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ORIGINAL ARTICLE

Anti-inflammatory potential of the probiotic dietary supplement Lactibiane Tolérance: In vitro and in vivo considerations

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Summary
Background & aims: Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. In inflammatory bowel disease (IBD), where major modifications of the intestinal microflora have been reported, there is an increasing interest in modulating the flora with probiotic products. This work addresses the anti-inflammatory potential of Lactibiane Tolérance, a probiotic dietary supplement (mixture of four strains) using in vitro and in vivo approaches.
Methods: Comparison of the four individual strains and the commercial product reconstituted from them was conducted by in vitro tests (cytokine release after 24 h stimulation of human peripheral blood mononuclear cells (PBMC)). The potential immunomodulatory characteristics of Lactibiane Tolérance were determined in vivo in an acute mice model of 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis. Assessment of colitis included blinded histological and macroscopic scores.
Results: We showed that Lactibiane Tolérance has anti-inflammatory properties in vitro by stimulating IL-10 production and in vivo by conferring a significant protective effect in the TNBS-induced colitis model (more than 50% decrease of colitis symptoms, $P<0.01$).

Abbreviations: CFU, colony-forming units; PBMC, peripheral blood mononuclear cells; SPF, specific-pathogen free; TMB, tetramethylbenzidine; TNBS, 2, 4, 6-trinitrobenzene sulfonic acid; UC, ulcerative colitis.

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Conclusions: These results demonstrate that a probiotic dietary supplement, Lactibiane Tolérance, can significantly prevent the initial injury of TNBS and could stimulate the initiation of clinical trials in IBD.

Introduction

Chronic inflammatory bowel diseases (IBDs) such as Crohn's disease and ulcerative colitis (UC) is a significant public health problem in Western societies affecting 150 and 200 in 100,000 individuals, respectively; yet its aetiology remains poorly understood.[1] IBD is characterized clinically by chronic inflammation in the large and/or small intestine, the symptoms of which include diarrhoea, abdominal pain, weight loss, and nausea. Recent insights into the nature of this disease, derived mainly from studies of experimental models of colonic inflammation, strongly suggest that it can result from a loss of immune tolerance to antigens in the bacterial microflora.[2,3] These considerations have focused attention on the bacterial microflora itself and the possibility that although some bacteria are potential inducers of disease, others, known as probiotic organisms, are able to prevent disease.[4] Probiotics have been defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host.[5] Results from animal models and human clinical trials have confirmed various therapeutic effects of selected strains of microbes in viral- and bacterial-induced intestinal infections and in antibiotic-induced diarrhoea as well as immune disorders.[6,7] The use of lactobacilli and bifidobacteria exhibiting probiotic properties to prevent and/or treat intestinal disorders is increasingly accepted.[8] The outcomes of clinical trials often appear difficult to compare because of differences in study designs, end points measured, probiotic doses or strains used. However, in many occasions, probiotic therapy was proven successful in clinical IBD trials, involving UC, Crohn's disease and pouchitis,[9,10] as well as in various experimental models for acute and chronic intestinal inflammation.[11–15] The exact mechanism(s) and molecular pathway(s) by which probiotics can ameliorate experimental colitis or IBD remains unknown, but evidence suggests that they are able to modulate the host immune response. Probiotic-related immunomodulation represents one of the major options in management of IBD[12] and it is becoming clear that both the systemic and mucosal level of the immune system can be modulated by orally delivered bacterial strains.[14,16–19] It has been shown that the oral administration of a single strain *Lactobacillus plantarum* 299v to IL-10 deficient mice attenuates the severity of established colitis.[20] Another probiotic preparation including eight strains maintained remission of refractory pouchitis after transient antibiotic therapy and this probiotic cocktail was also beneficial in the treatment of three distinct experimental colitis models of IL-10 deficient mice.[21–23] However, effects of probiotics on intestinal inflammation in rodent models can exert or not beneficial effects, depending on strains and models.[24] Similarly, human clinical studies dealing with probiotics and IBD were able or not to maintain remission. Taken together, these results suggest that some but not all probiotic bacteria or bacterial combinations may be effective in the treatment or prevention of IBD. Nonetheless, very few studies have been published on the efficiency of commercial preparations in the prevention of colitis.

The purpose of the present study was to evaluate the probiotic dietary supplement Lactibiane Tolérance for its immunomodulatory potential in a model of colitis induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS) and to study the relation with previously performed in vitro tests using human peripheral blood mononuclear cells (PBMC). Former research results already showed that the TNBS model used was relevant to discriminate "protective" strains or products, showing between 30% and 70% reduction of inflammatory score, from strains or products which did not significantly attenuate experimental colitis.[25]

Material and methods

Product

Lactibiane Tolérance (PiLeJe, France) is a dietary supplement containing 4×10^9 colony-forming units (CFU)/g of viable lyophilized lactic acid bacteria (mixture consisted of 12.5% *Bifidobacterium lactis* LA 303, 12.5% *Lactobacillus acidophilus* LA 201, 50% *Lactobacillus plantarum* LA 301 and 25% *Lactobacillus salivarius* LA 302, Package Reference 4BS1 03-2006).

In vitro tests using human PBMC

The typical purpose of this in vitro test is to identify potential immunomodulatory characteristics of Lactibiane Tolérance product and the four lactic acid bacteria strains composing Lactibiane Tolérance in order to obtain additional information on their potential probiotic effects.

PBMC preparation: Fresh human blood, obtained from four healthy subjects at the Centre Régional de Transmission Sanguine (CRTS) de Lille, was diluted at a 1:2 ratio with PBS-Ca (GIBCO) and purified on a Ficoll gradient (GIBCO). After centrifugation at 400*g* for 30 min at 20 °C the PBMC formed an interphase ring layer in the serum. PBMC were aspirated carefully, suspended to a final volume of 50 ml using PBS-Ca and washed three times in the same buffer with centrifugation steps at 350*g* for 10 min at 20 °C. PBMC were subsequently resuspended using complete RPMI medium (GIBCO), supplemented with 10% wt/volume fetal calf serum (inactivated at 56 °C for 30 min), 1% wt/volume L-glutamine (GIBCO), and gentamycin (150 μg/ml) (GIBCO). PBMC were counted under the microscope and adjusted at a concentration of 2×10^6 cells/ml and distributed (in 1 ml aliquots) in 24-well tissue culture plates (Corning, Inc.).

Bacteria preparation: Overnight *Lactobacillus* or *Bifidobacterium* cultures were washed twice with PBS buffer, pH 7.2, before being resuspended in PBS at a concentration of 2×10^9 CFU/ml. For PBMC testing, a laboratory mixture was composed which consisted of 12.5% *Bifidobacterium lactis* LA 303, 12.5% *Lactobacillus acidophilus* LA 201, 50% *Lactobacillus plantarum* LA 301 and 25% *Lactobacillus salivarius* LA 302, which mimics the composition of Lactibiane Tolérance. As a negative control, PBS buffer without bacteria was used.

PBMC incubation: From the bacterial suspensions described above, 10 μl were transferred into the wells of the PBMC plates, which were incubated at 37 °C in a 5% CO_2/95% air atmosphere. After 24 h incubation, the supernatant was aspirated, centrifuged at 2000 rpm (Eppendorf model) and the supernatant removed and stored at −20 °C. The control consisted of bacteria-free buffer.

Cytokine quantification: Cytokine expression levels were determined by ELISA. ELISA plates were coated with anti-cytokine antibody (overnight procedure) and the antibody was blocked with PBS/BSA 1%. A proper standard was prepared with known concentrations of cytokines, covering the detection range of 15.62–2000 pg/ml (incubate overnight). The anti-cytokine detection and quantification was performed with the streptavidine reaction on substrate (TMB; tetramethylbenzidine, Pharmingen). The commercial kits of Pharmingen have been used, according to the manufacturer's description. Mainly two cytokines were determined, the pro-inflammatory Th1 cytokine IL-12 and the anti-inflammatory Th2 cytokine IL-10.

In vivo test using a model of colitis

Animals: Animal experiments were performed in an accredited establishment (number A59107; animal facility of the Institut Pasteur de Lille, France) according to French government guidelines (number 86/609/CEE). Conventional adult female BALB/C mice (aged 7/8 weeks), with homogenous flora, breast-fed (Felasa, 1994) and maintained under specific pathogen-free (SPF) conditions, were purchased from Iffa Credo (Saint-Germain sur l'Arbresle, France). Mice were group housed (8–10/cage) and kept under filter top hoods behind a barrier under SPF conditions. Mice had free access to tap water and rodent chow and underwent at least 1 week of acclimatization before any intervention. Groups of 10 mice were used for each experimental group.

Preparation of bacterial cultures and administration to mice: For the TNBS experiments, a commercial preparation of Lactibiane Tolérance was used. The original package received was resuspended in 0.2 mol/l $NaHCO_3$ buffer pH 8.5, containing 2% glucose. Per application 100 μl samples in $NaHCO_3$ buffer (10^9 bacteria/ml) were administered by the oral route.

TNBS induction of acute colitis and study design: The design of the standard bacterial interventional study is represented in Fig. 1. Briefly, bacterial suspensions were given to experimental groups of 10 mice, from day 5 before induction of colitis to day 1 after induction. Mortality rate, macroscopic and histological scores of inflammation and body weight were assessed 48 h after colitis induction. Mice were anesthetized with 3 mg of ketamine (Imalgene 1000; Mérial Lyon, France), 46.7 μg of diazepam (Valium, Roche Diagnostics) and 15 μg of atropine (Aguettant Laboratory, Lyon, France) dissolved in 0.9% NaCl. TNBS (Fluka, France) at a dose of 120 mg/kg of body weight was dissolved in 0.9% NaCl/ethanol (50/50 volume/volume) and 50 μl were administered intra-rectally at 4 cm proximal to the anus, using a 3.5 F catheter (EO 3416-1; Biotrol, Chelles, France). "Negative control" mice received only 50% ethanol ("Ethanol-mice"). "Positive control" mice (also referred to as "TNBS-control" or "TNBS-treated" mice) were fed only with $NaHCO_3$ buffer, in comparison with "treated" mice, which were additionally

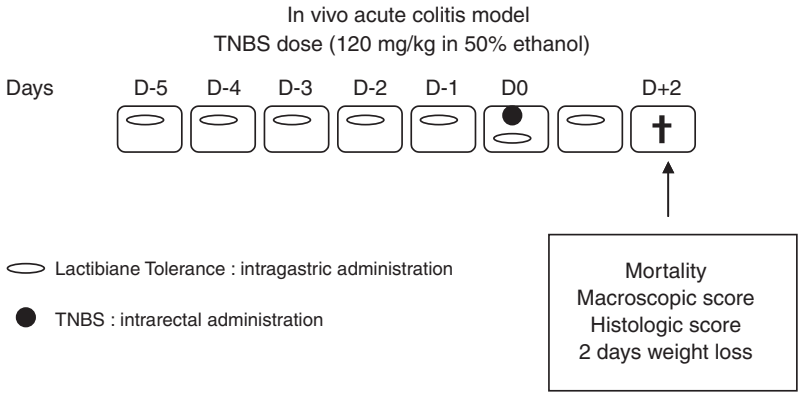

Figure 1 In vivo study design.

administered an amount of Lactibiane Tolérance corresponding to 10^8 bacteria per day. Animals were sacrificed by cervical dislocation 2 days after TNBS administration. Mice were weighed prior to TNBS administration and upon sacrifice and the number of mice developing diarrhoea was scored.

Macroscopic assessment of colitis: The colon was dissected free from fat and mesentery, was removed, carefully opened and cleaned with PBS. Colonic damage and inflammation were assessed according to the Wallace criteria.[26] These criteria for macroscopic scoring (scores ranging between 0 and 10) have been well established in both rats and mice studies,[27] and reflect (i) the intensity of inflammation, (ii) the thickening of the colon mucosa and (iii) the extent of the ulceration.

Histological assessment of colitis: For histological assessment, a colon sample located in the most damaged area was fixed in 4% paraformaldehyde acid and embedded in paraffin. Four-micrometer sections were stained with hematoxylin/eosin (Sigma, France) and examined blindly according to Ameho criteria.[28] The grading on a scale ranging from 0 to 6 takes into account the degree of inflammatory infiltrates, the presence of erosion, ulceration or necrosis, and the depth and surface of the lesion. Representative samples have been photographed.

Degree of protection: Calculating the degree of protection is a convenient way to compare groups of mice within and between experiments. To that extent results are expressed as a % reduction of the mean macroscopic inflammation of treated mice (Lactibiane Tolérance group) in relation to the mean score of non-treated mice (TNBS-control group): % relative protection $= 100 \times$ (average Wallace score ''positive control'' group$-$average Wallace score ''treatment'' group)/average Wallace score ''positive control'' group. Applying this ''relative protection'' to the average colitis level of each ''positive control'' group allows us to eliminate inevitable Wallace score variations between independent experiments. The weight loss of the mice was calculated per group, including all surviving mice, expressed as the average weight loss at the time of sacrifice and, to facilitate comparison between groups, presented as a percentage of the weight at the onset of the experiment.

Statistical analysis

Results were analyzed by the non-parametric one-way analysis of variance, Mann–Whitney *U*-test (XLSTAT software: http://www.xlstat.com). Differences were judged to be statistically significant when the *P* value was <0.05. Dead mice were not included in the statistical analyses.

Results

Cytokine secretion of mononuclear cells from healthy human

Strains of Lactibiane Tolérance were tested for their capacity to induce the secretion of IL-10 and IL-12 after 24 h of culture with healthy human PBMC (Fig. 2). *Lactobacillus salivarius* LA 302 (4983 pg/ml) and *Bifidobacterium lactis* LA 303 (1862 pg/ml) strongly induced IL-10 production. On the contrary, they had a low capability to stimulate the production of the pro-inflammatory IL-12 (118 and 78 pg/ml, respectively).

Prevention of colitis in mice with Lactibiane Tolérance

First, we characterized the development of colitis in animals subjected to TNBS injection. Two

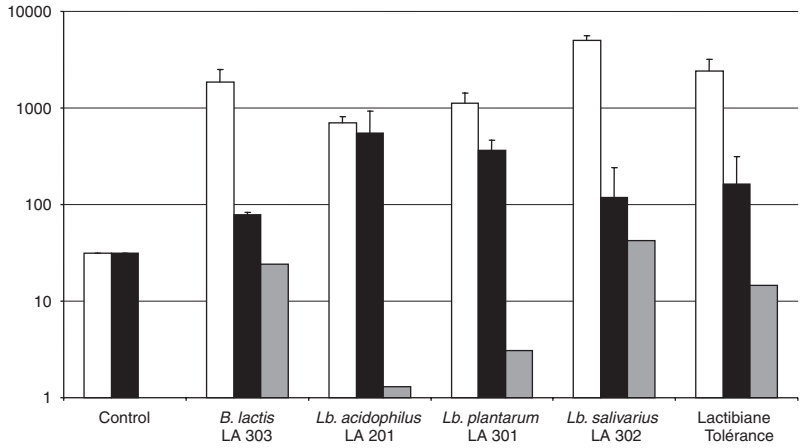

Figure 2 Cytokine induction level of the strains composing Lactibiane Tolérance: IL-10 production levels in pg/ml (□), IL-12 production levels in pg/ml (■), and (IL-10/IL-12) ratio (▨). Values are the average of four donors. The control is PBS buffer, without bacteria.

Table 1 Results of the colitis experiments.

	Mice group					
	Lactibiane Tolérance (Exp1)	Control (Exp1)	Lactibiane Tolérance (Exp2)	Control (Exp2)	Lactibiane Tolérance (Average)	Control (Average)
Wallace score						
Average	1.85	4.28	1.80	3.89	1.83	4.08
SD	1.80	1.33	1.55	1.90	1.67	1.61
SEM	0.57	0.42	0.49	0.60	0.53	0.51
% Protection	57.00		52.60			
P-value*	0.0057		0.0116			
Weight loss %						
Average	−18.33	−23.46	−14.06	−16.19	−16.19	−19.83
SD	7.71	3.25	7.14	5.72	7.42	4.49
SEM	2.44	1.03	2.26	1.81	2.35	1.42
P-value*	0.01		0.27			
Ameho score						
Average			1.80	4.44		
SD			1.75	1.67		
SEM			0.55	0.53		
% Protection			58.10			
P-value*			0.0036			
Mice with Diarrhoea %	20	60	10	40	15	50
Dead mice %	0	20	0	10	0	15

*Mann–Whitney test.

independent experiments (Exp1 and Exp2) have been performed. Table 1 represents the scoring of all relevant parameters, with proper statistical parameters.

Macroscopic score of colitis: Whereas control mice, killed 2 days after administration of 50% ethanol, had no macroscopic lesions in the colon, a severe colitis was measured 2 days after administration of TNBS, with an average macroscopic score (Wallace score) over both experiments of 4.08 ± 1.61 SD; SEM 0.51 (Table 1); dead mice have not been scored.

Histological score: Table 1 also lists the histological Ameho score for the treated mice (Exp2), which was found to be 4.44 ± 1.67 SD; SEM 0.53.

To determine the ability of Lactibiane Tolérance to prevent the experimental colitis, a daily dose containing 10^8 bacteria was administrated from day 5 before induction of colitis to day 1 after induction of colitis. Table 1 and Fig. 3 clearly show a significant reduction of symptoms of TNBS-induced colitis in mice due to the oral administration of Lactibiane Tolérance at the given concentration (Average Wallace score over both experiments $= 1.83 \pm 1.67$; SEM 0.53, *P*-level $= 0.01$ and histological Ameho score $= 1.80 \pm 1.75$; SEM 0.55, *P*-level $= 0.002$). Histological analysis correlated with these findings, showing considerable reduction of epithelial lesions, significant decrease of goblet cells and crypt loss and reduction of inflammatory infiltrates (mainly neutrophils) accompanied with a reduction of colon wall thickness to almost normal levels. Figures of the histological preparations, representing an intact, moderately damaged and severely damaged sample of the intestinal tissue, are represented in Fig. 4.

Lactibiane Tolérance not only led to a considerable attenuation of the colitis but also to a reduced weight loss as compared to non-treated controls (Table 1). In Exp1 the weight loss was significantly reduced in the treated group ($P = 0.01$) while in Exp2 Lactibiane Tolérance limited the weight loss, albeit not significantly (Table 1).

Lactibiane Tolérance also improved the other clinical parameters measured (number of mice with diarrhoea or number of mice that died in the experiment) (Table 1).

In order to compare the effect caused by Lactibiane Tolérance between both experiments, and to compare the effect to other probiotic strains or preparations, the percent protection was calculated as explained above (see also Foligné et al.[29]). The percent protection values obtained in this study with Lactibiane Tolérance are between 50% and 60% (details listed in Table 1).

Discussion

Clinical and experimental data suggest an important role for intestinal microflora in the pathogenesis of IBD, and probiotics have been shown to ameliorate pouchitis. We evaluated the effect of a dietary product on experimental colitis in mice and showed that Lactibiane Tolérance significantly ameliorated colitis induced by TNBS. These results are encouraging and suggest a protective effect for dietary probiotic supplements and should stimulate the initiation of clinical trials in IBD.

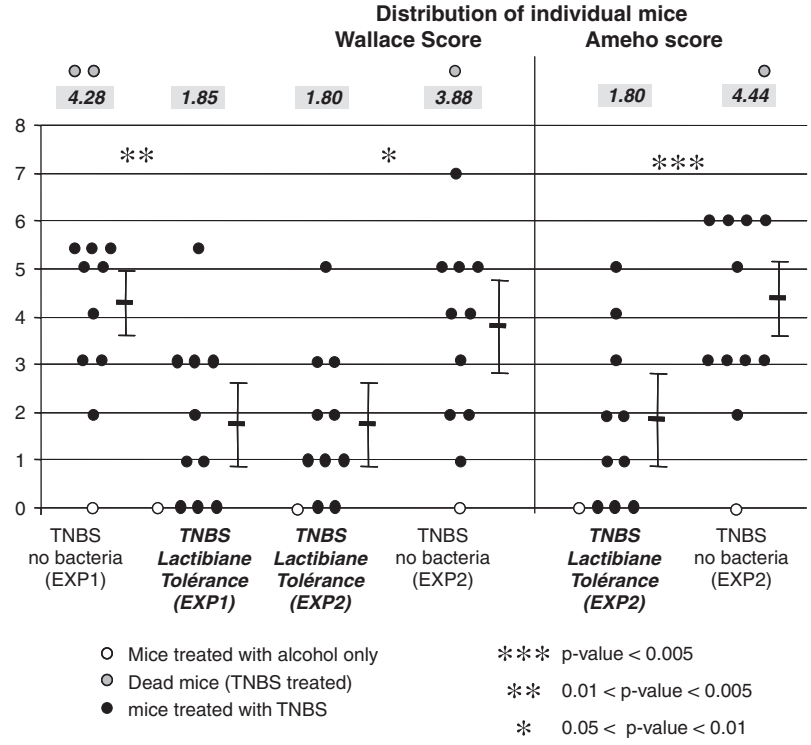

Figure 3 Individual mice scores and group means $\pm$ SD, obtained in both experiments and expressed by the Ameho and Wallace scores. In controls more than 10 mice may have been scored.

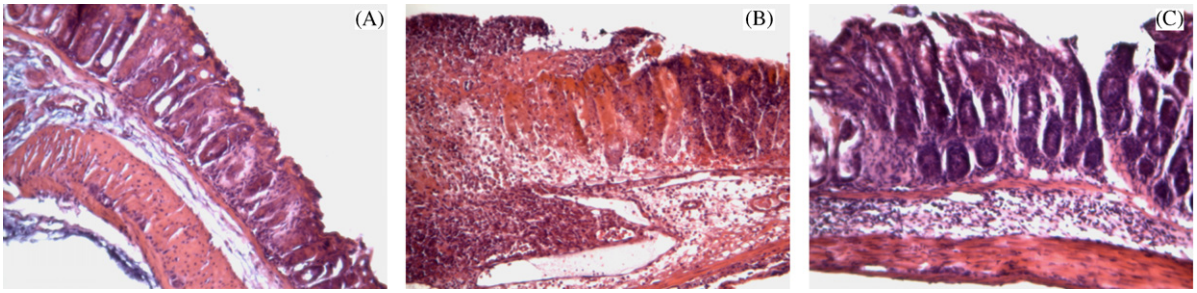

Figure 4 Representative cross sections of murine (balb/c) distal colons, magnification $\times$ 40. (A) Normal appearance of negative control mice. (B) Thickening of the colon wall accompanied with massive inflammatory infiltrate and muscular necrosis in mice 2 days after TNBS induction of colitis. (C) Lesser extent of histological damages after administration of Lactibiane Tolérance to TNBS-treated mice.

The pathogenesis of IBD is complex, involving environmental, genetic, microbial, and immune factors. Therefore, treatment should target components that either predispose to or mediate the chronic inflammatory response of IBD. A large number of chemical compounds have been used in an attempt to revert to the non-inflammatory state, as well as for the maintenance of the remission state. The manipulation of intestinal microbiota has received little attention, and the few studies addressing this option have been carried out with antibiotics. The external manipulation of its composition using probiotic organisms seems to be a promising therapeutic form.[4] Since animal models have provided strong evidence for the role of resident luminal bacteria in IBD pathogenesis, it is appropriate that probiotics are evaluated for their therapeutic efficacy.[24] A number of different rodent models and different probiotics (single or in combination, and in different doses) have been used to date. In some studies, probiotics exerted a beneficial effect in terms of protection against the severity of intestinal inflammation induced or enhancement of disease resolution. Protection against recurrence of the chronic inflammatory disease has also been reported.[30] Although in some studies the probiotic preparations did not have any beneficial effect, they never appeared to be harmful. The differences in therapeutic efficacy observed can be linked to the strain-specific differences observed for different lactic acid bacteria in relation to the immune system of their host.[25,31] Given this observed diversity, a proper screening of the candidate therapeutic strains for their immunomodulatory performance is required. In this study, we used an existing in vitro method based on cytokine inductions by human PBMC, validated by an animal model of colitis to show that Lactibiane Tolérance reduced the effects of TNBS-mediated colitis. These results suggest a potential role for this product in a therapeutic or prophylactic treatment of IBD.

There are a number of possible mechanisms by which probiotics can produce these beneficial effects in IBD. There is strong evidence to suggest that probiotics modulate both innate and acquired immunity. Probiotics could interact with indigenous bacteria and/or host mucosal cells to induce or modulate the immune response, modifying the number of CD4 T cells. It is well known that probiotics can actively interfere with anti-inflammatory and pro-inflammatory signalling pathways, inducing production of IL-10 and reducing INF-γ and TNF release. In this study, we measured the IL-10 and IL-12 ratio after PBMC induction as a potential in vitro marker for anti-inflammation. IL-10 is an essential cytokine with well-established immunoregulatory activity, confirmed in vitro and in vivo using several animal models. In humans, intravenous injection of IL-10 in healthy human volunteers was shown to be safe[32] and to have inhibitory effects on T cells as well as to suppress production of the pro-inflammatory cytokines TNF-α and IL-1β.[33]

Because of this ability to limit production of pro-inflammatory cytokines, IL-10 has been studied for the reduction of acute and chronic inflammatory processes such as acute lung injury, rheumatoid arthritis, and IBD. Already 10 years ago, the intravenous administration of human IL-10 in patients with steroid-unresponsive chronic active Crohn's disease was successful.[34] The availability of both recombinant murine and human IL-10 and the development of a variety of animal models for IBD have improved our understanding of the biological role of IL-10 and indicated a potential therapeutic application for this cytokine. E.g. in IL-10 knockout mice, which will develop chronic enterocolitis resembling IBD in humans, IL-10 administration resulted in the alleviation of IBD associated symptoms.[35–37] The fact that a similar effect has

been obtained with *Lactococcus lactis* strains that were genetically modified to produce murine IL-10[38,39] has been the rationale for the use of probiotic strains with high IL-10 induction potential for the reduction of inflammation in IBD patients. In addition to a high IL-10 induction potential, it is important to minimize the IL-12 induction by the lactic acid bacteria, when considering to select a strain for an anti-inflammatory application. IL-12 is a pro-inflammatory cytokine, mainly produced by phagocytic and antigen-presenting cells (APCs), e.g. as a quick reaction against bacteria, intracellular parasites or other infectious agents. In addition to an important role in the first line of defence against infection, IL-12 will limit or inhibit differentiation of Th2 T cells, itself acting as an immunoregulatory molecule in the Th1 response. IL-12 will induce INF-γ, will directly and indirectly activate natural killer cells, and thus enhance further release of pro-inflammatory cytokines which promote an antigen-specific immune response. This IL-12 production enhancing feedback mechanism, mediated by INF-γ, is potentially leading to uncontrolled cytokine production. Fortunately, IL-10, as a regulatory cytokine, is a potent inhibitor of IL-12 production by these phagocytic cells[40] and may suppress the emergence of an unbalanced Th1 response, such as the one seen in the gastrointestinal tract of IBD patients in a acute phase of inflammation[41]; hence the importance in selecting probiotic strains with a favorable IL-10/IL-12 ratio, and strains which preferably induce very low levels of IL-12. The importance of the ratio between these two cytokines was also recently demonstrated by Peran et al.[42] In this study, administration of a specific strain of *Lactobacillus salivarius* ssp. *salivarius* facilitates the recovery of the inflamed tissue in the TNBS model of rat colitis. This beneficial effect was partly associated to the ability of the strain to modify the cytokine profile in macrophages, reducing the amount of inflammatory cytokine IL-12, while increasing the amount of the anti-inflammatory cytokine IL-10. These results also underline the fact that not all probiotic strains have equal activities in reducing intestinal inflammation and that considering the ratio between IL-10 and IL-12 is important for the screening of probiotics in the field of IBD therapy. PBMC tests have been criticized as they do not take into account the complexity of the intestinal barrier, but they may reflect the responsiveness of human immune cells with obviously rapid and ethic advantages.[43] The use of PBMC from a diversity of healthy human donors to screen the immunomodulatory activity of candidate probiotic strains by direct stimulation appears to us to be a good predictive indicator of in vivo anti-inflammatory strains. Although we agree that the results do not explain the physiological mechanism(s) involved, the assay seems to mimic how the immune system may sense the bacterial strains and evokes their capacity to polarize the immune response. Pure theoretically, live strains with a high IL-10/IL-12 ratio will more easily slow down an early Th1 response. In this context, Hart et al., when assessing effects of different probiotic bacteria on DC function, recently demonstrated that the probiotic preparation VSL#3 (i) was a potent inducer of IL-10 by DC, (ii) could inhibit generation of Th1-cells and (iii) could diminish LPS-induced IL-12 release while maintaining IL-10 production.[44]

In our study, Lactibiane Tolérance has been shown to promote the anti-inflammatory cytokine IL-10 and inhibit the pro-inflammatory cytokine IL-12 in vitro in human PBMC. As shown by the PBMC results, the anti-inflammatory effect is mainly ensured by two of the four strains composing Lactibiane Tolérance, *Bifidobacterium lactis* LA 303 and *Lactobacillus salivarius* LA 302. The PBMC results are perfectly in agreement with the prevention of the initial injury by TNBS obtained in vivo, using the standardized mice model previously developed for the evaluation of lactobacilli, administering daily probiotic doses to mice which are proportional to the doses used in man.[29] A high degree of protection was obtained ($>40\%$, Table 1) with a significant reduction of symptoms for most mice, and in some cases even a complete abolishment of symptoms. The high reproducibility obtained with the TNBS model used supports its use on a long-term basis for the investigation and comparison of individual lactic acid bacteria strains, with the purpose to select the most promising strains for further clinical research (for further details on the model see also Foligné et al.[29]). The fact that the potential to induce high levels of IL-10, combined with a low IL-12 induction profile, has confirmed former observations and hypothesis on the importance of these two cytokines in inflammation control makes the strains *Bifidobacterium lactis* LA 303 and *Lactobacillus salivarius* LA 302 excellent candidates to study the mechanisms underlying this observed reduction of inflammation. Comparison with high IL-12 inducing strains (and or strains which produce low levels of IL-10) will further help to clarify the strain-specific differences observed in this paper. It is hoped that further knowledge of these mechanisms will allow an even more profound selection of the most powerful anti-inflammatory strains, which will significantly reduce symptoms of IBD patients.

Acknowledgments

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