REVIEW

Lessons from probiotic–host interaction studies in murine models of experimental colitis

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In inflammatory bowel diseases (IBD), it is known that besides genetic and environmental factors (e.g. diet, drugs, stress), the microbiota play an important role in the pathogenesis. Patients with IBD have an altered microbiota (dysbiosis) and therefore, probiotics, defined as 'live micro-organisms that when administered in adequate amounts can confer a health benefit on the host', have been suggested as nutritional supplements to restore these imbalances. The best response on probiotics among the different types of IBD appears to be in the case of ulcerative colitis. Although probiotics show promise in IBD in both clinical and animal studies, further mechanistic studies are necessary to optimize the use of probiotics as supporting therapy in IBD. Murine models of experimental colitis have been used for decades to study this pathology, and these models have been proven useful to search for new therapeutic approaches. The purpose of this review is to summarize probiotic–host interaction studies in murine models of experimental colitis and to evaluate how these models can further help in understanding these complex interactions. Unraveling the molecular mechanisms behind the beneficial effects will assist in better and possibly more efficient probiotic formulations.

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1 Introduction

Probiotics are defined as 'live micro-organisms that when administered in adequate amounts can confer a health benefit on the host' [1]. During the last decades, several clinical and experimental studies are published on the health-promoting capacities of probiotics, which are often applied as functional food products. An important challenge in probiotic research is defining assays to determine the health-promoting capacities of probiotics, especially in healthy subjects, as the effects are generally subtle as compared with pharmaceutical drugs or only observed in preventive set-ups. This complicates the set-up of appropriate clinical trials. In specific diseases, like acute diarrhea in children and antibiotic-associated diarrhea, the beneficial effects of probiotics are often more easy to recognize. Another interesting area for the application of probiotics is the group of inflammatory bowel diseases (IBD). IBD diseases such as Crohn's disease (CD) and ulcerative colitis (UC) are chronic idiopathic diseases that involve inflammation of the intestinal tract [2]. An increased prevalence of these diseases has been documented in developed countries. The pathogenesis of these diseases is not fully understood, but besides genetic, environmental and immunoregulatory factors, the microbiota plays a role as well. It is thought that the inflammation results from an aberrant mucosal immune response against the indigenous microbiota in genetically susceptible hosts [3]. Given the various side effects of the current IBD therapies, clinicians and patients

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Abbreviations: CD, Crohn's disease; **DCs,** dendritic cells; **DSS,** dextran sulfate sodium; **EcN,** *Escherichia coli* Nissle 1917; **IBD,** inflammatory bowel disease; **LTA,** lipoteichoic acid; **MAMPs,** microbe-associated molecular patterns; **PBMCs,** peripheral blood mononuclear cells; **PRRs,** pattern recognition receptors; **TLR,** Toll-like receptor; **TNBS,** trinitrobenzene sulfonic acid; **UC,** ulcerative colitis

have increased interest in probiotics as supporting therapeutic agents that can be taken as nutritional supplements or implemented in the daily diet (e.g. as fermented products) [4, 5]. It is thought that probiotics can exert beneficial effects by (i) restoring microbial imbalances, (ii) enhancing the epithelial barrier function and/or (iii) modulating the immune responses [6]. A crucial factor is the choice of the probiotic strain. To better guide the selection of the most appropriate probiotic strains for IBD patients, molecular knowledge on the probiotic bacterial strains used and on their evoked health benefits is needed. As the outcomes of clinical studies of IBD with probiotics are not always unambiguously positive [5, 7] and are limited in the amount of insights they can generate in relation to the mechanisms of probiotic action, the use of animal model systems with defined pathophysiology is crucial. In addition, studies with animal models need to be complemented with studies in in vitro cell models and molecular studies on the probiotic bacteria. This will facilitate our understanding on how probiotic bacteria interact with affected intestinal tissue and will help us define strategies on how probiotics can be optimized for maximal benefit. To be able to give a critical appraisal of probiotic research in murine experimental colitis, the focus of this review, we start this review by giving an overview of the work that has been performed in the most commonly used murine models for experimental colitis. However, it is not our purpose to give an extensive overview of all available murine colitis models reported in literature. For this, we refer to the review of Jurjus et al. [8].

2 Colitis models used to study probiotic–host interactions

Throughout the last years, several models of experimental colitis have been described to understand and find new therapeutic treatments for gastro-intestinal disorders [9]. These models were demonstrated to have various pathophysiological aspects of IBD, but no model completely mimics the human disease. Consequently, the obtained results need to be interpreted with caution [10, 11]. To date, the most widely applied colitis models in studies with probiotic bacteria are two chemically induced models, with dextran sulfate sodium (DSS) and trinitrobenzene sulfonic acid (TNBS), respectively, mainly because of their feasibility. A third model that is often used is the interleukin-10 (IL-10) knockout model. In addition, adoptive transfer models and *Citrobacter rodentium* induced colitis are also used in studies with probiotics.

2.1 DSS colitis

In this model, originally reported by Okayasu et al. [12], the epithelial layer is disrupted by administering DSS to mice, resulting in increased permeability and translocation of luminal bacteria and antigens. After this induction, mice start to develop an inflammation that is predominantly situated in the colon. The severity of this inflammation can vary depending on the concentration, the type of DSS and the amount of cycles administered to the mice [12]. The choice of the murine strain is another important factor that influences the final outcome of the induced inflammation, with remarkable differences between the two most common used strains BALB/c and C57/BL6 [13]. Although the mechanisms behind the colitis induction are not completely understood, it is known that B and T cells are not required for the initiation of the inflammation, as severe combined immunodeficient mice also develop colitis [14]. The acute DSS colitis model is therefore especially relevant to study probiotic effects on epithelial barrier function. In addition, Okayasu et al. [12] and Cooper et al. [15] showed that it is possible to induce chronic colitis by multiple cycles of DSS, which can be used to study the effect of probiotics on more chronic aspects of IBD. For an overview of probiotics tested in DSS colitis, see Table 1.

The probiotic strain *Escherichia coli* Nissle 1917 (EcN) has been demonstrated to maintain remission in patients with UC and to be equivalent to the standard treatment with mesalazine [16, 17]. Several researchers have therefore been applying EcN in experimental colitis models to find out more about its mode of action. In a study by Schultz et al. [18], administration of EcN ameliorated acute DSS colitis. A clear reduction was observed in the secretion of proinflammatory cytokines interferon-γ (IFN-γ) and IL-6. In another study, it was shown that not only live but also heat-killed EcN could relieve colitis symptoms [19]. Three other independent studies observed similar improvements on acute DSS colitis after the administration of EcN [20–22]. Interestingly, the study of Grabig et al. [20], using also Toll-like receptor (TLR)-2 and TLR-4 knockout mice, indicates that these TLRs are important in mediating the effects of the probiotic EcN.

The probiotic mixture VSL♯3 has also shown clinical potential in IBD (reviewed by [23]). This probiotic preparation contains eight bacterial species including four strains of lactobacilli (*Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*), three strains of bifidobacteria (*Bifidobacterium longum*, *Bifidobacterium breve* and *Bifidobacterium infantis*) and *Streptococcus salivarius* subsp. *thermophilus*. In an extensive study by Rachmilewitz et al. [24], VSL♯3 was administered to colitic mice as viable, γ-irradiated, or heat killed probiotic bacteria or purified DNA. Viable, γ-irradiated bacteria and purified DNA all ameliorated acute and chronic DSS colitis in a preventive and a therapeutic set-up. Although there has been much debate on whether probiotics should be 'viable' [25], this study seems to suggest a role for DNA as an immunostimulatory agent mediated through TLR-9. A reduction was seen in the disease activity index, the myeloperoxidase expression – a marker for neutrophil infiltration – and the histological score. VSL♯3 also seems to help protecting the epithelial barrier by maintaining the tight junction protein expression and by preventing

Table 1. Overview of experimental studies with probiotics in DSS-induced colitis

Probiotic strain	Outcome	Treatment[a)]	Mouse strain	Ref.
E. coli Nissle 1917	Significant improvement	S	C57/Bl6	[20]
	Significant improvement	S	C57/Bl6	[19]
	Significant improvement	P	BALB/c	[21]
	Significant improvement	P+S	BALB/c	[18]
	Significant improvement	S	BALB/c	[22]
VSL♯3	Significant improvement	P+S and Po	BALB;C57/BL6;129XB6F_2	[24]
	Significant improvement	S	BALB/c	[26]
	No difference	Po	BALB/c	[27]
L. casei Shirota	Significant improvement	S	BALB/c	[87]
	Significant improvement	P+S	BALB/c	[88]
L. casei DN-114 001	Significant improvement	P+S	BALB/c (WT and TLR-4$^{-/-}$)	[86]
L. casei BL23	Significant improvement	P+S	BALB/c	[101]
L. crispatus M247	Significant improvement	P	BALB/c	[94]
Enterococcus faecalis	Significant improvement	S	BALB/c	[102]
L. rhamnosus GG	Deterioration	P+S	C57/BL6	[30]
	Deterioration	P	C57/BL6	[31]
	No difference	P+S	BALB/c	[88]
L. plantarum NCIMB8826	Deterioration	P	C57/BL6	[31]
L. acidophilus NCFM	No difference	P	C57/BL6	[32]
L. paracasei	Significant improvement	P	C57/BL6	[31]
L. salivarius 433118	No difference	P+S	C57/BL6	[29]
E. coli M-17	Significant improvement	P+S	C57/BL6	[103]
L. brevis HY7401, *L. sp.* HY7801 and *B. longum* HY8004	Significant improvement	Po	ICR	[104]
Bacillus polyfermenticus	Significant improvement	S	CD-1	[43]
L. plantarum HY115	Significant improvement	S	ICR	[105]
L. brevis HY7401	Significant improvement	S	ICR	[105]
L. rhamnosus OLL2838	Improved barrier function	S	BALB/c	[96]
Four *Lactobacillus* and four *Bifidobacterium* species[b)]	Significant improvement	P+S	Swiss albino	[28]
Lactococcus lactis subsp. *cremoris* FC	Significant improvement	P+S	C57/BL6	[106]
Butyrivibrio fibrisolvens MDT-1	Significant improvement	P	ICR	[107]
L. delbrueckii	No difference	S	BALB/c	[108]
Enterococcus durans	Significant improvement	S	BALB/c	[108]
L. reuteri	Significant improvement	P+S	BALB/c	[109]
B. infantis	Significant improvement	P+S	BALB/c	[109]

a) Treatment protocol: P, preventive treatment, i.e. starting 1–14 days before colitis induction; S, simultaneous treatment, i.e. together with DSS administration and till the end of the experiment or Po, post-induction, i.e. after DSS administration.

b) *L. rhamnosus* GG, *L. plantarum* CIP102021, *L. casei* CIP107868 and *L. delbrueckii* subsp. *lactis* CIP101028; and *B. bifidum* CIP56.7T, *B. infantis* CIP64.67T, *B. lactis* CIP105265T, *B. adolescentis* CIP64.59T.

apoptosis of epithelial cells [26]. Of interest, in one study, no beneficial effect of VSL♯3 could be seen in a mild chronic DSS model [27]. However, in this study VSL♯3 was administered after the onset of colitis in contrast with the other studies where probiotic administration was started prior [24] or at the same time as the colitis induction [26].

Another probiotic mixture of four *Lactobacillus* and four *Bifidobacterium* species also reduced mucosal inflammation and damage in DSS colitis in mice [28]. In contrast, *Lactobacillus salivarius* subsp. *salivarius* 433118 (UCC118) did not have a beneficial effect on DSS colitis or on pathological or physiological parameters [29]. Other strains like *L. acidophilus* NCFM, *L. plantarum* NCIMB8826 and *L. rhamnosus* GG also did not attenuate colitis symptoms [30–32]. In the case of *L. rhamnosus* GG and *L. plantarum* NCIMB8826, the probiotic strains even seemed to deteriorate some disease symptoms. We have recently shown that while the wild-type *L. rhamnosus* GG strain seemed to aggravate the severity of the colitic parameters, a mutant lacking D-alanine on lipoteichoic acid (LTA), a key cell surface molecule, evoked a clear anti-inflammatory response [30]. Similarly, a LTA-deficient mutant of *L. acidophilus* NCFM also proved to be better in treating DSS colitis as compared with the wild-type strain [32]. This indicates that the presence and structure of LTA plays an important role in the anti-inflammatory capacity of lactobacilli (See Section 4.5).

2.2 TNBS colitis

In this hapten-induced colitis model, TNBS in ethanol is administered intrarectally. The inflammation is presumed

to be induced by a two-step process: first the ethanol disrupts the epithelial barrier function. Subsequently, TNBS haptenizes intestinal antigens and microbial proteins and thereby triggers the host immune system [33, 34]. In contrast with the DSS model, this model is useful to study T helper cell-dependent mucosal immune responses, which can vary depending on the mouse strain used [34]. An overview of probiotic studies in TNBS colitis is given in Table 2.

Oral administration of VSL#3 to mice during a remission period between a first and second course of colitis induced by TNBS resulted in a milder form of colitis by inducing the production of IL-10 and thereby increasing the number of TGF-β bearing regulatory $CD4^+$ T cells [35]. Two other non-commercial probiotic mixtures (*L. acidophilus* and *B. longum* or *L. plantarum*, *S. thermophilus* and *B. animalis* subsp. *lactis*) also induced regulatory T cells, resulting in a protective effect against TNBS colitis [36]. A mixture of *B. lactis* LA 303, *L. acidophilus* LA 201, *L. plantarum* LA 301 and *L. salivarius* LA 302 was similarly demonstrated to reduce colitis symptoms [37].

Lactobacillus salivarius Ls33 had a significant protective effect when used in TNBS colitic mice, while *L. plantarum* Lp115 and *L. acidophilus* NCFM did not [38]. This protective effect of *L. salivarius* Ls33 was also seen in a study by Foligne et al. [39]. In this study, ten probiotic strains were analyzed for their potential protective effect in TNBS colitis in mice. Besides *L. salivarius* Ls33, several other strains (see Table 2) were shown to have protective effects. All these strains showed a high in vitro IL-10/IL-12 ratio, in cytokine-induction experiments with peripheral blood mononuclear cells (PBMCs). This IL-10/IL-12 ratio is indicative for strains with anti (IL-10) versus proinflammatory (IL-12) capacity. In contrast, *L. acidophilus* NCFM, a strain with a low IL-10/IL-12 ratio in PBMCs, did not alleviate TNBS colitis, also indicating a correlation between the in vitro IL-10/IL-12 and in vivo efficacy in TNBS colitis. Others also found this correlation. *L. fermentum* strain ACA-DC 179 reduced colitis symptoms possibly by induction of the anti-inflammatory cytokine IL-10, whereas *S. macedonicus* ACA-DC 198, causing a lower IL-10/IL-12 ratio in vitro, was not able to reduce disease symptoms [40]. Another strain with a low IL10/IL12 ratio, *L. plantarum* NCIMB8826, also did not show a significant protective effect in TNBS colitis [41]. This is in agreement with the studies in the DSS model (see above).

Table 2. Overview of experimental studies with probiotics in TNBS-induced colitis

Probiotic strain	Outcome	Treatment[a)]	Mouse strain	Ref.
VSL#3	Significant improvement	Po	SJL/J	[35]
L. salivarius Ls33	Significant improvement	P	BALB/c	[38]
	Significant improvement	P+S	BALB/c; C57/BL6	[39]
L. plantarum Lp115	Significant improvement	P+S	BALB/c; C57/BL6	[39]
	No difference	P	BALB/c	[38]
L. acidophilus NCFM	No difference	P	BALB/c	[38]
	No difference	P+S	BALB/c; C57/BL6	[39]
B. lactis LA 303, *L. acidophilus* LA 201, *L. plantarum* LA 301 and *salivarius* LA 302	Significant improvement	P+S	BALB/c	[37]
L. plantarum NCIMB 8826	Significant improvement	P+S	BALB/c; C57/BL6	[39, 110]
	Significant improvement	P	BALB/c	[42]
	No difference	P+S	BALB/c	[41]
L. plantarum AK8-4	Significant improvement	P+S	ICR	[44]
B. longum HY8004	Significant improvement	P+S	ICR	[44]
B. bifidum S17	Significant improvement	P+S	C57/BL6	[45]
L. rhamnosus LR32	Significant improvement	P+S	BALB/c; C57/BL6	[39]
L. casei BL23	Significant improvement	P+S	BALB/c; C57/BL6	[39]
B. animalis subsp. *lactis* BL04	Significant improvement	P+S	BALB/c; C57/BL6	[39]
B. animalis subsp. *lactis* BI07	Significant improvement	P+S	BALB/c; C57/BL6	[39]
L. acidophilus IPL908	Significant improvement	P+S	BALB/c; C57/BL6	[39]
Oenococcus oeni IOEB9115	Significant improvement	P+S	BALB/c	[111]
Saccharomyces boulardii	Significant improvement	P+S	BALB/c	[112]
Bacillus polyfermenticus	Significant improvement	S	CD-1	[43]
L. fermentum CECT5716	Significant improvement	P	BALB/c	[46]
L. acidophilus and *B. longum*	Significant improvement	P+S	BALB/c	[36]
L. plantarum, *S. thermophilus*, and *B. animalis* subsp. *lactis*	Significant improvement	P+S	BALB/c	[36]
Faecalibacterium prausnitzii	Significant improvement	P+S	BALB/c	[47]
L. fermentum ACA-DC 179	Significant improvement	P	BALB/c	[40]
S. macedonicus ACA-DC 198	No difference	P	BALB/c	[40]

a) P, preventive treatment, i.e. before TNBS induction; S, simultaneous treatment, i.e. during TNBS induction and till the end of the experiment; Po, post-induction, i.e. after TNBS induction.

Intriguingly, also for *L. plantarum* NCIMB8826, a mutant lacking D-alanine substitutions on its LTA was able to better alleviate colitis symptoms compared with wild type. However, in a study by Pavan et al. [42], wild-type *L. plantarum* NCIMB8826 seemed to alleviate some colitis symptoms.

Several other TNBS-based studies have shown benefits of other probiotic strains with suggestions of possible modes of actions. *Bacillus polyfermenticus* ameliorated TNBS colitis by suppressing apoptosis and promoting epithelial cell proliferation and migration [43]. *B. longum* HY8004 and *L. plantarum* AK8-4 blocked the expression of the proinflammatory cytokines IL-1, TNF and COX-2 in the colon, inhibited colon shortening, myeloperoxidase production and intestinal bacterial degradation of extracellular matrix glycosaminoglycans [44]. *B. bifidum* S17, a strain with a good adherence capacity, also caused an anti-inflammatory effect [45]. In a study of Mané et al. [46], *L. fermentum* CECT 5716 not only seemed to protect against TNBS-induced colitis but also to have a therapeutic effect and to accelerate colitis recovery. The authors suggest that the effects seen are due to the antioxidant abilities of the strain and that the accelerated recovery is associated with enhanced TLR signaling induced by the probiotic [20, 24]. In another study, oral administration of *Faecalibacterium prausnitzii* reduced the severity of TNBS colitis [47]. Intriguingly, reduced prevalence of this normal resident of the human intestinal tract was correlated with a higher risk of post-operative recurrence of ileal CD [48, 49], but the molecular details underlying the anti-inflammatory capacity of this bacterium still need to be determined. At this stage, it is already clear that most studies report on the effects that can be observed mainly downstream of what must initially happen when the probiotic bacteria (or products thereof) come in contact with the host intestinal mucosa (see also future directions).

2.3 IL-10-deficient mice

IL-10-deficient mice develop chronic colitis as they age. In the absence of IL-10, a subset of antigen-specific regulatory T cells is reduced [50]. In these mice, elevated T_H1 cytokine responses are observed and the disease can be treated by neutralizing antibodies against IL-12p40 or by the external administration of recombinant IL-10 [51]. An intrinsic disadvantage of this model is that probiotic effects that are partially mediated by IL-10 cannot be observed. This could implicate that probiotic strains with a favorable IL-10/IL-12 ratio may not be that effective. Nevertheless, this mouse model is relevant for probiotic research, as mice develop spontaneously colitis, i.e. a more natural genesis as compared with the use of aggressive chemicals, and have various symptoms similar to human IBD. Various probiotic studies have indeed shown potential in the IL-$10^{-/-}$ knockout model via several mechanisms (Table 3).

Like in the DSS and TNBS model, VSL#3 shows promising results in IL-$10^{-/-}$ knockout mice [52–54]. In another study, administration of *L. plantarum* CGMCC1258 to IL-$10^{-/-}$ knockout mice alleviated colitis symptoms by decreasing intestinal permeability [55], by downregulating the expression of adhesion molecules (e.g. ICAM-1, MAdCAM-1) [56] and by restoring normal amino acid uptake by an oligopeptide cotransporter 1 [57, 58]. Another *L. plantarum* strain, i.e. 299 V, was also shown to improve colitic symptoms in a IL-$10^{-/-}$ knockout mice model, associated with reduced levels of IL-12, IFN-γ and immunoglobulin G_{2a} levels [59]. *L. salivarius* 433118 also attenuated colitis [4, 60–62]. For this strain, the anti-inflammatory effect seems to be associated with a reduction in proinflammatory T_H1 cytokines, restoring the T_H1/T_H2 balance. It was also shown that oral or subcutaneous administration did not influence the beneficial effect [62]. Another probiotic action seems to be neutralizing reactive oxygen species (ROS). In a study with *Lactobacillus gasseri* as a vehicle to produce manganese superoxide dismutase (MnSOD), an enhanced anti-inflammatory effect could be observed, which was associated with a reduction in neutrophil and macrophage infiltration [63]. Finally, in IL-$10^{-/-}$ knockout mice challenged with the pathogen *Helicobacter hepaticus*, a mixture of *L. paracasei* and *L. reuteri* also downregulated the proinflammatory cytokines, TNF and IL-12 and thereby reducing colitic inflammation [64].

2.4 Studies with other colitis models

The list of available colitis models is extending rapidly in the last decades. Besides the DSS, TNBS and the IL-$10^{-/-}$ knockout models, other models have also been used to study the beneficial effects of probiotics, such as adoptive transfer models. In these models, immunodeficient mouse strains, such as *Rag*$^{-/-}$ and severe combined immune deficiency (SCID) mice, develop colitis upon transfer with a subset of T lymphocytes. These adoptive transfer models are therefore useful to elucidate the role of pathogenic and regulatory T cells in mucosal immunity and intestinal inflammation [65]. As previously demonstrated in a TNBS colitis model, *B. bifidum* S17 also showed anti-inflammatory capacities in a $CD4^+CD45RB^{hi}$ transfer model in $Rag^{-/-}$ mice [45]. In a recent paper, Veiga et al. [66] demonstrate that *B. animalis* subsp. *lactis* improves *T-bet*$^{-/-}$*Rag*$^{-/-}$ colitis in mice by altering the microbiota and inhibiting colitogenic microbes. In a transfer model in SCID mice, *B. bifidum* BGN4 showed a beneficial effect by inhibiting $CD4^+$ lymphocyte infiltration and inflammatory cytokine production [67]. A mixture of *L. reuteri* DSM-12246 and *L. rhamnosus* 19070-2 reduced IL-4, a cytokine directing T_H2 differentiation, and had a reduced colitis score [68].

Another model uses *C. rodentium*, a mouse bacterial pathogen. When administered to mice, this strain induces a predominant T_H1 mucosal cytokine response resulting in mucosal lesions similar to IBD [69]. In this model, *L. acidophilus* NCFM was demonstrated to prevent

Table 3. Overview of experimental studies with probiotics in IL-10$^{-/-}$ knockout mice

Probiotic strain	Outcome	Treatment[a)]	Mouse strain	Ref.
VSL#3	Significant improvement	P	129/SvEv	[52]
	Significant improvement	P	NS[b)]	[53]
	Significant improvement	Po	129/SvEv	[54]
L. gasseri ATCC33323	Significant improvement	P	C57/BL6	[63]
L. plantarum CGMCC1258	Significant improvement	P	129/SvEv	[55–58]
L. salivarius 433118	Significant improvement	P	129/Ola × C57/BL6	[60, 62]
	Significant improvement	P	C57/BL6; C57/BL10	[61]
	No difference	P & Po	C57/BL6	[29]
B. infantis 35624	Significant improvement	P	129/Ola × C57/BL6	[60]
L. reuteri 6798 & *L. paracasei* 1602	Significant improvement	P	C57/BL6	[64]
L. plantarum 299V	Significant improvement	Po	C57BL6 × 129/Ola	[59]

a) P, preventive treatment, i.e. starting probiotic treatment immediately after weaning; or Po, post-onset of colitis symptoms.
b) NS, not specified.

Citrobacter-induced colitis [70]. Preinoculation of *L. acidophilus* NCFM proved to be more effective than co-inoculation of pathogen and probiotic.

3 Alternatives for in vivo studies

Although the understanding of the mechanisms of disease and the initial development of new therapies for IBD is largely dependent on animal studies, the use of alternative in vitro cell culture studies should be considered to downsize the number of animals tested. To investigate the immunomodulatory potential of probiotics, epithelial cell lines (like Caco-2 and HT-29), dendritic cells (DCs) and PBMCs are often used. One of the main issues concerning the use of these cells is to what extent results from in vitro cell cultures can be extrapolated to the in vivo situation. In this respect, working with ex vivo tissue material, which better represents the in vivo situation, would possibly be a better option, but this material is often difficult to maintain in vitro [71]. For this reason, most work has been performed with the use of cell lines or cells isolated from patients, complementary to the validation of the data in an in vivo model.

In this context, Foligne et al. [39] were able to find an interesting correlation, as mentioned before, between the in vitro IL-10/IL-12 ratio of different lactic acid bacteria in PBMCs and their anti-inflammatory character in a murine TNBS colitis model. Since then, this ratio has been suggested to be useful for the screening of new probiotic strains. In another study, Grangette et al. [41] show a clear relationship between the results in vitro with PBMCs from healthy donors and their in vivo TNBS colitis model. The authors used both live bacteria and purified LTA from wild-type *L. plantarum* NCIMB8826 and a mutant affected in D-alanylation. This mutant was shown to induce a higher expression of the anti-inflammatory cytokine IL-10 but lower expression of proinflammatory cytokines IL-12, IFN-γ and TNF. These results were also confirmed with the use of bone marrow cells isolated from mice. Moreover, it was found that the proinflammatory effect of purified LTA was dependent on TLR-2. Others like Mileti et al. [31] and Sokol et al. [47] found a similar correlation to link their in vitro and in vivo results. However, not for all strains a clear correlation can be found [31]. This makes it still difficult to extrapolate from the in vitro results indicating that in vivo studies still prove to be important. Of interest, an alternative in vitro model is proposed in a recent publication, in which the authors make use of a three-dimensional coculture of enterocytes, monocytes and DCs [72]. In this coculture model, human epithelial cells are seeded on top of a collagen-coated transwell. The monocytes and DCs are embedded in the collagen and thereby physically separated from direct contact with the immunomodulatory components being tested. This and future developments along this line hold great promise for the future.

4 Critical evaluation of the colitis models for probiotic research

Although the results from animal models cannot be merely extrapolated to humans, at this stage it is important to critically evaluate the various studies mentioned above and highlight the important issues for future research and indicate features to consider for the application of probiotics in relation to IBD and possibly other diseases as well.

4.1 Animal models versus clinical studies

Considering the various animal colitis studies with probiotics (Tables 1–3), it is clear that some probiotics have the potential to confer significant health-promoting effects. Remarkably, animal studies can be valid to gain more insight into probiotic–host interactions, as there are clearly some correlations between effects in humans and animal models. For example, VSL#3 and EcN are by far the most

used probiotics in IBD in clinical trials, with VSL#3 showing efficacy in pouchitis and EcN in the prevention of recurrence of UC [73]. In line with the clinical trials, both VSL#3 and EcN were shown to be efficient in treating DSS-induced colitis [18–22, 24, 26]. In addition, VSL#3 was also able to reduce colitis symptoms in the TNBS and the IL-$10^{-/-}$ knockout model [35, 52–54]. *L. rhamnosus* GG, another well-documented probiotic strain, seems to show promise in the prevention of pouchitis and the recurrence of UC, but its use is contraindicated in CD [74, 75]. Studies with *L. rhamnosus* GG in the DSS model underline the fact that caution should be taken when applying this strain in an active disease state [30]. Interestingly, a rather negative effect of *L. rhamnosus* GG was also observed in active colitis using a DSS model in rats [76]. This indicates that, at least in this case, clinical studies and animal studies in mice and rats lead to the same conclusion, i.e. that a particular probiotic strain may not be effective against all forms of IBD or experimental colitis, and that its use should only be considered in particular cases. The facts that the mice show similarity with humans regarding cellular receptors, including genes encoding TLRs and other immune receptors that interact with gut microbes (further discussed below) [77], and that the murine and human microbiota are similar at the division (superkingdom) level, with *Firmicutes* and *Bacteroidetes* dominating [78], further support the use of mouse models. Evidently, there are of course important differences among mice and humans in immune functioning and microbiota at the species and strain level. Therefore, further refinements, such as transplanting a human microbiota to mice, are being implemented [79]. As with all animal studies, the data can never be merely extended to the human situation and well-designed clinical trials will still prove to be important.

4.2 Importance of responsiveness of the host (determined by its genotype and microbiota)

An important finding from the various animal experiments is that the development of the disease itself depends to some extent on the mouse strain. For example, in the DSS model, different mouse strains have shown a differential susceptibility against the toxic compound [80]. Similarly, in the TNBS model, BALB/c and SJL/J mice are susceptible but C57/BL6 mice are resistant [9]. This is probably due to the different genetic backgrounds of these mouse strains. It is well documented that human subjects show inter-individual differences in terms of susceptibility for IBD [81]. For example, the genetic susceptibility for CD is in part determined by polymorphisms in NOD2/CARD15, which is a cytoplasmic receptor that recognizes muramyl dipeptide, a component of peptidoglycan in bacterial cell walls [82, 83], but various other susceptibility genes for IBD in humans are also found [84]. It can be hypothesized that these inter-individual differences at the genetic level will have a strong impact on the responsiveness of individual subjects to probiotic treatment, but this remains to be further documented. In addition, the individual microbiota (and dysbiosis) will also impact on the responsiveness of subjects to probiotic treatment [84]. Veiga et al. [66] recently reported data showing that the composition of the endogenous gut microbiota plays a key role in shaping host responses to probiotics by demonstrating a positive effect of *B. lactis* in a *T-bet*$^{-/-}$ *Rag2*$^{-/-}$ murine colitis model, in which the mice are normally deficient in bifidobacteria. Furthermore, the study from Carroll et al. [63] indicates that even the gender of the host can be of importance as they observed that male mice responded better to the probiotic treatment than their female counterparts.

4.3 Importance of epithelial barrier

As mentioned before, studies with the DSS-induced colitis model are crucial to gain more insights into the importance of the epithelial barrier in probiotic applications. The studies of Grangette et al. [41] with *L. plantarum,* Mileti et al. [31] and Claes et al. with *L. rhamnosus* [30] indicate that certain probiotic strains should not be administered when the epithelial barrier is severely impaired. It seems reasonable that due to the increased epithelial permeability by, e.g. DSS administration, luminal bacterial and antigen translocation is promoted. We hypothesize that this will subsequently lead to an inflammation cascade that cannot be overcome by probiotic treatment depending on the severity of the epithelial barrier disruption. In one study with the DSS model, the protective effect of VSL#3 was not observed, in contrast to other seemingly similar studies [27]. In this particular study, administration of DSS was continued throughout the experiment, which could possibly confer a constant burden to the epithelial cells making it impossible to repair the epithelial barrier. Interestingly, in SAMP mice, i.e. mice that develop spontaneous ileitis on aging, VSL#3 could prevent the onset of the disease by restitution of the epithelial barrier function but could not affect established disease [85]. Taken together, various studies suggest that probiotics are probably contraindicated when the epithelial barrier is severely disturbed. For this reason, timing of probiotic is of crucial importance.

4.4 Timing of probiotic treatment

As to when probiotics should be administered is being debated for a long time. To address this question, it is important to consider various factors, for example whether the patient has UC, CD or pouchitis, in an active phase or in remission. As mentioned before, almost all clinical trials with probiotics in active UC and CD have been disappointing. This seems to indicate that most probiotic strains are probably not able to downregulate active inflammation. In

contrast, clinical trials with patients in remission, especially in UC and pouchitis, have been more promising [7]. Therefore, it is the author's opinion that probiotics are best administered either in preventive set-ups or when patients are in remission. From Tables 1–3, it can be observed that most studies in murine colitis indeed also use a preventive set-up. Nevertheless, there are studies that prove that in some cases probiotics can be effective to threat experimental colitis.

4.5 Importance of probiotic strain and its cell surface

Apart from VSL#3 and EcN, other commercially available strains, like *L. casei* Shirota (Yakult) and *L. casei* DN-114 001 (Actimel) with clinical benefits [7, 74], were also demonstrated to have a beneficial effect in colitis models [86–88]. On the other hand, *L. rhamnosus* GG, another clinically well-documented strain with proven beneficial effects in antibiotic-associated diarrhea and allergy [89], does not seem to have protective effects in DSS-induced colitis [30, 31, 88]. These results seem to be in concordance with clinical trials of IBD, as *L. rhamnosus* GG, like most other probiotics, has not shown a beneficial effect in active CD [7] and active pouchitis (see above). In this context, two other probiotic strains, *L. plantarum* NCIMB8826 and *L. acidophilus* NCFM, also seem to be contraindicated in IBD [31, 32, 38, 39, 41]. Interestingly, deleting or modifying LTA in these strains by a single gene mutation seems to make the strains more anti-inflammatory [30, 32, 41]. The current hypothesis is that LTA can confound the anti-inflammatory effects of these lactobacilli. LTA can be seen as the proinflammatory Gram-positive counterpart of Gram-negative lipopolysaccharides (LPS) [90, 91]. However, the exact role of LTA still needs to be further defined, as billions of Gram-positive bacteria – with LTA in their cell wall – live in the gastrointestinal tract and apparently do not induce inflammation. Nevertheless, the fact that a single gene mutation in LTA can change the pro/anti-inflammatory aspect of a probiotic strain [30, 32, 41] indicates the importance of the molecular characterization of probiotics, especially cell surface components. For example, Matsumoto et al. [88] demonstrated that a specific polysaccharide component of *L. casei* Shirota plays an important role in probiotic efficacy. Bacterial cell surface molecules such as LTA, polysaccharides and surface proteins are potential microbe-associated molecular patterns (MAMPs) that can interact with host pattern recognition receptors (PRRs) in the gastro-intestinal mucosa and induce a signaling cascade that mount in host responses such as cytokine production [92]. Of these PRRs, TLRs are best documented for their role in detecting probiotic strains. For instance, as mentioned above, by using knockout mice, it was shown that TLR2 and TLR4 are important in mediating the effects of the probiotic EcN [20]. However, the exact surface molecules and MAMPs of EcN and other probiotics that are involved remain to be determined (see below). Moreover, it should be kept in mind that the mucosal immune response to a probiotic is the well-choreographed sum of the signals induced by the interaction of multiple surface molecules and host receptors [92].

4.6 Importance of the formulation and the viability of the probiotic strains

Different formulations of probiotics have been used in mouse models for experimental colitis. Intragastric gavage is by far the most commonly used method to administer the probiotics as it insures a specific dose is taken up by the mice. Alternatives for the administration include the supplementation of the probiotics in the drinking water, mixing them in the mouse chow, administering them intrarectally, by intraperitoneal or subcutaneous injection. Remarkably, even intraperitoneal and subcutaneous administration resulted in a protective effect, implying a systemic beneficial effect of the probiotic treatment [47, 62, 93]. According to Grabig et al. [20], intrarectal administration of EcN was even better than oral administration but so far this has not been validated by other groups or for various probiotic strains. Concerning the dose administered, it is generally accepted that a minimum amount of bacteria is necessary. Doses of as low as 2×10^5 CFUs have been shown to have effects in mice [86], but in general 10^8 CFU/mouse/day are used, which is equivalent to about 5×10^9 CFU/kg. In a study by Castagliuolo et al. [94], administration of 10^{10} CFU/mouse/day of *L. crispatus* caused weight loss and the appearance of loose stools associated with a macroscopically enlarged cecum, while lower doses did not have an effect on healthy mice, indicating that more is not always better.

It has been proposed that live bacteria are not required for the beneficial effect of probiotics [19, 24]. The use of heat-killed probiotic bacteria has given different results. Heat-killed *L. crispatus* M247, *L. reuteri*, *L. rhamnosus* OLL2838 and VSL#3 lost their protective effect in experimental colitis models [24, 94–96]. Remarkably, γ-irradiated but not heat-killed VSL#3 retained its probiotic effect and it was demonstrated that DNA was responsible for the observed attenuation of experimental colitis by signaling through TLR-9 [24]. By the heat treatment, bacterial cell surface molecules, like LTA, are released and could be more exposed to the host immune system, thereby inducing an increased proinflammatory response that masks the beneficial effects seen with live probiotics where, e.g. LTA is generally 'safely' embedded in the cell membrane. Nevertheless, the exact impact of heat treatment on the pro/anti-inflammatory capacity of probiotic bacteria remains to be further investigated. In addition to probiotic MAMPs that engage host PRRs and in this way induce beneficial host immune responses, other mechanisms can be involved in mediating the beneficial effects of probiotic bacteria. For

instance, Veiga et al. [66] have also shown that live and metabolically active *B. animalis* subsp. *lactis* bacteria are required for the optimal reduction of intestinal inflammation in the *T-bet*$^{-/-}$*Rag2*$^{-/-}$ mouse model of colitis. Their results indicate that the lactic acid production of these probiotics stimulates lactate-consuming and butyrate-producing bacteria, resulting in an increase in SCFAs such as butyrate. These SCFAs not only inhibit colitogenic *Enterobacteriaceae* [66] but also have an anti-inflammatory signaling by interaction with G-protein-coupled receptors such as GPR43 [97]. Taken together, in accordance with the WHO/FAO definition, probiotics should best be applied as live bacteria.

5 Future directions

One important problem in unraveling the modes of probiotic action in colitis is the fact that there are still no specific biomarkers of intestinal inflammation available to accurately quantify the host responses. Consequently, a multitude of markers (cytokines, etc.) are used in different animal trials, making comparisons between trials difficult. In addition, the probiotic molecules that directly confer the health benefits should be further characterized. Nevertheless, important progress has been made in recent years. In *L. acidophilus* NCFM, the S-layer protein SlpA was demonstrated to induce high levels of IL-10 and low levels of IL-12 in DCs by binding to the PRR DC-SIGN and modulating T-cell functions [98]. It remains, however, elusive to what extent *L. acidophilus* NCFM-stimulated DCs, which produce high levels of IL-10 and IL-6, are capable of skewing T cells toward Th17 or Treg cells. Of interest, the induction of IL-10-dependent, TGF-β-bearing regulatory cells has also been suggested to be one of the mechanisms of probiotic action of VSL#3 [35]. The VSL#3 molecules that are involved remain to be characterized, but a link has been made to its DNA [99]. In addition, two secreted proteins p40 and p75 of *L. rhamnosus* GG were characterized as having epithelial barrier enhancing effects [100]. Studying the potential of these purified molecules compared with the action of live whole cell probiotics seems to be appealing for the future. However, before being able to point out the specific microbial components of the different probiotic strains, more details about the molecular mode of probiotic action are still required.

6 Concluding remarks

The data from both clinical trials and animal models demonstrate the potential of probiotics as supporting treatments in some (milder) forms of IBD. In this review, listing the recent probiotic studies in murine experimental colitis emphasizes the vast amount of data already generated. However, due to large differences in the experimental set-up, the models used and the criteria considered for analysis, it is still difficult to draw general conclusions, not to say to make specific recommendations on the use of probiotics as a supplementary therapy in the case of IBD. To better guide the selection of the most appropriate probiotic strains, more molecular knowledge on the probiotic bacterial strains and their interaction with the host is needed. It is clear that animal colitis models complemented with well-designed in vitro studies will remain important to further study probiotic–host interactions. Ultimately, we hope that disclosing the molecular factors supporting probiotic action will contribute to delineating the optimal conditions driving the best performance of probiotics and to the screening and selection of novel probiotic strains on well-defined molecular criteria.

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7 References

[1] FAO/WHO, *Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report*. FAO/WHO, Rome, Italy 2001.

[2] Podolsky, D. K., Inflammatory bowel disease. *N. Engl. J. Med.* 2002, *347*, 417–429.

[3] Sartor, R. B., Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. *Nat. Clin. Pract.* 2006, *3*, 390–407.

[4] Sheil, B., Shanahan, F., O'Mahony, L., Probiotic effects on inflammatory bowel disease. *J. Nutr.* 2007, *137*, 819S–824S.

[5] Sartor, R. B., Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics. *Gastroenterology* 2004, *126*, 1620–1633.

[6] Lebeer, S., Vanderleyden, J., De Keersmaecker, S. C., Genes and molecules of lactobacilli supporting probiotic action. *Microbiol. Mol. Biol. Rev.* 2008, *72*, 728–764.

[7] Isaacs, K., Herfarth, H., Role of probiotic therapy in IBD. *Inflamm. Bowel Dis.* 2008, *14*, 1597–1605.

[8] Jurjus, A. R., Khoury, N. N., Reimund, J. M., Animal models of inflammatory bowel disease. *J. Pharmacol. Toxicol. Methods* 2004, *50*, 81–92.

[9] Kawada, M., Arihiro, A., Mizoguchi, E., Insights from advances in research of chemically induced experimental models of human inflammatory bowel disease. *World J. Gastroenterol.* 2007, *13*, 5581–5593.

[10] Elson, C. O., Cong, Y., McCracken, V. J., Dimmitt, R. A. et al., Experimental models of inflammatory bowel disease

reveal innate, adaptive, and regulatory mechanisms of host dialogue with the microbiota. *Immunol. Rev.* 2005, *206*, 260–276.

[11] Arseneau, K. O., Pizarro, T. T., Cominelli, F., Discovering the cause of inflammatory bowel disease: lessons from animal models. *Curr. Opin. Gastroenterol.* 2000, *16*, 310–317.

[12] Okayasu, I., Hatakeyama, S., Yamada, M., Ohkusa, T. et al., A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. *Gastroenterology* 1990, *98*, 694–702.

[13] Melgar, S., Karlsson, A., Michaelsson, E., Acute colitis induced by dextran sulfate sodium progresses to chronicity in C57BL/6 but not in BALB/c mice: correlation between symptoms and inflammation. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2005, *288*, G1328–G1338.

[14] Dieleman, L. A., Ridwan, B. U., Tennyson, G. S., Beagley, K. W. et al., Dextran sulfate sodium-induced colitis occurs in severe combined immunodeficient mice. *Gastroenterology* 1994, *107*, 1643–1652.

[15] Cooper, H. S., Murthy, S. N., Shah, R. S., Sedergran, D. J., Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab. Invest.* 1993, *69*, 238–249.

[16] Kruis, W., Fric, P., Pokrotnieks, J., Lukas, M. et al., Maintaining remission of ulcerative colitis with the probiotic *Escherichia coli* Nissle 1917 is as effective as with standard mesalazine. *Gut* 2004, *53*, 1617–1623.

[17] Rembacken, B. J., Snelling, A. M., Hawkey, P. M., Chalmers, D. M., Axon, A. T., Non-pathogenic *Escherichia coli* versus mesalazine for the treatment of ulcerative colitis: a randomised trial. *Lancet* 1999, *354*, 635–639.

[18] Schultz, M., Strauch, U. G., Linde, H. J., Watzl, S. et al., Preventive effects of *Escherichia coli* strain Nissle 1917 on acute and chronic intestinal inflammation in two different murine models of colitis. *Clin. Diagn. Lab. Immunol.* 2004, *11*, 372–378.

[19] Kamada, N., Inoue, N., Hisamatsu, T., Okamoto, S. et al., Nonpathogenic *Escherichia coli* strain Nissle1917 prevents murine acute and chronic colitis. *Inflamm. Bowel Dis.* 2005, *11*, 455–463.

[20] Grabig, A., Paclik, D., Guzy, C., Dankof, A. et al., *Escherichia coli* strain Nissle 1917 ameliorates experimental colitis via toll-like receptor 2- and toll-like receptor 4-dependent pathways. *Infect. Immun.* 2006, *74*, 4075–4082.

[21] Kokesova, A., Frolova, L., Kverka, M., Sokol, D. et al., Oral administration of probiotic bacteria (*E. coli* Nissle, *E. coli* O83, *Lactobacillus casei*) influences the severity of dextran sodium sulfate-induced colitis in BALB/c mice. *Folia Microbiol.* 2006, *51*, 478–484.

[22] Ukena, S. N., Singh, A., Dringenberg, U., Engelhardt, R. et al., Probiotic *Escherichia coli* Nissle 1917 inhibits leaky gut by enhancing mucosal integrity. *PLoS One* 2007, *2*, e1308.

[23] Chapman, T. M., Plosker, G. L., Figgitt, D. P., VSL♯3 probiotic mixture: a review of its use in chronic inflammatory bowel diseases. *Drugs* 2006, *66*, 1371–1387.

[24] Rachmilewitz, D., Katakura, K., Karmeli, F., Hayashi, T. et al., Toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis. *Gastroenterology* 2004, *126*, 520–528.

[25] Reid, G., Guarner, F., Gibson, G., Tompkins, T. et al., Discussion on toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis. *Gastroenterology* 2004, *127*, 366–367; author reply 367.

[26] Mennigen, R., Nolte, K., Rijcken, E., Utech, M. et al., Probiotic mixture VSL♯3 protects the epithelial barrier by maintaining tight junction protein expression and preventing apoptosis in a murine model of colitis. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2009, *296*, G1140–G1149.

[27] Gaudier, E., Michel, C., Segain, J. P., Cherbut, C., Hoebler, C., The VSL♯3 probiotic mixture modifies microflora but does not heal chronic dextran-sodium sulfate-induced colitis or reinforce the mucus barrier in mice. *J. Nutr.* 2005, *135*, 2753–2761.

[28] Nanda Kumar, N. S., Balamurugan, R., Jayakanthan, K., Pulimood, A. et al., Probiotic administration alters the gut flora and attenuates colitis in mice administered dextran sodium sulfate. *J. Gastroenterol. Hepatol.* 2008, *23*, 1834–1839.

[29] Feighery, L. M., Smith, P., O'Mahony, L., Fallon, P. G., Brayden, D. J., Effects of *Lactobacillus salivarius* 433118 on intestinal inflammation, immunity status and in vitro colon function in two mouse models of inflammatory bowel disease. *Dig. Dis. Sci.* 2008, *53*, 2495–2506.

[30] Claes, I. J., Lebeer, S., Shen, C., Verhoeven, T. L. et al., Impact of lipoteichoic acid modification on the performance of the probiotic *Lactobacillus rhamnosus* GG in experimental colitis. *Clin. Exp. Immunol.* 2010, *162*, 306–314.

[31] Mileti, E., Matteoli, G., Iliev, I. D., Rescigno, M., Comparison of the immunomodulatory properties of three probiotic strains of lactobacilli using complex culture systems: prediction for in vivo efficacy. *PLoS ONE* 2009, *4*, e7056.

[32] Mohamadzadeh, M., Pfeiler, E. A., Brown, J. B., Zadeh, M. et al., Microbes and Health Sackler Colloquium: regulation of induced colonic inflammation by *Lactobacillus acidophilus* deficient in lipoteichoic acid. *Proc. Natl. Acad. Sci. USA* 2011, *108*, Suppl.1, 4623–4630.

[33] Morris, G. P., Beck, P. L., Herridge, M. S., Depew, W. T. et al., Hapten-induced model of chronic inflammation and ulceration in the rat colon. *Gastroenterology* 1989, *96*, 795–803.

[34] Neurath, M. F., Fuss, I., Kelsall, B. L., Stuber, E., Strober, W., Antibodies to interleukin 12 abrogate established experimental colitis in mice. *J. Exp. Med.* 1995, *182*, 1281–1290.

[35] 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-beta-bearing regulatory cells. *J. Immunol.* 2005, *174*, 3237–3246.

[36] Roselli, M., Finamore, A., Nuccitelli, S., Carnevali, P. et al., Prevention of TNBS-induced colitis by different *Lactobacillus* and *Bifidobacterium* strains is associated with an

expansion of gammadeltaT and regulatory T cells of intestinal intraepithelial lymphocytes. *Inflamm. Bowel Dis.* 2009, *15*, 1526–1536.

[37] Drouault-Holowacz, S., Foligne, B., Dennin, V., Goudercourt, D. et al., Anti-inflammatory potential of the probiotic dietary supplement lactibiane tolerance: in vitro and in vivo considerations. *Clin. Nutr.* 2006, *25*, 994–1003.

[38] Daniel, C., Poiret, S., Goudercourt, D., Dennin, V. et al., Selecting lactic acid bacteria for their safety and functionality by use of a mouse colitis model. *Appl. Environ. Microbiol.* 2006, *72*, 5799–5805.

[39] Foligne, B., Nutten, S., Grangette, C., Dennin, V. et al., Correlation between in vitro and in vivo immunomodulatory properties of lactic acid bacteria. *World J. Gastroenterol.* 2007, *13*, 236–243.

[40] Zoumpopoulou, G., Foligne, B., Christodoulou, K., Grangette, C. et al., *Lactobacillus fermentum* ACA-DC 179 displays probiotic potential in vitro and protects against trinitrobenzene sulfonic acid (TNBS)-induced colitis and *Salmonella* infection in murine models. *Int. J. Food Microbiol.* 2008, *121*, 18–26.

[41] Grangette, C., Nutten, S., Palumbo, E., Morath, S. et al., Enhanced antiinflammatory capacity of a *Lactobacillus plantarum* mutant synthesizing modified teichoic acids. *Proc. Natl. Acad. Sci. USA* 2005, *102*, 10321–10326.

[42] Pavan, S., Desreumaux, P., Mercenier, A., Use of mouse models to evaluate the persistence, safety, and immune modulation capacities of lactic acid bacteria. *Clin. Diagn. Lab. Immunol.* 2003, *10*, 696–701.

[43] Im, E., Choi, Y. J., Pothoulakis, C., Rhee, S. H., *Bacillus polyfermenticus* ameliorates colonic inflammation by promoting cytoprotective effects in colitic mice. *J. Nutr.* 2009, *139*, 1848–1854.

[44] Lee, B., Lee, J. H., Lee, H. S., Bae, E. A. et al., Glycosaminoglycan degradation-inhibitory lactic acid bacteria ameliorate 2,4,6-trinitrobenzenesulfonic acid-induced colitis in mice. *J. Microbiol. Biotechnol.* 2009, *19*, 616–621.

[45] Preising, J., Philippe, D., Gleinser, M., Wei, H. et al., Selection of bifidobacteria based on adhesion and anti-inflammatory capacity in vitro for amelioration of murine colitis. *Appl. Environ. Microbiol.* 2010, *76*, 3048–3051.

[46] Mane, J., Loren, V., Pedrosa, E., Ojanguren, I. et al., *Lactobacillus fermentum* CECT 5716 prevents and reverts intestinal damage on TNBS-induced colitis in mice. *Inflamm. Bowel Dis.* 2009, *15*, 1155–1163.

[47] Sokol, H., Pigneur, B., Watterlot, L., Lakhdari, O. et al., *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc. Natl. Acad. Sci. USA* 2008, *105*, 16731–16736.

[48] Manichanh, C., Rigottier-Gois, L., Bonnaud, E., Gloux, K. et al., Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. *Gut* 2006, *55*, 205–211.

[49] Sokol, H., Seksik, P., Rigottier-Gois, L., Lay, C. et al., Specificities of the fecal microbiota in inflammatory bowel disease. *Inflamm. Bowel Dis.* 2006, *12*, 106–111.

[50] Davidson, N. J., Leach, M. W., Fort, M. M., Thompson-Snipes, L. et al., T helper cell 1-type CD4+T cells, but not B cells, mediate colitis in interleukin 10-deficient mice. *J. Exp. Med.* 1996, *184*, 241–251.

[51] Berg, D. J., Davidson, N., Kuhn, R., Muller, W. et al., Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4(+) TH1-like responses. *J. Clin. Invest.* 1996, *98*, 1010–1020.

[52] Hormannsperger, G., Clavel, T., Hoffmann, M., Reiff, C. et al., Posttranslational inhibition of proinflammatory chemokine secretion in intestinal epithelial cells: implications for specific IBD indications. *J. Clin. Gastroenterol.* 2010, *44*, S10–S15.

[53] Reiff, C., Delday, M., Rucklidge, G., Reid, M. 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, *15*, 1721–1736.

[54] Soo, I., Madsen, K. L., Tejpar, Q., Sydora, B. C. et al., VSL♯3 probiotic upregulates intestinal mucosal alkaline sphingomyelinase and reduces inflammation. *Can. J. Gastroenterol.* 2008, *22*, 237–242.

[55] Liu, Z., Zhang, P., Ma, Y., Chen, H. et al., *Lactobacillus plantarum* prevents the development of colitis in IL-10-deficient mouse by reducing the intestinal permeability. *Mol. Biol. Rep.* 2011, *38*, 1353–1361.

[56] Chu, Z. X., Chen, H. Q., Ma, Y. L., Zhou, Y. K. et al., *Lactobacillus plantarum* prevents the upregulation of adhesion molecule expression in an experimental colitis model. *Dig. Dis. Sci.* 2010, *55*, 2505–2513.

[57] Chen, H. Q., Shen, T. Y., Zhou, Y. K., Zhang, M. et al., *Lactobacillus plantarum* consumption increases PepT1-mediated amino acid absorption by enhancing protein kinase C activity in spontaneously colitic mice. *J. Nutr.* 2010, *140*, 2201–2206.

[58] Chen, H. Q., Yang, J., Zhang, M., Zhou, Y. K. et al., *Lactobacillus plantarum* ameliorates colonic epithelial barrier dysfunction by modulating the apical junctional complex and PepT1 in IL-10 knockout mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2010, *299*, G1287–G1297.

[59] Schultz, M., Veltkamp, C., Dieleman, L. A., Grenther, W. B. et al., *Lactobacillus plantarum* 299 V in the treatment and prevention of spontaneous colitis in interleukin-10-deficient mice. *Inflamm. Bowel Dis.* 2002, *8*, 71–80.

[60] McCarthy, J., O'Mahony, L., O'Callaghan, L., Sheil, B. et al., Double blind, placebo controlled trial of two probiotic strains in interleukin 10 knockout mice and mechanistic link with cytokine balance. *Gut* 2003, *52*, 975–980.

[61] O'Mahony, L., Feeney, M., O'Halloran, S., Murphy, L. et al., Probiotic impact on microbial flora, inflammation and tumour development in IL-10 knockout mice. *Aliment. Pharmacol. Ther.* 2001, *15*, 1219–1225.

[62] Sheil, B., McCarthy, J., O'Mahony, L., Bennett, M. W. et al., Is the mucosal route of administration essential for probiotic function? Subcutaneous administration is

associated with attenuation of murine colitis and arthritis. *Gut* 2004, *53*, 694–700.

[63] Carroll, I. M., Andrus, J. M., Bruno-Barcena, J. M., Klaenhammer, T. R. et al., Anti-inflammatory properties of *Lactobacillus gasseri* expressing manganese superoxide dismutase using the interleukin 10-deficient mouse model of colitis. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2007, *293*, G729–G738.

[64] Pena, J. A., Rogers, A. B., Ge, Z., Ng, V. et al., Probiotic *Lactobacillus* spp. diminish *Helicobacter hepaticus*-induced inflammatory bowel disease in interleukin-10-deficient mice. *Infect. Immun.* 2005, *73*, 912–920.

[65] Pizarro, T. T., Arseneau, K. O., Bamias, G., Cominelli, F., Mouse models for the study of Crohn's disease. *Trends Mol. Med.* 2003, *9*, 218–222.

[66] Veiga, P., Gallini, C. A., Beal, C., Michaud, M. et al., *Bifidobacterium animalis* subsp. *lactis* fermented milk product reduces inflammation by altering a niche for colitogenic microbes. *Proc. Natl. Acad. Sci. USA* 2010, *107*, 18132–18137.

[67] Kim, N., Kunisawa, J., Kweon, M. N., Eog Ji, G., Kiyono, H., Oral feeding of *Bifidobacterium bifidum* (BGN4) prevents CD4(+) CD45RB(high) T cell-mediated inflammatory bowel disease by inhibition of disordered T cell activation. *Clin. Immunol.* 2007, *123*, 30–39.

[68] Moller, P. L., Paerregaard, A., Gad, M., Kristensen, N. N., Claesson, M. H., Colitic SCID mice fed *Lactobacillus* spp. show an ameliorated gut histopathology and an altered cytokine profile by local T cells. *Inflamm. Bowel Dis.* 2005, *11*, 814–819.

[69] Higgins, L. M., Frankel, G., Douce, G., Dougan, G., MacDonald, T. T., *Citrobacter rodentium* infection in mice elicits a mucosal Th1 cytokine response and lesions similar to those in murine inflammatory bowel disease. *Infect. Immun.* 1999, *67*, 3031–3039.

[70] Chen, C. C., Louie, S., Shi, H. N., Walker, W. A., Preinoculation with the probiotic *Lactobacillus acidophilus* early in life effectively inhibits murine *Citrobacter rodentium* colitis. *Pediatr. Res.* 2005, *58*, 1185–1191.

[71] Buset, M., Lipkin, M., Winawer, S., Swaroop, S., Friedman, E., Inhibition of human colonic epithelial cell proliferation in vivo and in vitro by calcium. *Cancer Res.* 1986, *46*, 5426–5430.

[72] Leonard, F., Collnot, E. M., Lehr, C. M., A three-dimensional coculture of enterocytes, monocytes and dendritic cells to model inflamed intestinal mucosa in vitro. *Mol. Pharm.* 2010, *7*, 2103–2119.

[73] Seksik, P., Dray, X., Sokol, H., Marteau, P., Is there any place for alimentary probiotics, prebiotics or synbiotics, for patients with inflammatory bowel disease? *Mol. Nutr. Food Res.* 2008, *52*, 906–912.

[74] Vanderpool, C., Yan, F., Polk, D. B., Mechanisms of probiotic action: implications for therapeutic applications in inflammatory bowel diseases. *Inflamm. Bowel Dis.* 2008, *14*, 1585–1596.

[75] Shen, J., Ran, H. Z., Yin, M. H., Zhou, T. X., Xiao, D. S., Meta-analysis: the effect and adverse events of lactobacilli versus placebo in maintenance therapy for Crohn disease. *Intern. Med. J.* 2009, *39*, 103–109.

[76] Geier, M. S., Butler, R. N., Giffard, P. M., Howarth, G. S., *Lactobacillus fermentum* BR11, a potential new probiotic, alleviates symptoms of colitis induced by dextran sulfate sodium (DSS) in rats. *Int. J. Food Microbiol.* 2007, *114*, 267–274.

[77] Waterston, R. H., Lindblad-Toh, K., Birney, E., Rogers, J. et al., Initial sequencing and comparative analysis of the mouse genome. *Nature* 2002, *420*, 520–562.

[78] Ley, R. E., Turnbaugh, P. J., Klein, S., Gordon, J. I., Microbial ecology: human gut microbes associated with obesity. *Nature* 2006, *444*, 1022–1023.

[79] Wagner, R. D., Johnson, S. J., Kurniasih Rubin, D., Probiotic bacteria are antagonistic to *Salmonella enterica* and *Campylobacter jejuni* and influence host lymphocyte responses in human microbiota-associated immunodeficient and immunocompetent mice. *Mol. Nutr. Food Res.* 2009, *53*, 377–388.

[80] Mahler, M., Bristol, I. J., Leiter, E. H., Workman, A. E. et al., Differential susceptibility of inbred mouse strains to dextran sulfate sodium-induced colitis. *Am. J. Physiol.* 1998, *274*, G544–G551.

[81] Preidis, G. A., Versalovic, J., Targeting the human microbiome with antibiotics, probiotics, and prebiotics: gastroenterology enters the metagenomics era. *Gastroenterology* 2009, *136*, 2015–2031.

[82] Hugot, J. P., Chamaillard, M., Zouali, H., Lesage, S. et al., Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001, *411*, 599–603.

[83] Ogura, Y., Bonen, D. K., Inohara, N., Nicolae, D. L. et al., A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001, *411*, 603–606.

[84] Packey, C. D., Sartor, R. B., Commensal bacteria, traditional and opportunistic pathogens, dysbiosis and bacterial killing in inflammatory bowel diseases. *Curr. Opin. Infect. Dis.* 2009, *22*, 292–301.

[85] Pagnini, C., Saeed, R., Bamias, G., Arseneau, K. O. et al., Probiotics promote gut health through stimulation of epithelial innate immunity. *Proc. Natl. Acad. Sci. USA* 2010, *107*, 454–459.

[86] Chung, Y. W., Choi, J. H., Oh, T. Y., Eun, C. S., Han, D. S., *Lactobacillus casei* prevents the development of dextran sulphate sodium-induced colitis in Toll-like receptor 4 mutant mice. *Clin. Exp. Immunol.* 2008, *151*, 182–189.

[87] Herias, M. V., Koninkx, J. F., Vos, J. G., Huis in't Veld, J. H., van Dijk, J. E., Probiotic effects of *Lactobacillus casei* on DSS-induced ulcerative colitis in mice. *Int. J. Food Microbiol.* 2005, *103*, 143–155.

[88] Matsumoto, S., Hara, T., Nagaoka, M., Mike, A. et al., A component of polysaccharide peptidoglycan complex on *Lactobacillus* induced an improvement of murine model of inflammatory bowel disease and colitis-associated cancer. *Immunology* 2009, *128*, e170–e180.

[89] Doron, S., Snydman, D. R., Gorbach, S. L., *Lactobacillus* GG: bacteriology and clinical applications. *Gastroenterol. Clin. North Am.* 2005, *34*, 483–498, ix.

[90] Ginsburg, I., Role of lipoteichoic acid in infection and inflammation. *Lancet Infect. Dis.* 2002, *2*, 171–179.

[91] von Aulock, S., Morath, S., Hareng, L., Knapp, S. et al., Lipoteichoic acid from *Staphylococcus aureus* is a potent stimulus for neutrophil recruitment. *Immunobiology* 2003, *208*, 413–422.

[92] Lebeer, S., Vanderleyden, J., De Keersmaecker, S. C., Host interactions of probiotic bacterial surface molecules: comparison with commensals and pathogens. *Nat. Rev. Microbiol.* 2010, *8*, 171–184.

[93] Foligne, B., Grangette, C., Pot, B., Probiotics in IBD: mucosal and systemic routes of administration may promote similar effects. *Gut* 2005, *54*, 727–728.

[94] Castagliuolo, I., Galeazzi, F., Ferrari, S., Elli, M. et al., Beneficial effect of auto-aggregating *Lactobacillus crispatus* on experimentally induced colitis in mice. *FEMS Immunol. Med. Microbiol.* 2005, *43*, 197–204.

[95] Ma, D., Forsythe, P., Bienenstock, J., Live *Lactobacillus reuteri* is essential for the inhibitory effect on tumor necrosis factor alpha-induced interleukin-8 expression. *Infect. Immun.* 2004, *72*, 5308–5314.

[96] Miyauchi, E., Morita, H., Tanabe, S., *Lactobacillus rhamnosus* alleviates intestinal barrier dysfunction in part by increasing expression of zonula occludens-1 and myosin light-chain kinase in vivo. *J. Dairy Sci.* 2009, *92*, 2400–2408.

[97] Maslowski, K. M., Vieira, A. T., Ng, A., Kranich, J. et al., Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* 2009, *461*, 1282–1286.

[98] Konstantinov, S. R., Smidt, H., de Vos, W. M., Bruijns, S. C. et al., S layer protein A of *Lactobacillus acidophilus* NCFM regulates immature dendritic cell and T cell functions. *Proc. Natl. Acad. Sci. USA* 2008, *105*, 19474–19479.

[99] Lammers, K. M., Brigidi, P., Vitali, B., Gionchetti, P. et al., Immunomodulatory effects of probiotic bacteria DNA: IL-1 and IL-10 response in human peripheral blood mononuclear cells. *FEMS Immunol. Med. Microbiol.* 2003, *38*, 165–172.

[100] Yan, F., Cao, H., Cover, T. L., Whitehead, R. et al., Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. *Gastroenterology* 2007, *132*, 562–575.

[101] Rochat, T., Bermudez-Humaran, L., Gratadoux, J. J., Fourage, C. et al., Anti-inflammatory effects of *Lactobacillus casei* BL23 producing or not a manganese-dependant catalase on DSS-induced colitis in mice. *Microb. Cell Fact.* 2007, *6*, 22.

[102] Chen, L. L., Wang, X. H., Cui, Y., Lian, G. H. et al., Therapeutic effects of four strains of probiotics on experimental colitis in mice. *World J. Gastroenterol.* 2009, *15*, 321–327.

[103] Fitzpatrick, L. R., Small, J., Hoerr, R. A., Bostwick, E. F. et al., In vitro and in vivo effects of the probiotic *Escherichia coli* strain M-17: immunomodulation and attenuation of murine colitis. *Br. J. Nutr.* 2008, *100*, 530–541.

[104] Hong, Y. S., Ahn, Y. T., Park, J. C., Lee, J. H. et al., (1)H NMR-based metabonomic assessment of probiotic effects in a colitis mouse model. *Arch. Pharm. Res.* 2010, *33*, 1091–1101.

[105] Lee, H. S., Han, S. Y., Bae, E. A., Huh, C. S. et al., Lactic acid bacteria inhibit proinflammatory cytokine expression and bacterial glycosaminoglycan degradation activity in dextran sulfate sodium-induced colitic mice. *Int. Immunopharmacol.* 2008, *8*, 574–580.

[106] Nishitani, Y., Tanoue, T., Yamada, K., Ishida, T. et al., *Lactococcus lactis* subsp. cremoris FC alleviates symptoms of colitis induced by dextran sulfate sodium in mice. *Int. Immunopharmacol.* 2009, *9*, 1444–1451.

[107] Ohkawara, S., Furuya, H., Nagashima, K., Asanuma, N., Hino, T., Effect of oral administration of *Butyrivibrio fibrisolvens* MDT-1 on experimental enterocolitis in mice. *Clin. Vaccine Immunol.* 2006, *13*, 1231–1236.

[108] Raz, I., Gollop, N., Polak-Charcon, S., Schwartz, B., Isolation and characterisation of new putative probiotic bacteria from human colonic flora. *Br. J. Nutr.* 2007, *97*, 725–734.

[109] van der Kleij, H., O'Mahony, C., Shanahan, F., O'Mahony, L., Bienenstock, J., Protective effects of *Lactobacillus reuteri* and *Bifidobacterium infantis* in murine models for colitis do not involve the vagus nerve. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2008, *295*, R1131–R1137.

[110] Foligne, B., Nutten, S., Steidler, L., Dennin, V. et al., Recommendations for improved use of the murine TNBS-induced colitis model in evaluating anti-inflammatory properties of lactic acid bacteria: technical and microbiological aspects. *Dig. Dis. Sci.* 2006, *51*, 390–400.

[111] Foligne, B., Dewulf, J., Breton, J., Claisse, O. et al., Probiotic properties of non-conventional lactic acid bacteria: immunomodulation by *Oenococcus oeni*. *Int. J. Food Microbiol.* 2010, *140*, 136–145.

[112] Foligne, B., Dewulf, J., Vandekerckove, P., Pignede, G., Pot, B., Probiotic yeasts: anti-inflammatory potential of various non-pathogenic strains in experimental colitis in mice. *World J. Gastroenterol.* 2010, *16*, 2134–2145.

