium prausnitzii* : from microbiology to diagnostics and prognostics. ISME J. 2017 Apr 1;11(4):841–52.

41. Torres J, Bonovas S, Doherty G, Kucharzik T, Gisbert JP, Raine T, et al. ECCO Guidelines on Therapeutics in Crohn's Disease: Medical Treatment. J Crohns Colitis. 2020 Jan 1;14(1):4–22.

42. Raine T, Bonovas S, Burisch J, Kucharzik T, Adamina M, Annese V, et al. ECCO Guidelines on Therapeutics in Ulcerative Colitis: Medical Treatment. J Crohns Colitis. 2022 Jan 28;16(1):2–17.

43. Argollo M, Kotze PG, Kakkadasam P, D'Haens G. Optimizing biologic therapy in IBD: how essential is therapeutic drug monitoring? Nat Rev Gastroenterol Hepatol. 2020 Nov;17(11):702–10.

44. Kapizioni C, Desoki R, Lam D, Balendran K, Al-Sulais E, Subramanian S, et al. Biologic Therapy for Inflammatory Bowel Disease: Real-World Comparative Effectiveness and Impact of Drug Sequencing in 13 222 Patients within the UK IBD BioResource. J Crohns Colitis. 2023 Dec 2;jjad203.

45. Paramsothy S, Rosenstein AK, Mehandru S, Colombel JF. The current state of the art for biological therapies and new small molecules in inflammatory bowel disease. Mucosal Immunol. 2018 Nov;11(6):1558–70.

46. Yu H, MacIsaac D, Wong JJ, Sellers ZM, Wren AA, Bensen R, et al. Market share and costs of biologic therapies for inflammatory bowel disease in the USA. Aliment Pharmacol Ther. 2018 Feb;47(3):364–70.

47. Khoudari G, Mansoor E, Click B, Alkhayyat M, Saleh MA, Sinh P, et al. Rates of Intestinal Resection and Colectomy in Inflammatory Bowel Disease Patients After Initiation of Biologics: A Cohort Study. Clin Gastroenterol Hepatol. 2022 May;20(5):e974–83.

48. Lowe SC, Sauk JS, Limketkai BN, Kwaan MR. Declining Rates of Surgery for Inflammatory Bowel Disease in the Era of Biologic Therapy. J Gastrointest Surg. 2021 Jan;25(1):211–9.

49. Santiago P, Braga-Neto MB, Loftus EV. Novel Therapies for Patients With Inflammatory Bowel Disease. Gastroenterol Hepatol. 2022 Aug;18(8):453–65.

50. Pigneur B, Sokol H. Fecal microbiota transplantation in inflammatory bowel disease: the quest for the holy grail. Mucosal Immunol. 2016 Nov;9(6):1360–5.

51. Suez J, Zmora N, Segal E, Elinav E. The pros, cons, and many unknowns of probiotics. Nat Med. 2019 May;25(5):716–29.

52. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014 Aug;11(8):506–14.

53. Bron PA, van Baarlen P, Kleerebezem M. Emerging molecular insights into the interaction between probiotics and the host intestinal mucosa. Nat Rev Microbiol. 2012 Jan;10(1):66–78.

54. Metchnikoff É. The prolongation of life: optimistic studies [Internet]. Mitchell PC, editor. London: William Heinemann; 1907 [cited 2024 Apr 14]. Available from: https://wellcomecollection.org/works/a5439955

55. Sanders ME, Merenstein D, Merrifield CA, Hutkins R. Probiotics for human use. Nutr Bull. 2018 Sep;43(3):212–25.

56. Pan M, Morovic W, Hidalgo-Cantabrana C, Roberts A, Walden KKO, Goh YJ, et al. Genomic and epigenetic landscapes drive CRISPR-based genome editing in *Bifidobacterium*. Proc Natl Acad Sci. 2022 Jul 26;119(30):e2205068119.

57. Do H, Li ZR, Tripathi PK, Mitra S, Guerra S, Dash A, et al. Engineered probiotic overcomes pathogen defences using signal interference and antibiotic production to treat infection in mice. Nat Microbiol. 2024 Feb;9(2):502–13.

58. Khan MT, Dwibedi C, Sundh D, Pradhan M, Kraft JD, Caesar R, et al. Synergy and oxygen adaptation for development of next-generation probiotics. Nature. 2023 Aug;620(7973):381–5.

59. Chlebicz-Wójcik A, Śliżewska K. Probiotics, Prebiotics, and Synbiotics in the Irritable Bowel Syndrome Treatment: A Review. Biomolecules. 2021 Aug;11(8):1154.

60. Ng SC, Hart AL, Kamm MA, Stagg AJ, Knight SC. Mechanisms of Action of Probiotics: Recent Advances. Inflamm Bowel Dis. 2009 Feb 1;15(2):300–10.

61. Ma T, Shen X, Shi X, Sakandar HA, Quan K, Li Y, et al. Targeting gut microbiota and metabolism as the major probiotic mechanism - An evidence-based review. Trends Food Sci Technol. 2023 Aug;138:178–98.

62. Sanders ME, Merenstein DJ, Reid G, Gibson GR, Rastall RA. Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. Nat Rev Gastroenterol Hepatol. 2019 Oct;16(10):605–16.

63. Fukuda S, Toh H, Hase K, Oshima K, Nakanishi Y, Yoshimura K, et al. Bifidobacteria can protect from enteropathogenic infection through production of acetate. Nature. 2011 Jan;469(7331):543–7.

64. Mao N, Cubillos-Ruiz A, Cameron DE, Collins JJ. Probiotic strains detect and suppress cholera in mice. Sci Transl Med. 2018 Jun 13;10(445):eaao2586.

65. Pereira FC, Wasmund K, Cobankovic I, Jehmlich N, Herbold CW, Lee KS, et al. Rational design of a microbial consortium of mucosal sugar utilizers reduces Clostridiodes difficile colonization. Nat Commun. 2020 Oct 9;11(1):5104.

66. Suez J, Zmora N, Zilberman-Schapira G, Mor U, Dori-Bachash M, Bashiardes S, et al. Post-Antibiotic Gut Mucosal Microbiome Reconstitution Is Impaired by Probiotics and Improved by Autologous FMT. Cell. 2018 Sep 6;174(6):1406-1423.e16.

67. Kristensen NB, Bryrup T, Allin KH, Nielsen T, Hansen TH, Pedersen O. Alterations in fecal microbiota composition by probiotic supplementation in healthy adults: a systematic review of randomized controlled trials. Genome Med. 2016 May 10;8(1):52.

68. Zmora N, Zilberman-Schapira G, Suez J, Mor U, Dori-Bachash M, Bashiardes S, et al. Personalized Gut Mucosal Colonization Resistance to Empiric Probiotics Is Associated with Unique Host and Microbiome Features. Cell. 2018 Sep 6;174(6):1388-1405.e21.

69. Montassier E, Valdés-Mas R, Batard E, Zmora N, Dori-Bachash M, Suez J, et al. Probiotics impact the antibiotic resistance gene reservoir along the human GI tract in a person-specific and antibiotic-dependent manner. Nat Microbiol. 2021 Aug;6(8):1043–54.

70. Liu Q, Yu Z, Tian F, Zhao J, Zhang H, Zhai Q, et al. Surface components and metabolites of probiotics for regulation of intestinal epithelial barrier. Microb Cell Factories. 2020 Feb 5;19:23.

71. Gou HZ, Zhang YL, Ren LF, Li ZJ, Zhang L. How do intestinal probiotics restore the intestinal barrier? Front Microbiol. 2022 Jul 14;13:929346.

72. Patel S, Shukla R, Goyal A. Probiotics in valorization of innate immunity across various animal models. J Funct Foods. 2015 Apr 1;14:549–61.

73. Min S, Than N, Shin YC, Hu G, Shin W, Ambrosini YM, et al. Live probiotic bacteria administered in a pathomimetic Leaky Gut Chip ameliorate impaired epithelial barrier and mucosal inflammation. Sci Rep. 2022 Dec 31;12(1):22641.

74. Beterams A, De Paepe K, Maes L, Wise IJ, De Keersmaecker H, Rajkovic A, et al. Versatile human in vitro triple coculture model coincubated with adhered gut microbes reproducibly mimics pro-inflammatory host-microbe interactions in the colon. FASEB J. 2021 Nov 1;35(12):e21992.

75. Yang C, Merlin D. Unveiling Colitis: A Journey through the Dextran Sodium Sulfate-induced Model. Inflamm Bowel Dis. 2024 Jan 27;izad312.

76. Liu HY, Gu F, Zhu C, Yuan L, Zhu C, Zhu M, et al. Epithelial Heat Shock Proteins Mediate the Protective Effects of Limosilactobacillus reuteri in Dextran Sulfate Sodium-Induced Colitis. Front Immunol. 2022 Mar 7;13:865982.

77. Kiousi DE, Rathosi M, Tsifintaris M, Chondrou P, Galanis A. Pro-biomics: Omics Technologies To Unravel the Role of Probiotics in Health and Disease. Adv Nutr. 2021 Feb 24;12(5):1802–20.

78. Burton KJ, Pimentel G, Zangger N, Vionnet N, Drai J, McTernan PG, et al. Modulation of the peripheral blood transcriptome by the ingestion of probiotic yoghurt and acidified milk in healthy, young men. Passos GA, editor. PLOS ONE. 2018 Feb 28;13(2):e0192947.

79. Heldner A, Heath MD, Schnautz B, Kotz S, Chaker A, Kramer MF, et al. Ex Vivo Immunomodulatory Effects of Lactobacillus-, Lacticaseibacillus-, and Bifidobacterium-Containing Synbiotics on Human Peripheral Blood Mononuclear Cells and Monocyte-Derived Dendritic Cells in the Context of Grass Pollen Allergy. Probiotics Antimicrob Proteins. 2023 Aug;15(4):868–79.

80. Ghavami SB, Yadegar A, Aghdaei HA, Sorrentino D, Farmani M, Mir AS, et al. Immunomodulation and Generation of Tolerogenic Dendritic Cells by Probiotic Bacteria in Patients with Inflammatory Bowel Disease. Int J Mol Sci. 2020 Jan;21(17):6266.

81. Thomas S, Metzke D, Schmitz J, Dörffel Y, Baumgart DC. Anti-inflammatory effects of *Saccharomyces boulardii* mediated by myeloid dendritic cells from patients with Crohn's disease and ulcerative colitis. Am J Physiol-Gastrointest Liver Physiol. 2011 Dec;301(6):G1083–92.

82. Folz J, Culver RN, Morales JM, Grembi J, Triadafilopoulos G, Relman DA, et al. Human metabolome variation along the upper intestinal tract. Nat Metab. 2023 May;5(5):777–88.

83. Horowitz JE, Warner N, Staples J, Crowley E, Gosalia N, Murchie R, et al. Mutation spectrum of NOD2 reveals recessive inheritance as a main driver of Early Onset Crohn's Disease. Sci Rep. 2021 Mar 10;11(1):5595.

84. Malik A, Sharma D, Aguirre-Gamboa R, McGrath S, Zabala S, Weber C, et al. Epithelial IFNγ signalling and compartmentalized antigen presentation orchestrate gut immunity. Nature. 2023 Nov 30;623(7989):1044–52.

85. Abraham C, Cho JH. Inflammatory Bowel Disease. N Engl J Med. 2009 Nov 19;361(21):2066–78.

86. Jalili-Firoozinezhad S, Gazzaniga FS, Calamari EL, Camacho DM, Fadel CW, Bein A, et al. A complex human gut microbiome cultured in an anaerobic intestine-on-a-chip. Nat Biomed Eng. 2019 May 13;3(7):520–31.

87. Fitzpatrick LR, Small J, Hoerr RA, Bostwick EF, Maines L, Koltun WA. In vitro and in vivo effects of the probiotic Escherichia coli strain M-17: immunomodulation and attenuation of murine colitis. Br J Nutr. 2008 Sep;100(03):530–41.

88. Li Y, Yang S, Lun J, Gao J, Gao X, Gong Z, et al. Inhibitory Effects of the Lactobacillus rhamnosus GG Effector Protein HM0539 on Inflammatory Response Through the TLR4/MyD88/NF-κB Axis. Front Immunol. 2020 Oct 5;11:551449.

89. Sato N, Garcia-Castillo V, Yuzawa M, Islam MdA, Albarracin L, Tomokiyo M, et al. Immunobiotic Lactobacillus jensenii TL2937 Alleviates Dextran Sodium Sulfate-Induced Colitis by Differentially Modulating the Transcriptomic Response of Intestinal Epithelial Cells. Front Immunol. 2020 Sep 17;11:2174.

90. Llopis M, Antolin M, Carol M, Borruel N, Casellas F, Martinez C, et al. Lactobacillus casei downregulates commensals' inflammatory signals in Crohn's disease mucosa: Inflamm Bowel Dis. 2009 Feb;15(2):275–83.

91. Shi J, Xie Q, Yue Y, Chen Q, Zhao L, Evivie SE, et al. Gut microbiota modulation and anti-inflammatory properties of mixed lactobacilli in dextran sodium sulfate-induced colitis in mice. Food Funct. 2021 Apr 21;12(11):5130–43.

92. Wong WY, Chan BD, Sham TT, Lee MML, Chan CO, Chau CT, et al. Lactobacillus casei Strain Shirota Ameliorates Dextran Sulfate Sodium-Induced Colitis in Mice by Increasing Taurine-Conjugated Bile Acids and Inhibiting NF-κB Signaling via Stabilization of IκBα. Front Nutr. 2022;9:816836.

93. Choi SH, Lee SH, Kim MG, Lee HJ, Kim GB. Lactobacillus plantarum CAU1055 ameliorates inflammation in lipopolysaccharide-induced RAW264.7 cells and a dextran sulfate sodium–induced colitis animal model. J Dairy Sci. 2019 Aug;102(8):6718–25.

94. Chung YW, Choi JH, Oh TY, Eun CS, Han DS. *Lactobacillus casei* prevents the development of dextran sulphate sodium-induced colitis in Toll-like receptor 4 mutant mice. Clin Exp Immunol. 2007 Dec 7;151(1):182–9.

95. Fan L, Qi Y, Qu S, Chen X, Li A, Hendi M, et al. *B. adolescentis* ameliorates chronic colitis by regulating Treg/Th2 response and gut microbiota remodeling. Gut Microbes. 2021 Jan 1;13(1):1826746.

96. Chen Y, Yang B, Stanton C, Ross RP, Zhao J, Zhang H, et al. *Bifidobacterium pseudocatenulatum* Ameliorates DSS-Induced Colitis by Maintaining Intestinal Mechanical Barrier, Blocking Proinflammatory Cytokines, Inhibiting TLR4/NF-κB Signaling, and Altering Gut Microbiota. J Agric Food Chem. 2021 Feb 10;69(5):1496–512.

97. Wang N, Wang S, Xu B, Liu F, Huo G, Li B. Alleviation Effects of Bifidobacterium animalis subsp. lactis XLTG11 on Dextran Sulfate Sodium-Induced Colitis in Mice. Microorganisms. 2021 Oct 3;9(10):2093.

98. Chen Y, Jin Y, Stanton C, Paul Ross R, Zhao J, Zhang H, et al. Alleviation effects of Bifidobacterium breve on DSS-induced colitis depends on intestinal tract barrier maintenance and gut microbiota modulation. Eur J Nutr. 2021 Feb;60(1):369–87.

99. Martín R, Chain F, Miquel S, Lu J, Gratadoux JJ, Sokol H, et al. The Commensal Bacterium Faecalibacterium prausnitzii Is Protective in DNBS-induced Chronic Moderate and Severe Colitis Models: Inflamm Bowel Dis. 2014 Mar;20(3):417–30.

100. Ahl D, Liu H, Schreiber O, Roos S, Phillipson M, Holm L. *Lactobacillus reuteri* increases mucus thickness and ameliorates dextran sulphate sodium-induced colitis in mice. Acta Physiol. 2016 Aug;217(4):300–10.

101. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermúdez-Humarán LG, Gratadoux JJ, et al. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci. 2008 Oct 28;105(43):16731–6.

102. Shi J, Du P, Xie Q, Wang N, Li H, Smith EE, et al. Protective effects of tryptophan-catabolizing *Lactobacillus plantarum* KLDS 1.0386 against dextran sodium sulfate-induced colitis in mice. Food Funct. 2020 Nov 2;11(12):10736–47.

103. Kumar M, Kissoon-Singh V, Coria AL, Moreau F, Chadee K. Probiotic mixture VSL#3 reduces colonic inflammation and improves intestinal barrier function in Muc2 mucin-deficient mice. Am J Physiol-Gastrointest Liver Physiol. 2017 Jan 1;312(1):G34–45.

104. Yan Y, Lei Y, Qu Y, Fan Z, Zhang T, Xu Y, et al. Bacteroides uniformis-induced perturbations in colonic microbiota and bile acid levels inhibit TH17 differentiation and ameliorate colitis developments. Npj Biofilms Microbiomes. 2023 Aug 14;9(1):56.

105. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. Signal Transduct Target Ther. 2017 Jul 14;2(1):17023.

106. Gao C, Major A, Rendon D, Lugo M, Jackson V, Shi Z, et al. Histamine H2 Receptor-Mediated Suppression of Intestinal Inflammation by Probiotic Lactobacillus reuteri. McFall-Ngai MJ, editor. mBio. 2015 Dec 31;6(6):e01358-15.

107. Quévrain E, Maubert MA, Michon C, Chain F, Marquant R, Tailhades J, et al. Identification of an anti-inflammatory protein from *Faecalibacterium prausnitzii* , a commensal bacterium deficient in Crohn's disease. Gut. 2016 Mar;65(3):415–25.

108. Breyner NM, Michon C, De Sousa CS, Vilas Boas PB, Chain F, Azevedo VA, et al. Microbial Anti-Inflammatory Molecule (MAM) from Faecalibacterium prausnitzii Shows a Protective Effect on DNBS and DSS-Induced Colitis Model in Mice through Inhibition of NF-κB Pathway. Front Microbiol. 2017 Feb 1;8:114.

109. Al-Sadi R, Nighot P, Nighot M, Haque M, Rawat M, Ma TY. Lactobacillus acidophilus Induces a Strain-specific and Toll-Like Receptor 2–Dependent Enhancement of Intestinal Epithelial Tight Junction Barrier and Protection Against Intestinal Inflammation. Am J Pathol. 2021 May;191(5):872–84.

110. Kil BJ, Pyung YJ, Park H, Kang JW, Yun CH, Huh CS. Probiotic potential of Saccharomyces cerevisiae GILA with alleviating intestinal inflammation in a dextran sulfate sodium induced colitis mouse model. Sci Rep. 2023 Apr 24;13(1):6687.

111. Zhang Y, Zhao X, Zhu Y, Ma J, Ma H, Zhang H. Probiotic Mixture Protects Dextran Sulfate Sodium-Induced Colitis by Altering Tight Junction Protein Expressions and Increasing Tregs. Mediators Inflamm. 2018 Apr 15;2018:1–11.

112. Liu Y, Yin F, Huang L, Teng H, Shen T, Qin H. Long-term and continuous administration of *Bacillus subtilis* during remission effectively maintains the remission of inflammatory bowel disease by protecting

intestinal integrity, regulating epithelial proliferation, and reshaping microbial structure and function. Food Funct. 2021 Feb 17;12(5):2201–10.

113. Burn GL, Foti A, Marsman G, Patel DF, Zychlinsky A. The Neutrophil. Immunity. 2021 Jul;54(7):1377–91.

114. Wu Y, Jha R, Li A, Liu H, Zhang Z, Zhang C, et al. Probiotics (Lactobacillus plantarum HNU082) Supplementation Relieves Ulcerative Colitis by Affecting Intestinal Barrier Functions, Immunity-Related Gene Expression, Gut Microbiota, and Metabolic Pathways in Mice. Tang X, editor. Microbiol Spectr. 2022 Dec 21;10(6):e01651-22.

115. Chen H, Lei P, Ji H, Ma J, Fang Y, Yu H, et al. Escherichia coli Nissle 1917 ghosts alleviate inflammatory bowel disease in zebrafish. Life Sci. 2023 Sep;329:121956.

116. Huang X, Ai F, Ji C, Tu P, Gao Y, Wu Y, et al. A Rapid Screening Method of Candidate Probiotics for Inflammatory Bowel Diseases and the Anti-inflammatory Effect of the Selected Strain Bacillus smithii XY1. Front Microbiol. 2021 Dec 17;12:760385.

117. Park JS, Joe I, Rhee PD, Jeong CS, Jeong G. A lactic acid bacterium isolated from kimchi ameliorates intestinal inflammation in DSS-induced colitis. J Microbiol. 2017 Apr;55(4):304–10.

118. Souza ÉL, Elian SD, Paula LM, Garcia CC, Vieira AT, Teixeira MM, et al. Escherichia coli strain Nissle 1917 ameliorates experimental colitis by modulating intestinal permeability, the inflammatory response and clinical signs in a faecal transplantation model. J Med Microbiol. 2016 Mar 1;65(3):201–10.

119. Sha S, Xu B, Kong X, Wei N, Liu J, Wu K. Preventive effects of Escherichia coli strain Nissle 1917 with different courses and different doses on intestinal inflammation in murine model of colitis. Inflamm Res. 2014 Oct;63(10):873–83.

120. Foligne B, Zoumpopoulou G, Dewulf J, Ben Younes A, Chareyre F, Sirard JC, et al. A Key Role of Dendritic Cells in Probiotic Functionality. PLoS ONE. 2007 Mar 21;2(3):e313.

121. Rossi O, Van Berkel LA, Chain F, Tanweer Khan M, Taverne N, Sokol H, et al. Faecalibacterium prausnitzii A2-165 has a high capacity to induce IL-10 in human and murine dendritic cells and modulates T cell responses. Sci Rep. 2016 Jan 4;6(1):18507.

122. Drury B, Hardisty G, Gray RD, Ho G tzer. Neutrophil Extracellular Traps in Inflammatory Bowel Disease: Pathogenic Mechanisms and Clinical Translation. Cell Mol Gastroenterol Hepatol. 2021 Mar 6;12(1):321–33.

123. Vong L, Lorentz RJ, Assa A, Glogauer M, Sherman PM. Probiotic *Lactobacillus rhamnosus* Inhibits the Formation of Neutrophil Extracellular Traps. J Immunol. 2014 Feb 15;192(4):1870–7.

124. Hegarty LM, Jones GR, Bain CC. Macrophages in intestinal homeostasis and inflammatory bowel disease. Nat Rev Gastroenterol Hepatol. 2023 Aug;20(8):538–53.

125. Na YR, Stakenborg M, Seok SH, Matteoli G. Macrophages in intestinal inflammation and resolution: a potential therapeutic target in IBD. Nat Rev Gastroenterol Hepatol. 2019 Sep;16(9):531–43.

126. Nayar S, Morrison JK, Giri M, Gettler K, Chuang L shiang, Walker LA, et al. A myeloid–stromal niche and gp130 rescue in NOD2-driven Crohn's disease. Nature. 2021 May 13;593(7858):275–81.

127. Dias AMM, Douhard R, Hermetet F, Regimbeau M, Lopez TE, Gonzalez D, et al. Lactobacillus stress protein GroEL prevents colonic inflammation. J Gastroenterol. 2021 May;56(5):442–55.

128. Isidro RA, Lopez A, Cruz ML, Gonzalez Torres MI, Chompre G, Isidro AA, et al. The Probiotic VSL#3 Modulates Colonic Macrophages, Inflammation, and Microflora in Acute Trinitrobenzene Sulfonic Acid Colitis. J Histochem Cytochem. 2017 Aug;65(8):445–61.

129. Kim DH, Kim S, Lee JH, Kim JH, Che X, Ma HW, et al. *Lactobacillus acidophilus* suppresses intestinal inflammation by inhibiting endoplasmic reticulum stress. J Gastroenterol Hepatol. 2019 Jan;34(1):178–85.

130. Wang Y, Liu H, Zhao J. Macrophage Polarization Induced by Probiotic Bacteria: a Concise Review. Probiotics Antimicrob Proteins. 2020 Sep 1;12(3):798–808.

131. Liang L, Yang C, Liu L, Mai G, Li H, Wu L, et al. Commensal bacteria-derived extracellular vesicles suppress ulcerative colitis through regulating the macrophages polarization and remodeling the gut microbiota. Microb Cell Factories. 2022 Dec;21(1):88.

132. Jang SE, Han MJ, Kim SY, Kim DH. Lactobacillus plantarum CLP-0611 ameliorates colitis in mice by polarizing M1 to M2-like macrophages. Int Immunopharmacol. 2014 Jul;21(1):186–92.

133. Jang SE, Hyam SR, Han MJ, Kim SY, Lee BG, Kim DH. *Lactobacillus brevis* G-101 ameliorates colitis in mice by inhibiting NF-κB, MAPK and AKT pathways and by polarizing M1 macrophages to M2-like macrophages. J Appl Microbiol. 2013 Sep;115(3):888–96.

134. Chen S, Saeed AFUH, Liu Q, Jiang Q, Xu H, Xiao GG, et al. Macrophages in immunoregulation and therapeutics. Signal Transduct Target Ther. 2023 May 22;8(1):207.

135. Jang S, Min S. Amelioration of colitis in mice by *Leuconostoc lactis* EJ-1 by M1 to M2 macrophage polarization. Microbiol Immunol. 2020 Feb;64(2):133–42.

136. Bassaganya-Riera J, Viladomiu M, Pedragosa M, De Simone C, Carbo A, Shaykhutdinov R, et al. Probiotic Bacteria Produce Conjugated Linoleic Acid Locally in the Gut That Targets Macrophage PPAR γ to Suppress Colitis. Heimesaat MM, editor. PLoS ONE. 2012 Feb 21;7(2):e31238.

137. Sun S, Xu X, Liang L, Wang X, Bai X, Zhu L, et al. Lactic Acid-Producing Probiotic Saccharomyces cerevisiae Attenuates Ulcerative Colitis via Suppressing Macrophage Pyroptosis and Modulating Gut Microbiota. Front Immunol. 2021 Nov 24;12:777665.

138. Xu L, Liu B, Huang L, Li Z, Cheng Y, Tian Y, et al. Probiotic Consortia and Their Metabolites Ameliorate the Symptoms of Inflammatory Bowel Diseases in a Colitis Mouse Model. Shen Z, editor. Microbiol Spectr. 2022 Aug 31;10(4):e00657-22.

139. Arthur JC, Gharaibeh RZ, Uronis JM, Perez-Chanona E, Sha W, Tomkovich S, et al. VSL#3 probiotic modifies mucosal microbial composition but does not reduce colitis-associated colorectal cancer. Sci Rep. 2013 Oct 8;3(1):2868.

140. Pathmakanthan S, Li CK, Cowie J, Hawkey CJ. *Lactobacillus plantarum* 299: Beneficial *in vitro* immunomodulation in cells extracted from inflamed human colon. J Gastroenterol Hepatol. 2004 Feb;19(2):166–73.

141. Liu M, Ding J, Zhang H, Shen J, Hao Y, Zhang X, et al. *Lactobacillus casei* LH23 modulates the immune response and ameliorates DSS-induced colitis *via* suppressing JNK/p-38 signal pathways and enhancing histone H3K9 acetylation. Food Funct. 2020 May 11;11(6):5473–85.

142. Collin M, Bigley V. Human dendritic cell subsets: an update. Immunology. 2018 May;154(1):3–20.

143. Wculek SK, Cueto FJ, Mujal AM, Melero I, Krummel MF, Sancho D. Dendritic cells in cancer immunology and immunotherapy. Nat Rev Immunol. 2020 Jan;20(1):7–24.

144. Luciani C, Hager FT, Cerovic V, Lelouard H. Dendritic cell functions in the inductive and effector sites of intestinal immunity. Mucosal Immunol. 2022 Jan;15(1):40–50.

145. Despalatović BR, Babić M, Bratanić A, Tonkić A, Vilović K. Difference in Presence and Number of CD83+ Dendritic Cells in Patients with Ulcerative Colitis and Crohn's Disease. Sci Rep. 2020 Jun 22;10(1):10055.

146. Jiménez-Cortegana C, Palomares F, Alba G, Santa-María C, De La Cruz-Merino L, Sánchez-Margalet V, et al. Dendritic cells: the yin and yang in disease progression. Front Immunol. 2024 Jan 4;14:1321051.

147. Bates JM, Flanagan K, Mo L, Ota N, Ding J, Ho S, et al. Dendritic cell CD83 homotypic interactions regulate inflammation and promote mucosal homeostasis. Mucosal Immunol. 2015 Mar;8(2):414–28.

148. Baradaran Ghavami S, Asadzadeh Aghdaei H, Sorrentino D, Shahrokh S, Farmani M, Ashrafian F, et al. Probiotic-Induced Tolerogenic Dendritic Cells: A Novel Therapy for Inflammatory Bowel Disease? Int J Mol Sci. 2021 Jan;22(15):8274.

149. Mitsialis V, Wall S, Liu P, Ordovas-Montanes J, Parmet T, Vukovic M, et al. Single-Cell Analyses of Colon and Blood Reveal Distinct Immune Cell Signatures of Ulcerative Colitis and Crohn's Disease. Gastroenterology. 2020 Aug;159(2):591-608.e10.

150. Chapuy L, Sarfati M. Single-Cell Protein and RNA Expression Analysis of Mononuclear Phagocytes in Intestinal Mucosa and Mesenteric Lymph Nodes of Ulcerative Colitis and Crohn's Disease Patients. Cells. 2020 Mar 27;9(4):813.

151. Volarevic V, Zdravkovic N, Harrell CR, Arsenijevic N, Fellabaum C, Djonov V, et al. Galectin-3 Regulates Indoleamine-2,3-dioxygenase-Dependent Cross-Talk between Colon-Infiltrating Dendritic Cells and T Regulatory Cells and May Represent a Valuable Biomarker for Monitoring the Progression of Ulcerative Colitis. Cells. 2019 Jul 12;8(7):709.

152. Mann ER, Bernardo D, Ng SC, Rigby RJ, Al-Hassi HO, Landy J, et al. Human Gut Dendritic Cells Drive Aberrant Gut-specific T-cell Responses in Ulcerative Colitis, Characterized by Increased IL-4 Production and Loss of IL-22 and IFNγ: Inflamm Bowel Dis. 2014 Dec;20(12):2299–307.

153. Steinbach EC, Plevy SE. The Role of Macrophages and Dendritic Cells in the Initiation of Inflammation in IBD: Inflamm Bowel Dis. 2014 Jan;20(1):166–75.

154. Sazonovs A, Stevens CR, Venkataraman GR, Yuan K, Avila B, Abreu MT, et al. Large-scale sequencing identifies multiple genes and rare variants associated with Crohn's disease susceptibility. Nat Genet. 2022 Sep;54(9):1275–83.

155. Fernandez EM, Valenti V, Rockel C, Hermann C, Pot B, Boneca IG, et al. Anti-inflammatory capacity of selected lactobacilli in experimental colitis is driven by NOD2-mediated recognition of a specific peptidoglycan-derived muropeptide. Gut. 2011 Aug 1;60(8):1050–9.

156. Lamubol J, Ohto N, Kuwahara H, Mizuno M. *Lactiplantibacillus plantarum* 22A-3-induced TGF-β1 secretion from intestinal epithelial cells stimulated CD103 $^{+}$ DC and Foxp3 $^{+}$ Treg differentiation and amelioration of colitis in mice. Food Funct. 2021 Jul 12;12(17):8044–55.

157. Li Q, Sun X, Yu K, Lv J, Miao C, Yang J, et al. Enterobacter ludwigii protects DSS-induced colitis through choline-mediated immune tolerance. Cell Rep. 2022 Aug;40(9):111308.

158. Al-Hassi HO, Mann ER, Sanchez B, English NR, Peake STC, Landy J, et al. Altered human gut dendritic cell properties in ulcerative colitis are reversed by *Lactobacillus plantarum* extracellular encrypted peptide STp. Mol Nutr Food Res. 2014 May;58(5):1132–43.

159. Mann ER, You J, Horneffer-van Der Sluis V, Bernardo D, Omar Al-Hassi H, Landy J, et al. Dysregulated Circulating Dendritic Cell Function in Ulcerative Colitis Is Partially Restored by Probiotic Strain *Lactobacillus casei* Shirota. Mediators Inflamm. 2013 Jul 18;2013:1–12.

160. Ng SC, Plamondon S, Kamm MA, Hart AL, Al-Hassi HO, Guenther T, et al. Immunosuppressive effects via human intestinal dendritic cells of probiotic bacteria and steroids in the treatment of acute ulcerative colitis: Inflamm Bowel Dis. 2010 Aug;16(8):1286–98.

161. Okada Y, Tsuzuki Y, Sugihara N, Nishii S, Shibuya N, Mizoguchi A, et al. Novel probiotic yeast from Miso promotes regulatory dendritic cell IL-10 production and attenuates DSS-induced colitis in mice. J Gastroenterol. 2021 Sep;56(9):829–42.

162. Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. Nat Rev Immunol. 2020 Nov;20(11):651–68.

163. Ramanan D, Pratama A, Zhu Y, Venezia O, Sassone-Corsi M, Chowdhary K, et al. Regulatory T cells in the face of the intestinal microbiota. Nat Rev Immunol. 2023 Nov;23(11):749–62.

164. Giuffrida P, Di Sabatino A. Targeting T cells in inflammatory bowel disease. Pharmacol Res. 2020 Sep;159:105040.

165. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature. 2013 Dec;504(7480):451–5.

166. Britton GJ, Contijoch EJ, Mogno I, Vennaro OH, Llewellyn SR, Ng R, et al. Microbiotas from Humans with Inflammatory Bowel Disease Alter the Balance of Gut Th17 and RORγt+ Regulatory T Cells and Exacerbate Colitis in Mice. Immunity. 2019 Jan;50(1):212-224.e4.

167. Henrick BM, Rodriguez L, Lakshmikanth T, Pou C, Henckel E, Arzoomand A, et al. Bifidobacteria-mediated immune system imprinting early in life. Cell. 2021 Jul 22;184(15):3884-3898.e11.

168. Di Giacinto C, Marinaro M, Sanchez M, Strober W, Boirivant M. Probiotics Ameliorate Recurrent Th1-Mediated Murine Colitis by Inducing IL-10 and IL-10-Dependent TGF-β-Bearing Regulatory Cells. J Immunol. 2005 Mar 15;174(6):3237–46.

169. Kwon HK, Lee CG, So JS, Chae CS, Hwang JS, Sahoo A, et al. Generation of regulatory dendritic cells and $CD4^+$ $Foxp3^+$ T cells by probiotics administration suppresses immune disorders. Proc Natl Acad Sci. 2010 Feb 2;107(5):2159–64.

170. Roselli M, Finamore A, Nuccitelli S, Carnevali P, Brigidi P, Vitali B, et al. Prevention of TNBS-induced colitis by different Lactobacillus and Bifidobacterium strains is associated with an expansion of γδT and regulatory T cells of intestinal intraepithelial lymphocytes: Inflamm Bowel Dis. 2009 Oct;15(10):1526–36.

171. Jia L, Wu R, Han N, Fu J, Luo Z, Guo L, et al. *Porphyromonas gingivalis* and *Lactobacillus rhamnosus* GG regulate the Th17/Treg balance in colitis via TLR4 and TLR2. Clin Transl Immunol. 2020 Jan;9(11):e1213.

172. Luerce TD, Gomes-Santos AC, Rocha CS, Moreira TG, Cruz DN, Lemos L, et al. Anti-inflammatory effects of Lactococcus lactis NCDO 2118 during the remission period of chemically induced colitis. Gut Pathog. 2014 Jul;6(1):33.

173. Park JS, Choi JW, Jhun J, Kwon JY, Lee BI, Yang CW, et al. *Lactobacillus acidophilus* Improves Intestinal Inflammation in an Acute Colitis Mouse Model by Regulation of Th17 and Treg Cell Balance and Fibrosis Development. J Med Food. 2018 Mar;21(3):215–24.

174. Kanda T, Nishida A, Ohno M, Imaeda H, Shimada T, Inatomi O, et al. Enterococcus durans TN-3 Induces Regulatory T Cells and Suppresses the Development of Dextran Sulfate Sodium (DSS)-Induced Experimental Colitis. Singh UP, editor. PLOS ONE. 2016 Jul 20;11(7):e0159705.

175. Qiu X, Zhang M, Yang X, Hong N, Yu C. Faecalibacterium prausnitzii upregulates regulatory T cells and anti-inflammatory cytokines in treating TNBS-induced colitis. J Crohns Colitis. 2013 Dec;7(11):e558–68.

176. Rodríguez-Nogales A, Algieri F, Garrido-Mesa J, Vezza T, Utrilla MP, Chueca N, et al. Differential intestinal anti-inflammatory effects of *Lactobacillus fermentum* and *Lactobacillus salivarius* in DSS mouse colitis: impact on microRNAs expression and microbiota composition. Mol Nutr Food Res. 2017 Nov;61(11):1700144.

177. Verma R, Lee C, Jeun EJ, Yi J, Kim KS, Ghosh A, et al. Cell surface polysaccharides of *Bifidobacterium bifidum* induce the generation of Foxp3 $^{+}$ regulatory T cells. Sci Immunol. 2018 Oct 26;3(28):eaat6975.

178. Zheng B, Van Bergenhenegouwen J, Overbeek S, Van De Kant HJG, Garssen J, Folkerts G, et al. Bifidobacterium breve Attenuates Murine Dextran Sodium Sulfate-Induced Colitis and Increases Regulatory T Cell Responses. Fuss IJ, editor. PLoS ONE. 2014 May 2;9(5):e95441.

179. Hidalgo-Cantabrana C, Algieri F, Rodriguez-Nogales A, Vezza T, Martínez-Camblor P, Margolles A, et al. Effect of a Ropy Exopolysaccharide-Producing Bifidobacterium animalis subsp. lactis Strain Orally Administered on DSS-Induced Colitis Mice Model. Front Microbiol. 2016 Jun 9;7:868.

180. Yu R, Zuo F, Ma H, Chen S. Exopolysaccharide-Producing Bifidobacterium adolescentis Strains with Similar Adhesion Property Induce Differential Regulation of Inflammatory Immune Response in Treg/Th17 Axis of DSS-Colitis Mice. Nutrients. 2019 Apr 4;11(4):782.

181. Lorea Baroja M, Kirjavainen PV, Hekmat S, Reid G. Anti-inflammatory effects of probiotic yogurt in inflammatory bowel disease patients. Clin Exp Immunol. 2007 Jul 23;149(3):470–9.

182. Sun Z, Huang S, Yan X, Zhang X, Hao Y, Jiang L, et al. Living, Heat-Killed Limosilactobacillus mucosae and Its Cell-Free Supernatant Differentially Regulate Colonic Serotonin Receptors and Immune Response in Experimental Colitis. Nutrients. 2024 Feb 6;16(4):468.

183. Zhao HM, Huang XY, Zuo ZQ, Pan QH, Ao MY, Zhou F, et al. Probiotics increase T regulatory cells and reduce severity of experimental colitis in mice. World J Gastroenterol. 2013 Feb 7;19(5):742.

184. Qu D, Yu L, Tian F, Zhang H, Chen W, Gu Z, et al. *Bifidobacterium bifidum* FJSWX19M5 alleviated 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced chronic colitis by mitigating gut barrier injury and increasing regulatory T cells. Food Funct. 2022 Nov 28;14(1):181–94.

185. Jeong JJ, Lee HJ, Jang SE, Han MJ, Kim DH. *Lactobacillus plantarum* C29 alleviates NF-κB activation and Th17/Treg imbalance in mice with TNBS-induced colitis. Food Agric Immunol. 2018 Jan 1;29(1):577–89.

186. Curciarello R, Canziani KE, Salto I, Barbiera Romero E, Rocca A, Doldan I, et al. Probiotic Lactobacilli Isolated from Kefir Promote Down-Regulation of Inflammatory Lamina Propria T Cells from Patients with Active IBD. Front Pharmacol. 2021 Apr 15;12:658026.

187. Kim DH, Kim S, Ahn JB, Kim JH, Ma HW, Seo DH, et al. Lactobacillus plantarum CBT LP3 ameliorates colitis via modulating T cells in mice. Int J Med Microbiol. 2020 Feb;310(2):151391.

188. Kuczma MP, Szurek EA, Cebula A, Chassaing B, Jung YJ, Kang SM, et al. Commensal epitopes drive differentiation of colonic T_{regs}. Sci Adv. 2020 Apr 17;6(16):eaaz3186.

189. Liu X jing, Yu R, Zou K fang. Probiotic Mixture VSL#3 Alleviates Dextran Sulfate Sodium-induced Colitis in Mice by Downregulating T Follicular Helper Cells. Curr Med Sci. 2019 Jun;39(3):371–8.

190. von Schillde MA, Hörmannsperger G, Weiher M, Alpert CA, Hahne H, Bäuerl C, et al. Lactocepin Secreted By Lactobacillus Exerts Anti-Inflammatory Effects By Selectively Degrading Proinflammatory Chemokines. Cell Host Microbe. 2012 Apr;11(4):387–96.

191. Reiff C, Delday M, Rucklidge G, Reid M, Duncan G, Wohlgemuth S, et al. Balancing inflammatory, lipid, and xenobiotic signaling pathways by VSL#3, a biotherapeutic agent, in the treatment of inflammatory bowel disease: Inflamm Bowel Dis. 2009 Nov;15(11):1721–36.

192. Carol M, Borruel N, Antolin M, Llopis M, Casellas F, Guarner F, et al. Modulation of apoptosis in intestinal lymphocytes by a probiotic bacteria in Crohn's disease. J Leukoc Biol. 2006 May 1;79(5):917–22.

193. Spencer J, Bemark M. Human intestinal B cells in inflammatory diseases. Nat Rev Gastroenterol Hepatol. 2023 Apr;20(4):254–65.

194. Bemark M, Pitcher MJ, Dionisi C, Spencer J. Gut-associated lymphoid tissue: a microbiota-driven hub of B cell immunity. Trends Immunol. 2024 Mar;45(3):211–23.

195. Spencer J, Sollid LM. The human intestinal B-cell response. Mucosal Immunol. 2016 Sep;9(5):1113–24.

196. Uzzan M, Martin JC, Mesin L, Livanos AE, Castro-Dopico T, Huang R, et al. Ulcerative colitis is characterized by a plasmablast-skewed humoral response associated with disease activity. Nat Med. 2022 Apr;28(4):766–79.

197. Kawamoto S, Maruya M, Kato LM, Suda W, Atarashi K, Doi Y, et al. Foxp3+ T Cells Regulate Immunoglobulin A Selection and Facilitate Diversification of Bacterial Species Responsible for Immune Homeostasis. Immunity. 2014 Jul;41(1):152–65.

198. Castro-Dopico T, Dennison TW, Ferdinand JR, Mathews RJ, Fleming A, Clift D, et al. Anti-commensal IgG Drives Intestinal Inflammation and Type 17 Immunity in Ulcerative Colitis. Immunity. 2019 Apr;50(4):1099-1114.e10.

199. Hong M, Ye BD, Yang SK, Jung S, Lee HS, Kim BM, et al. Immunochip Meta-Analysis of Inflammatory Bowel Disease Identifies Three Novel Loci and Four Novel Associations in Previously Reported Loci. J Crohns Colitis. 2018 May 25;12(6):730–41.

200. Castro-Dopico T, Colombel JF, Mehandru S. Targeting B cells for inflammatory bowel disease treatment: back to the future. Curr Opin Pharmacol. 2020 Dec;55:90–8.

201. Leiper K, Martin K, Ellis A, Subramanian S, Watson AJ, Christmas SE, et al. Randomised placebo-controlled trial of rituximab (anti-CD20) in active ulcerative colitis. Gut. 2011 Nov 1;60(11):1520–6.

202. Varma P, Falconer J, Aga A, Prince HM, Pianko S. Rituximab-induced Crohn's disease. Scand J Gastroenterol. 2017 May 4;52(5):606–8.

203. Yang R, Shan S, Shi J, Li H, An N, Li S, et al. *Coprococcus eutactus* , a Potent Probiotic, Alleviates Colitis via Acetate-Mediated IgA Response and Microbiota Restoration. J Agric Food Chem. 2023 Feb 22;71(7):3273–84.

204. Liu HY, Giraud A, Seignez C, Ahl D, Guo F, Sedin J, et al. Distinct B cell subsets in Peyer's patches convey probiotic effects by Limosilactobacillus reuteri. Microbiome. 2021 Oct 3;9(1):198.

205. Shalon D, Culver RN, Grembi JA, Folz J, Treit PV, Shi H, et al. Profiling the human intestinal environment under physiological conditions. Nature. 2023 May;617(7961):581–91.

206. O'Toole PW, Marchesi JR, Hill C. Next-generation probiotics: the spectrum from probiotics to live biotherapeutics. Nat Microbiol. 2017 Apr 25;2(5):1–6.

207. Merenstein D, Pot B, Leyer G, Ouwehand AC, Preidis GA, Elkins CA, et al. Emerging issues in probiotic safety: 2023 perspectives. Gut Microbes. 2023 Mar 15;15(1):2185034.