BASIC RESEARCH

Correlation between *in vitro* and *in vivo* immunomodulatory properties of lactic acid bacteria

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Abstract

AIM: To investigate the correlation between the *in vitro* immune profile of probiotic strains and their ability to prevent experimental colitis in mice.

METHODS: *In vitro* immunomodulation was assessed by measuring interleukin (IL)-12p70, IL-10, tumor necrosis factor alpha (TNFα) and interferon γ (IFNγ) release by human peripheral blood mononuclear cells (PBMCs) after 24 h stimulation with 13 live bacterial strains. A murine model of acute TNBS-colitis was next used to evaluate the prophylactic protective capacity of the same set of strains.

RESULTS: A strain-specific *in vivo* protection was observed. The strains displaying an *in vitro* capacity to induce higher levels of the anti-inflammatory cytokine IL-10 and lower levels of the inflammatory cytokine IL-12, offered the best protection in the *in vivo* colitis model. In contrast, strains leading to a low IL-10/IL-12 cytokine ratio could not significantly attenuate colitis symptoms.

CONCLUSION: These results show that we could predict the *in vivo* protective capacity of the studied lactic acid bacteria (LAB) based on the cytokine profile we established *in vitro*. The PBMC-based assay we used may thus serve as a useful primary indicator to narrow down the number of candidate strains to be tested in murine models for their anti-inflammatory potential.

INTRODUCTION

Probiotic lactobacilli and bifidobacteria are increasingly recognized as a way to prevent and/or treat intestinal disorders[1]. Probiotic treatment has been successful in a limited number of clinical inflammatory bowel disease (IBD) trials[2,3], as well as in various experimental rodent models for acute and chronic intestinal inflammation[4]. Cytokines are key regulators of inflammation in IBD, and several pro-inflammatory and immune regulatory cytokines are dysregulated in the mucosa of IBD patients. Probiotic–mediated immunomodulation represents an interesting option in the management of IBD[5] and it was shown that both the systemic and mucosal immune systems can be modulated by orally delivered bacteria[6-8]. However, not all candidate probiotics have been proven equally efficient due to the differences in survival and persistence of the strain in the gastro-intestinal tract, and/or to strain-specific interactions of the probiotic with the host immune system[9-11]. The selection of a successful protective strain may therefore rely on the proper screening of a large number of candidate strains for their technological and immunomodulatory performance.

However, it remains challenging to set up *in vitro* tests with a fair predictive value that would allow us to narrow down the number of candidate strains to be tested in animal models. Until now, results of *in vitro* studies have rarely been linked to *in vivo* effects[12,13]. This could possibly be explained by the variety of parameters that may interfere in the systematic comparison of strains such as the bacterial preparations used (viability, growth phase, dose and timing of administration), possible time effects (early *versus* late

immune responses), or physiological status and type of eukaryotic cells used. When testing human peripheral blood mononuclear cells (PBMCs), the *in vitro* experiment may also be influenced by the method of PBMC preparation as well as the variable responsiveness of the donors[10,14,15]. Once identified, however, these parameters/factors can be controlled by using standardized methodologies[16], allowing, on the one hand, to classify strains according to the *in vitro* differences in their interaction with human immunocompetent cells and, on the other hand, to confirm *in vivo* the "protective capacity" of the best candidate strains (showing between 30% and 70% reduction of the inflammatory score)[16,17]. In this paper we addressed the question whether prophylaxis by oral consumption of live non-pathogenic lactic acid bacteria (LAB) in experimental colitis actually matches their *in vitro* stimulation profile on human PBMCs. Cytokine profiles released *in vitro* by human PBMC stimulated with 13 bacterial strains were compared with the protection they offered in a murine trinitrobenzene sulfonate (TNBS) model of acute colitis. The results of this study demonstrate that the *in vitro* immune profiling of the strains is indeed predictive of their *in vivo* protective effect in a mouse colitis model. These findings support the idea that promising LAB strains for IBD alleviation may be discriminated from non-protective ones using *in vitro* and *in vivo* assays.

MATERIALS AND METHODS

Bacterial strains and growth conditions
Bacterial strains and their origin are shown in Table 1. *Lactobacillus* strains were grown under limited aeration at 37℃ in MRS medium (Difco) and *Bifidobacterium* strains were grown anaerobically in MRS supplemented with 0.05% L-cysteine-hydrochloride (Sigma). *Lactococcus lactis* MG1363 was grown at 30℃ in M17 medium supplemented with 0.5% glucose. *E. coli* and *S. gordonii* were grown at 37℃ in LB and BHI medium (Difco), respectively. The number of live bacteria (CFU) was deduced from the absorbance at 600 nm (A_{600}), using a calibration curve for each strain. For immune cell stimulation, bacterial cells were grown till stationary phase, washed and resuspended at 1×10^9 CFU/mL in phosphate buffered saline (PBS) containing 20% glycerol and stored at -80℃ until used for assays. For *in vivo* experiments, bacteria were grown for 18 h, washed twice in sterile PBS (pH 7.2) and resuspended at 1×10^9 CFU/mL in 0.2 mol/L NaHCO$_3$ buffer (pH 8.8) containing 2% glucose.

PBMC isolation
PBMCs were isolated from peripheral blood of healthy donors as previously described[18]. Briefly, after a Ficoll gradient centrifugation (Pharmacia, Uppsala, Sweden), mononuclear cells were collected, washed in RPMI 1640 medium (Live technologies, Paisley, Scotland) and adjusted to 2×10^6 cells/mL in RPMI 1640 supplemented with gentamicin (150 μg/mL), L-glutamine (2 mmol/L), and 10% foetal calf serum (FCS) (Gibco-BRL).

Induction of cytokine release
PBMCs (2×10^6 cells/mL) were seeded in 24-well tissue

Table 1 Strains used with their origin

Bacterial species /subspecies	Strain designation	Type of isolate, source and/or reference
Lactobacillus salivarius subsp *salivarius*	Ls33	Commercial strain
Lactobacillus rhamnosus	Lr32	Commercial strain
Lactobacillus casei	Bl23	ATCC[1] 393, plasmid-cured
Lactobacillus acidophilus	NCFM	Human, commercial strain
Lactobacillus acidophilus	IPL[3] 908	Commercial isolate
Lactobacillus plantarum	NCIMB 8826	Human, NCIMB[2] collection
Lactobacillus plantarum	Lp115	Commercial strain
Bifidobacterium animalis subsp *lactis*	BL04	Commercial strain
Bifidobacterium animalis subsp *lactis*	BI07	Commercial strain
Bifidobacterium bifidum	BB02	Commercial strain
Lactococcus lactis	MG1363	Cheese starter derivative[42]
Streptococcus gordonii	V288 (Challis)	ATCC[1] 35105
Escherichia coli (non-pathogenic)	TG1	Cloning strain[43]

[1]ATCC: American type culture collection, Manassas, (VA), USA; [2]NCIMB: National collection of industrial and marine bacteria, Terry Research Station, Aberdeen, Scotland; [3]Institut Pasteur Lille, Lille, France.

culture plates (Corning, NY). Twenty microliters of a thawed bacterial suspension at 10^9 CFU/mL were added (bacteria:cell ratio of 10:1). PBS containing 20% glycerol was used as a negative (non-stimulated) control. On the basis of preliminary time-course studies, 24 h stimulation corresponded to the best time point for cytokine responses of bacteria stimulated-PBMCs. After 24 h stimulation at 37℃ in an atmosphere of air with 5% CO₂, culture supernatants were collected, clarified by centrifugation and stored at -20℃ until cytokine analysis. Neither medium acidification nor bacterial proliferation was observed. Cytokines were measured by ELISA using BD pharmingen antibody pairs (BD Biosciences, San Jose, Ca, USA) for tumor necrosis factor alpha (TNFα), interleukin (IL)-10, interferon γ (IFNγ) and IL-12p70, according to the manufacturer's recommendations.

Induction of colitis and inflammation scoring
Animal experiments were performed in an accredited establishment (number 59-35009; Institut Pasteur de Lille, France) and approved guidelines, according to French Ethical Committee and European Union Normatives (number 86/609/CEE). BALB/c and C57/Bl6 mice (female, 8 wk) were obtained from Charles River (St Germain sur l'Arbresle, France). A standardized murine TNBS colitis model was used in which sublethal levels of inflammation were induced[16]. Briefly, a 50 μL solution of 100 mg/kg (BALB/c mice) or 180 mg/kg (C57Bl6 mice) TNBS (Sigma) in 50% ethanol was slowly administered in the colon *via* a 3.5 F catheter. Bacterial suspensions (100 μL), containing 1×10^9 CFU/mL in NaHCO₃ buffer (or buffer alone for controls) were administered intragastrically to mice each day, starting 5 d before until d 1 after TNBS administration. The mice were weighed and killed 48 h after TNBS administration. Colons were removed, washed and opened. Inflammation grading was performed by two
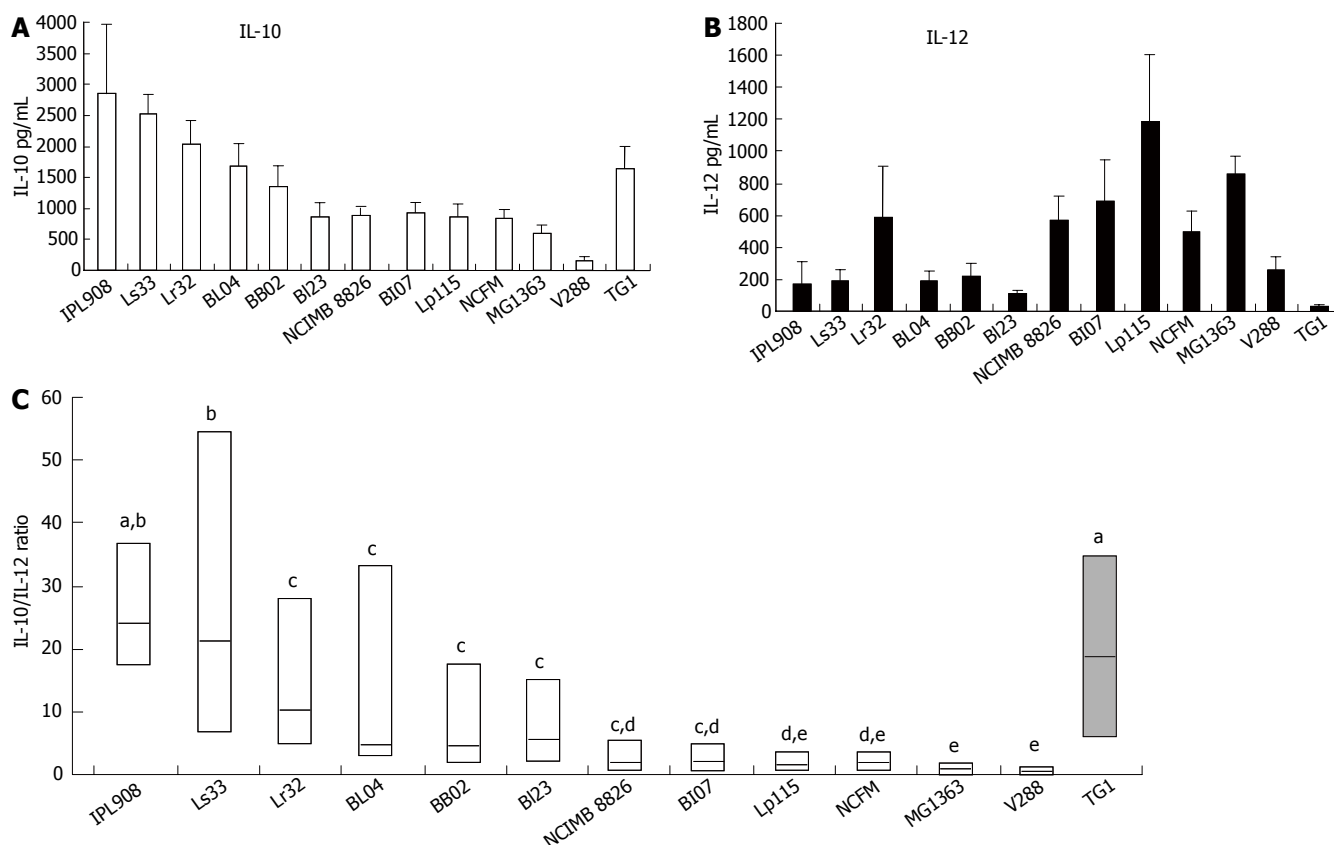
Figure 1 Strain-specific patterns of IL-10 (**A**) and IL-12p70 (**B**) release for various bacterial strains and IL-10/IL-12 ratios (**C**) for 6 to 12 independent healthy donors. Bars represent the mean ± SE values in pg/mL for 6 to 12 independent healthy donors. Ranked box and whisker plots show the median values and first to third quartiles in boxes. Different letters indicate significant differences according to Mann-Whitney U test ($P < 0.05$).

blinded observers, using the Wallace scoring method[19]. Results are expressed as % protection, corresponding to the reduction of the mean macroscopic inflammation score of bacteria-treated mice ($n = 10$) in comparison to the mean score of TNBS-treated control mice (NaHCO₃ buffer-treated mice, $n = 10$)[16]. Histological analysis was performed on hematoxylin/eosin-stained 5 μm tissue sections from colon samples fixed in 10% formalin and embedded in paraffin.

Statistical analysis

Results were analyzed by the non-parametric one-way analysis of variance and Mann-Whitney U test. Differences were judged to be statistically significant when the P value was < 0.05. For *in vivo* experiments, only protection levels exceeding 30% (positive and negative) were considered to be relevant, as previously described[16]. For the calculation of the IL-10/IL-12 ratio, all undetectable IL-12 values (below 50 pg/mL) were arbitrarily set at 50 pg/mL level to normalize aberrant quotients. Association of variables was analyzed by the P value-assigned Spearman rank correlation coefficient.

RESULTS

Cytokine release by PBMCs is strain specific

The *in vitro* immunostimulation by 13 live bacterial strains (Table 1) of PBMCs collected from 6 to 12 independent donors, revealed distinct and typical patterns of cytokine release. TNFα release was quite uniform for the different LAB investigated, while IFNγ showed variations tending to parallel IL-12 profiles (data not shown). IL-10 and IL-12 levels displayed a strain-specific pattern (Figure 1A and B). Variations in IL-10 concentrations were substantial with values ranging between 200 and 3000 pg/mL depending on the bacterial strain. For IL-12, we also observed significant variations between strains, covering a range of cytokine levels of 50 to 1200 pg/mL. As IL-10 and IL-12 appeared to be the most discriminative cytokines, we used the IL-10/IL-12 ratio (Figure 1C) to distinguish between strains exhibiting a "pro-" *versus* "anti-inflammatory" profile (low *versus* high IL-10/IL-12 ratio, respectively). This approach was found to be useful to identify strains with marked opposite profiles, but did not allow discrimination of strains with median cytokine ratios.

The variation in absolute cytokine concentrations released by PBMCs derived from different donors was examined by conducting successive experiments with a limited set of 6 strains (*E. coli* TG1, *L. salivarius* Ls33, *L. casei* Bl23, *L. plantarum* NCIMB 8826, *Lc. lactis* MG1363 and *L. acidophilus* NCFM). In general, for a variety of individual donors, the ranking of strains was quite reproducible: the most potent "anti-inflammatory" strains induced the highest IL-10 responses in all donors, while other strains were stronger IL-12 inducers in most donors. As an example, Figure 2A and B shows the IL-10 and IL-12 expression profiles for four donors in response to the six "reference" strains used. Relative differences
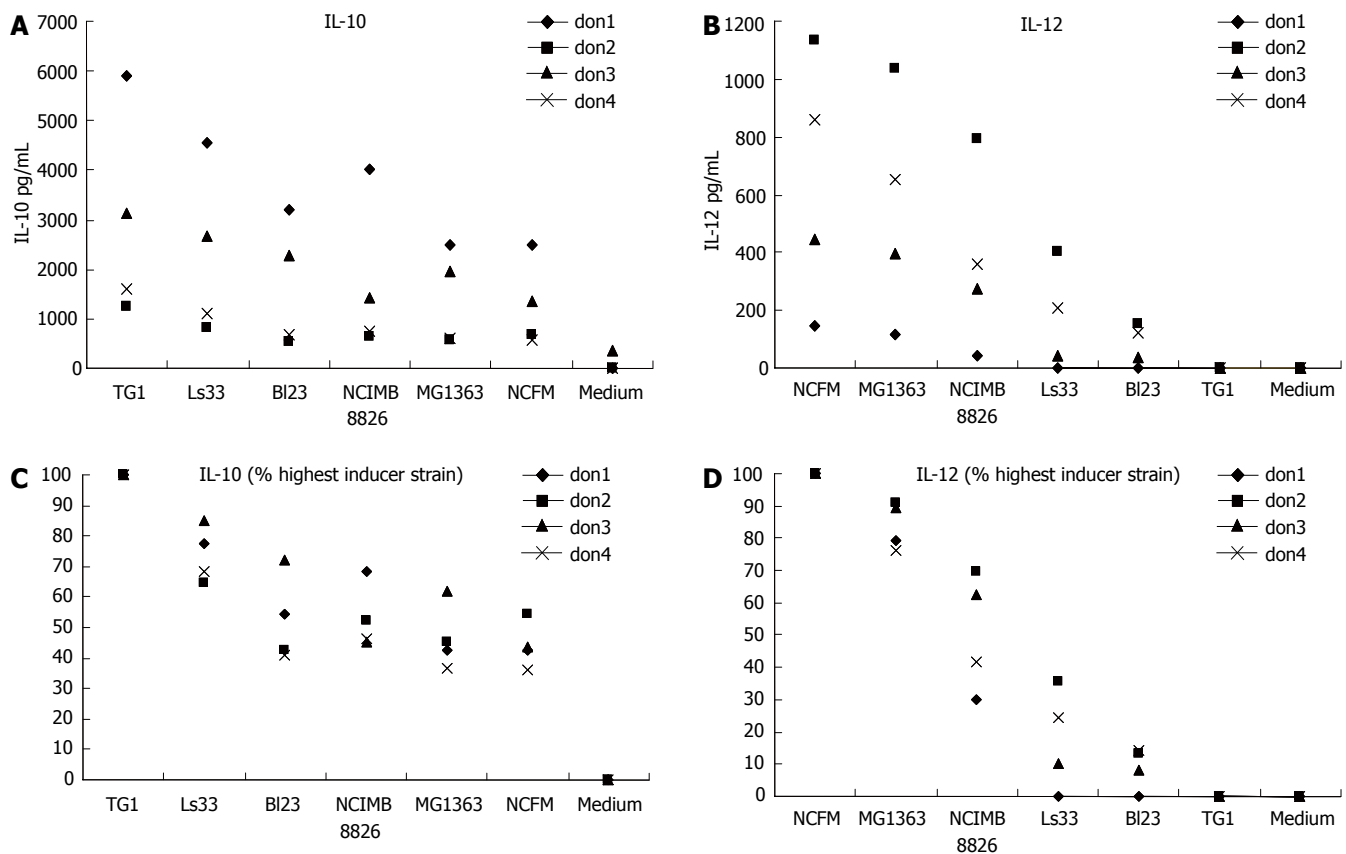
Figure 2 IL-10 (**A**) and IL-12p70 (**B**) release in 4 distinct individual human PBMC donors and the expression level of IL-10 (**C**) and IL-12p70 (**D**). Individual values are represented in pg/mL while IL-10 and IL-12p70 levels are expressed as % of the highest inducer strain. [a]$P < 0.05$.

between two strains, expressed as a percentage of the highest inducer, taken as internal control, were quite constant for all donors (Figures 2C and D). For example, we consistently found 35% and 82% difference ($P < 0.05$) between the strains *L. salivarius* Ls33 and *L. acidophilus* NCFM, for IL-10 and IL-12 induction, respectively. Based on these observations, both strains could be compared in a semi-quantitative way, using their average IL-10 and IL-12 release patterns upon stimulation of PBMCs from four different donors. To that extent we calculated a full matrix of *P*-values for the IL-10/IL-12 ratios obtained from several overlapping studies with reference strains as well as new isolates, for at least 4 PBMC donors, which allowed us to rank strains from an "anti-inflammatory" to a "pro-inflammatory" profile.

When applied to the 13 strains used in this study, this methodology established a semi-quantitative ranking, which could classify strains *L. salivarius* Ls33, *L. casei* Bl23, *L. rhamnosus* Lr32, *L. acidophilus* IPL908 as more anti-inflammatory than the three bifidobacteria and the two *L. plantarum* strains. *Lc. lactis* MG1363, *S. gordonii* V288 and *L. acidophilus* NCFM® strains showed a slightly pro-inflammatory profile with a very low IL-10/IL-12 ratio.

Protection of TNBS-induced colitis was strain-specific

We investigated the protective effect of the 13 strains studied *in vitro* against TNBS-induced colitis in mice (Figure 3). Ls33, Lr32, Bl23, IPL908 and BL04 strains consistently led to a considerable attenuation of colitis (data represent the result of usually 2 to 4 distinct experiments),

with reduced weight loss, improved clinical parameters (rectal bleeding, stool consistency, *i.e.* liquid pasty stool and diarrhoea, lethargy; data not shown) and reduced macroscopic inflammation scores. Considering the % protection as the reduction of the mean macroscopic inflammation score of bacteria-treated mice ($n = 10$) in comparison to the mean score of TNBS-treated control mice, the *Lactobacillus plantarum* strains and the BB02 and Bl07 bifiidobacterial strains induced moderate but significant levels of protection. In contrast, no improvement in colitis was observed for the strains *L. acidophilus* NCFM, *Lc. lactis* MG1363 or *S. gordonii* V288 and for the non-pathogenic *E. coli* TG1. None of these strains, however, aggravated the symptoms of colitis. Histological analysis corroborated these findings, showing dramatic improvement in epithelial lesions of the animals receiving protective strains, with a significant decrease in goblet cells and crypt loss (data not shown), and reduced inflammatory infiltrates (mainly neutrophils) accompanied with a reduction of the colon wall thickness to almost normal levels (Figure 4).

Additional experiments confirmed this strain-specific protection in mice with a different genetic background (C57/Bl6 mice). The protection observed in BALB/c mice with *L. salivarius* Ls33 (56.5% ± 7.2%, $P < 0.01$) was confirmed in C57/Bl6 mice (47%, $P < 0.01$), whereas the *L. acidophilus* NCFM® and *E. coli* strains alleviated colitis neither in BALB/c mice (-12.5% ± 2.7%, NS; and +19.4% ± 3.7%, NS, respectively) nor in C57/Bl6 (+26%, NS; and -6.4%, NS, respectively).
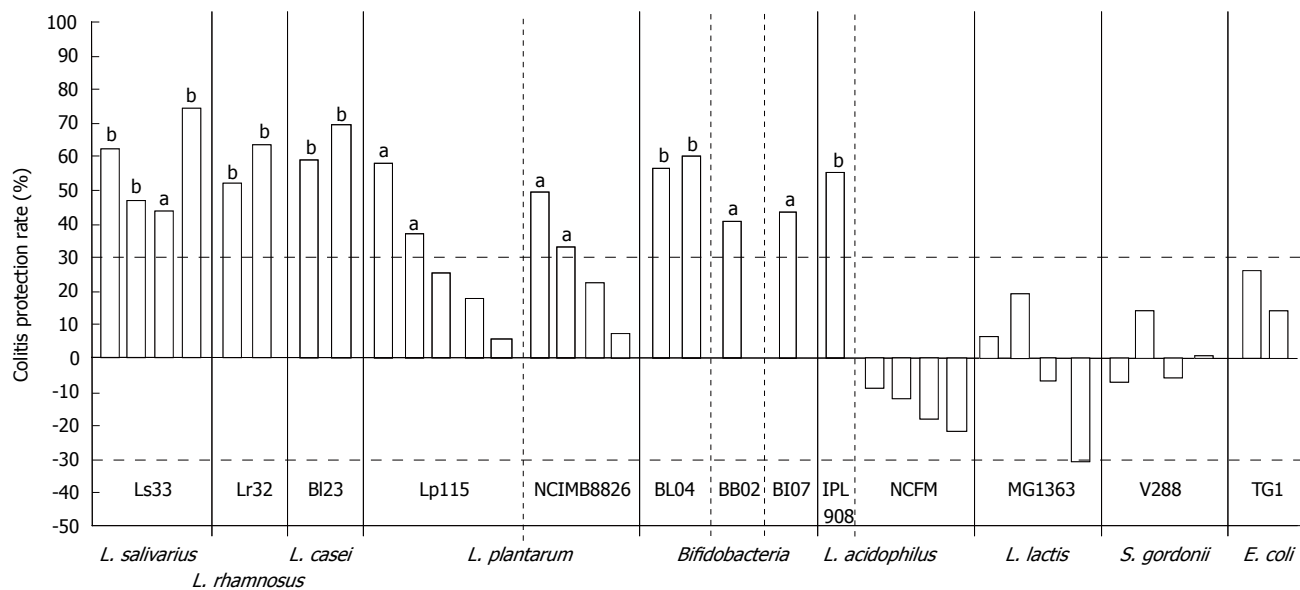
Figure 3 Protective effect of LAB strains against TNBS-induced colitis in BALB/c mice. Results are expressed as a % reduction of the mean macroscopic inflammation of mice treated with LAB as compared to the mean score of non-treated mice. Colitis index was assessed 48 h after TNBS administration. Each bar represents an independent experiment and corresponds to the ratio of control and LAB-treated mice groups ($n = 10$). [a]$P < 0.05$, [b]$P < 0.01$ vs TNBS-control group. The horizontal dashed lines indicate the 30% threshold of the uncertain statistical significance.

In vivo / In vitro correlation

Considering both *in vitro* and *in vivo* results, it was evident that strains displaying the highest *in vitro* anti-inflammatory profile (a high IL-10/IL-12 ratio) were the most protective in the *in vivo* colitis model, while those leading to intermediary *in vitro* IL-10/IL-12 ratios showed limited protection. In contrast, bacteria characterized by a low anti-inflammatory potential (low IL-10/IL-12 ratio) did not improve inflammation at all. As a result, with the exception of the Gram-negative *E. coli*, the ranking of all Gram-positive bacteria investigated, based on the *in vitro* cytokine profiling, closely matched the ranking based on the improvement of colitis symptoms. Although this link could not be expressed as an "exact linear" association between % protection and IL-10/IL-12 ratio, it was found to be highly significant using the Spearman rank correlation coefficient ($r_s = 0.825$, $P < 0.001$) (Figure 5).

DISCUSSION

Immunomodulation through probiotics represents one of the current treatment options for IBD[5,20] and specific strains may stimulate immunomodulatory mediators, inhibit pro-inflammatory cytokines and influence the phenotypes of immunocompetent cells with subsequent events such as migration of dendritic cells and induction of regulatory T cells[15,21]. The mechanisms of action of probiotics are most probably multi-factorial, involving a variety of effector signals, cell types and receptors[22], and strains may differ in their respective ability to trigger these signals considering both immunocompetent and intestinal epithelial cells[23]. It has been proposed that some probiotics are able to prevent or restore intestinal homeostasis after an immune dysregulation, improving mucosal barrier functions as well as down-regulating inflammatory responses[24,25].
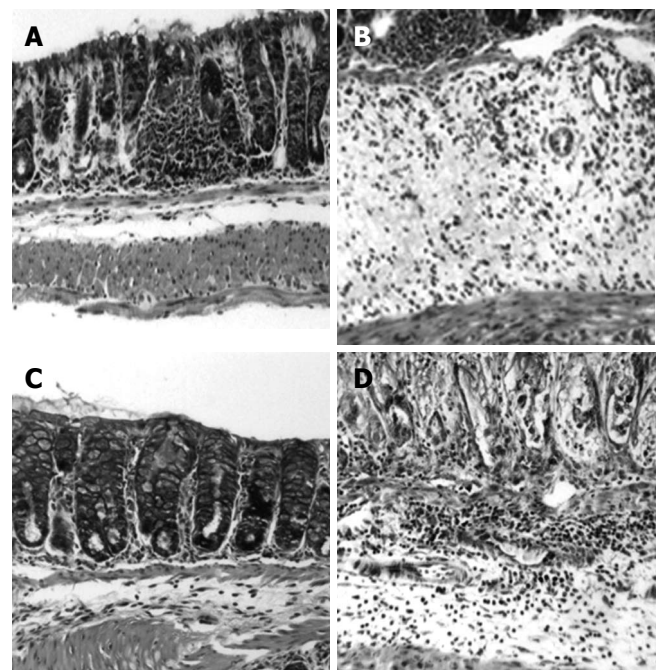
In this study, we aimed at developing a simple and

Figure 4 Hematoxylin/eosin staining of representative cross-sections of murine (BALB/c) distal colon (HE, x 20). **A**: Normal appearance of the colon from a negative control mice; **B**: Thickening of the colon wall accompanied with massive inflammatory infiltrate and muscular necrosis 2 d after TNBS-induction of colitis; **C**: Reduction of histological damage in *L. salivarius* Ls33-treated mice after TNBS administration; **D**: Lack of histological improvement of colitis in *L. acidophilus* NCFM-treated mice after TNBS administration.

standardized *in vitro* test allowing preliminary classification of candidate probiotic strains according to their immune modulation capacity that would be predictive of their *in vivo* effect. To this end, we ranked 13 LAB strains with reference to the IL10/IL12 cytokine ratio induced on human PBMCs and further assessed their protective effect against TNBS-induced colitis in mice.

We observed strong strain-specific variations of the *in vitro* cytokine induction profiles after stimulation of immunocompetent cells, which confirms the results reported in previous studies[9-11,15,26]. We also noted that differences between blood donors did not prevent us ranking the strains based on their cytokine responses as the relative responses of PBMCs to various LAB were consistent from one donor to another. PBMCs from healthy donors can thus be used to screen the immunomodulatory activity of candidate probiotic strains and this test/assay appears to be a good indicator of *in vivo* anti-inflammatory strains. Despite the fact that this assay does not clarify the physiological mechanism(s) involved, it seems to mimic how the immune system may sense the bacterial strains and consequently polarise the immune response. Strains leading to a high IL-10/IL-12 ratio would more easily slow down an early Th1 response.

In accordance with this hypothesis, we found differences in the *in vivo* protective capacity of the 13 studied strains against TNBS-induced inflammation[27]. This strain-specificity has already been described in other experimental models of colitis[28-31]. The variations we observed between different LAB strains in this respect cannot simply be explained by differences in persistence, especially when daily administrations maintain the level of bacteria in the gastro-intestinal tract. Of note, we have previously verified whether the anti-inflammatory profiles could in any way be linked to *in vitro* properties such as gastric juice (pepsin/pancreatin) and bile salt resistance, epithelial cell adhesion and *in vivo* intestinal persistence (unpublished data). However, no link was found between them.

Above all, we have demonstrated in this study that the ranking of strains obtained on the basis of an *in vitro* IL-10/IL-12 cytokine induction ratio closely matches the ranking of the *in vivo* ability of the strains to attenuate experimental colitis. The concordance between the *in vitro* and *in vivo* assays has been illustrated in the case of a cell-wall mutant of *L. plantarum*[18]. The importance of the IL-10/IL-12 ratio has also recently been highlighted by Peran *et al*[32] who showed that administration of a specific strain of *Lactobacillus salivarius* subsp. *salivarius* could improve the recovery of inflamed tissue in a TNBS model of rat colitis. This strain was selected among 30 LAB strains for eliciting the best IL-10/IL-12 and IL-10/TNFα ratios. Unfortunately, no strains exhibiting a moderate or low IL-10/IL-12 profile were included in the *in vivo* study, which could have validated the proposed screening strategy. Similarly, *in vitro* tests on immunocompetent cells have been used successfully to study the effect of distinct bacterial fractions (intact cell walls, protoplast and polysaccharide-peptidoglycans) of the strain *Lactobacillus casei* Shirota[33]. Using the ability of this strain to inhibit the LPS-induced IL-6 in mice bone marrow derived-DCs and PBMCs isolated from ulcerative colitis patients, the authors could establish that the polysaccharide-peptidoglycan complex was the most efficient compound to improve chronic colitis and ileitis in their mice model. Another example attesting similar links between *in vitro* macrophage stimulation and related protection in a murine TNBS model of colitis was reported for the anti-inflammatory
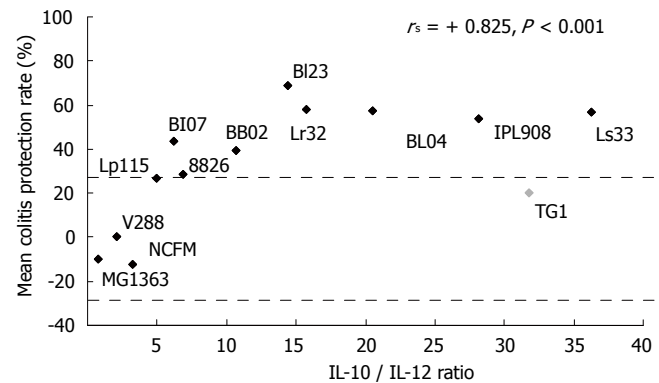

Figure 5 Correlation between the average protection against experimental colitis and the mean IL-10/IL-12 ratios obtained *in vitro*, for all investigated strains. Spearman coefficient ($r_s = 0.825$, $P < 0.001$). The horizontal dashed lines indicate the 30% threshold of unprotectiveness.

drug "catapolside"[34], leading to clinical trials in IBD patients in Korea.

Recently, the PBMC IL-10/IL-12 ratio has also been used to follow the immunomodulatory properties of probiotics in a clinical study on irritable bowel syndrome (IBS)[31]. Despite different aetiology and characteristics, IBD and IBS share some aspects of immune dysregulation, and IBS has been documented to be associated with low-grade inflammation. Concomitantly to alleviation of the main IBS symptoms, the authors showed normalization of the PBMC IL-10/IL-12 ratio in patients receiving probiotics *versus* placebo, showing that *in vivo* probiotic efficiency can be correlated to specific *in vitro* assays.

Despite the positive correlation observed between *in vivo* protection and *in vitro* cytokine ratio, some restrictions are clear. First of all, the link is not valid for Gram-negative bacteria. Gram-negative bacteria, including both non-pathogenic and pathogenic strains, are well known to be potent inducers of monocytic IL-10, while Gram-positive species preferentially stimulate IL-12 [10]. These observations do not exclude Gram-negative strains from being anti-inflammatory. For example, bacteria such as the *E. coli* strain Nissle[35] or virulence-attenuated *Yersinia pseudotuberculosis* mutants[36] have been shown to be able to reduce colonic lesions and inflammatory mediators in murine models of experimental colitis. Secondly, it is not excluded that *Lactobacillus* strains may moderate colitis by non immune-related modes of action, as for example by acting on barrier integrity or influencing the oxidative pathway[37]. Alternatively, LAB strains with a rather pro-inflammatory profile may have other health benefits which substantiate their probiotic status[38,39].

The methods described in this paper can be used to screen a larger set of potential probiotic strains for their immunomodulatory properties, since they are relatively simple and quite reproducible. We are currently building a reference database including both *in vitro* cytokine profiles, as well as protection levels measured in the TNBS mice model for selected reference strains. This database encompasses strains and blends used in former clinical trials in IBD (i.e. *L. rhamnosus* GG, VSL#3 and *E. coli* strain Nissle[2,3,40,41]), which could further support

and validate the predictive value of the screening strategy presented in this paper.

In conclusion, our results linked *in vitro* and *in vivo* anti-inflammatory properties of a series of LAB and confirm that potential probiotic strains can be pre-screened *in vitro* for their immunomodulating potential, before animal and clinical investigations. This allows pre-selection of probiotics able to modulate the host immune system in a specific way while reducing considerably the use of animals for screening purposes. Besides these ethical considerations, the comparative study of strains carefully selected for either pro- or anti-inflammatory properties will assist further investigation of the mechanism(s) by which specific probiotics signal to the host.

COMMENTS

Background
Evidence exists for the protective role of selected probiotic strains in inflammatory bowel disease. Probiotic strains have clearly been shown to differ in their *in vitro* interaction with immunocompetent cells, especially in terms of cytokine responses. No clear link has been established so far between the *in vitro* immunomodulation potential (e.g. on PBMC's) of the probiotic strain and its ability to prevent experimental colitis in mice (e.g. in TNBS- induced colitis). The use of simple *in vitro* methods to select the most efficient strains for possible clinical trials will improve the quality and reduce the costs of this type of research.

Research frontiers
Improving gastrointestinal function is important for IBD patients and fundamental research is needed to develop new treatments for such pathogenesis. Probiotics are generally accepted to be one of these alternative methods. Few original research papers, however, have evaluated the potential of this approach by focusing on the level of the bacterial strain or strains used.

Probiotic-mediated immunomodulation represents an interesting option in the management of IBD and there is evidence that the immune system can be modulated by orally delivered bacteria. However, in some animal and clinical studies, probiotics did not show the expected beneficial effect. The selection of a successful protective strain may rely on a proper screening of a large number of candidate strains for their technological and immunomodulatory performance, as recently published in *WJG*. Together with proper *in vitro* analytical methods, the use of a reliable animal model is therefore indispensable. Using a well-standardized animal model, we have demonstrated that consistent differences in anti-inflammatory potential of several orally administered lactic acid bacteria could be observed in TNBS-induced colitis.

In the present study we linked for the first time the results of *in vitro* and *in vivo* anti-inflammatory readouts for 13 selected LAB, and confirmed herewith the strain-specific nature of the potential of probiotics to modulate the host's immune system and in assisting in the alleviation of intestinal disorders, IBD in particular.

Innovations and breakthroughs
Linking *in vitro* and *in vivo* anti-inflammatory properties of a series of LAB can confirm the interest of screening potential probiotic strains using *in vitro* assays, before launching animal and clinical investigations. This allows not only the pre-selection of probiotics able to modulate the host's immune system in a specific way while reducing considerably the use of animals for screening purposes, but also results in a classification of microorganisms that could be useful for the study or comparison of other probiotic properties. So, besides ethical considerations, the comparative study of strains carefully selected for either pro- or anti-inflammatory properties will assist further investigation of the mechanism(s) by which specific probiotics signal to the host.

Applications
The use of probiotic strains in IBD treatment has been controversial, because of some major drawbacks. The first is the type of inflammatory disease targeted (Crohn's disease on the one hand, with low success levels, *versus* ulcerative colitis and pouchitis where treatment was more successful). The second is that a comparison between a therapeutic preventive application in combination with or without traditional medication, is not always clear. The third is that the choice of strains or the mixture thereof is often made rather artificially and driven by available commercial preparations which are not necessarily developed or suitable for this type of medical application. Using the described selection procedure, the influence of well-selected strains (e.g. with expected positive and negative performance as anti-inflammatory agent) in different conditions (with or without traditional medication) of a selected animal model (prophylactic or therapeutic) can be evaluated unequivocally. The results should not only allow to select the best possible conditions for the follow-up clinical study but will also help to understand the underlying mechanisms and factors that influence the efficiency of the application.

Terminology
The TNBS mouse model of colitis is a hapten-induced experimental model (TNBS = 2, 4, 6-trinitrobenzene sulfonic acid), which has proven to be a very useful model for studying certain forms of human inflammatory bowel disease. This model has e.g. been used to show that an IL-12-driven, Th1 T cell-mediated inflammation of the colon is not only prevented by systemic administration of anti-IL-12 antibody, but can also be treated this way. Consequently anti-IL-12 is currently used in the treatment of Crohn's disease. Other studies with this model have established that mucosal inflammation and/or its prevention depend partially on a balance between pro-inflammatory Th1 T cell responses and anti-inflammatory TGF-β and IL-10 responses.

Peer review
This is an interesting study showing that *in vitro* assays using human PBMCs can partially translate to murine models of colitis giving an indication of the level of expected protection of this particular model of colitis using IL-10/IL-12 ratios. It would be interesting to discover whether this held out if colitis was induced before administration of the probiotic (perhaps a more physiological approach for therapy). However, this does offer some insight into prophylactic approaches for managing patients in remission.

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