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Impact of lipoteichoic acid modification on the performance of the probiotic *Lactobacillus rhamnosus* GG in experimental colitis

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Summary

While some probiotic strains might have adjuvant effects in the therapy for inflammatory bowel diseases (IBD), these effects remain controversial and cannot be generalized. In this study, a *dltD* mutant of the model probiotic *Lactobacillus rhamnosus* GG (LGG), having a drastic modification in its lipoteichoic acid (LTA) molecules, was analysed for its effects in an experimental colitis model. Dextran sulphate sodium (DSS) was used to induce either moderate to severe or mild chronic colitis in mice. Mice received either phosphate-buffered saline (PBS), LGG wild-type or the *dltD* mutant via the drinking water. Macroscopic parameters, histological abnormalities, cytokine and Toll-like receptor (TLR) expression were analysed to assess disease activity. LGG wild-type did not show efficacy in the different experimental colitis set-ups. This wild-type strain even seemed to exacerbate the severity of colitic parameters in the moderate to severe colitis model compared to untreated mice. In contrast, mice treated with the *dltD* mutant showed an improvement of some colitic parameters compared to LGG wild-type-treated mice in both experimental models. In addition, treatment with the *dltD* mutant correlated with a significant down-regulation of Toll-like receptor-2 expression and of downstream proinflammatory cytokine expression in the colitic mice. These results show that molecular cell surface characteristics of probiotics are crucial when probiotics are considered for use as supporting therapy in IBD.

Keywords: dextran sulphate sodium, inflammatory bowel disease, lipoteichoic acid, probiotic–host interaction, Toll-like receptors

Introduction

Inflammatory bowel diseases (IBD), such as Crohn's disease (CD) and ulcerative colitis (UC), are chronic illnesses that involve inflammation of the intestinal tract [1]. An increased prevalence of these diseases has been documented in developed countries. It is estimated that more than 3 million people are affected in North America and Europe [2,3]. The pathogenesis of these diseases is not fully understood, but besides genetic, environmental and immunoregulatory factors, the enteric microbiota seem to play an important role. It is thought that the inflammation results from an aberrant mucosal immune response against the indigenous microbiota in genetically susceptible hosts [4]. Additionally, it has been found that IBD is linked to an altered microbiota composition (dysbiosis) [5]. Among the mechanisms by which bacteria may promote inflammatory signalling, recent evidence suggests that microbe-associated molecular patterns (MAMPs) derived from intestinal bacteria may modulate IBD via stimulation of their respective innate immune receptors, including Toll-like receptors (TLRs) [6]. This is reflected, for example, by the dysregulation of several TLRs and susceptibility genes, such as nucleotide-binding oligomerization domain-containing 2 (NOD2), in colitis [7].

Some probiotics, which are defined as 'live microorganisms that when administered in adequate amounts can confer a health benefit on the host' [8], have been suggested to help in restoring the imbalances associated with IBD [9,10]. Therefore, probiotics might be useful as supporting therapeutic agents, although the results of clinical trials were not always unambiguous [10–12]. A crucial factor might be the choice of the probiotic strain. One of the best-documented and model probiotic strains is *Lactobacillus rhamnosus* GG (LGG) [13]. Well-substantiated health effects include prevention of acute diarrhoea in children [14], prevention of antibiotic-associated diarrhoea [15–17], prevention of atopic disease [18] and treatment of recurrent *Clostridium difficile*-associated colitis [19]. In IBD patients,

most promising clinical effects with LGG are in prevention of pouchitis [20] and maintenance of remission in UC [21], while clinical studies with LGG in patients with CD did not result in positive outcomes [22–24].

Some molecules of LGG have been suggested to be important for the probiotic effects based on *in vitro* studies. For example, two secreted proteins of LGG were demonstrated to prevent cytokine-induced apoptosis in intestinal epithelial cells [25]. In addition, LGG DNA was shown recently to induce anti-inflammatory signalling via Toll-like receptor (TLR)-9 in polarized intestinal epithelial cells [26]. However, the lack of efficacy of LGG in several clinical trials with IBD patients [22–24,27] and in animal models of colitis [28,29] suggests that LGG contains factors that confound its anti-inflammatory effects *in vivo.*

Lipoteichoic acid (LTA) is a macroamphiphilic molecule anchored in the cytoplasmic membrane through its glycolipid moiety. It consists of a glycerol-phosphate or ribitol-phosphate chain decorated with D-alanine ester or glycosyl substitutions, and extending into the cell wall [30]. It is generally regarded as a proinflammatory bacterial molecule. LTA can be seen as the Gram-positive counterpart of Gram-negative lipopolysaccharides (LPS) [31,32], as both molecules stimulate macrophages to secrete proinflammatory cytokines *in vitro*, although LTA is generally less active [33]. The *in vivo* importance of the proinflammatory potential of LTA of gut bacteria is less clear. In healthy conditions, LTA does not cause excessive inflammation in the gut, as intestinal epithelial cells have developed special mechanisms to tolerate the continuous exposure to LTA of commensals in the gut lumen, such as down-regulation of TLR expression [34,35]. In the inflamed and more permeable gut of IBD patients LTA can, however, be hypothesized to activate macrophages and other inflammatory cells [36], although this needs to be substantiated further.

In the present work, we investigated the impact of a dedicated gene-knock-out mutation (*dltD*) on the anti-inflammatory efficacy of the probiotic strain LGG in a murine experimental colitis model. This LGG *dltD* mutant was constructed and characterized previously [37]. Its LTA molecules were shown to be completely devoid of D-alanine esters, drastically altering the LTA structure *in situ* on live LGG bacterial cells [37]. We induced colitis in mice by administration of dextran sulphate sodium (DSS) to focus on the involvement of epithelial barrier disruption and innate immunity.

Materials and methods

Animals

Pathogen-free female BALB/c and C57/BL6 mice, 6–8 weeks old, weighing 16–22 g, were obtained from Harlan (Zeist, the Netherlands). The mice were housed in conventional filter-top cages and had free access to commercial feed and water. All experiments were performed under the approval of the K. U. Leuven Animal Experimentation Ethics Committee (Project approval number 027/2008).

Bacterial strains, media and growth conditions

Lactobacillus rhamnosus GG (ATCC53103) (LGG) and its derivatives CMPG5540 (*dltD* mutant; tetracycline resistant) [37] and CMPG5340 (wild-type control strain used in the *in vivo* persistence analysis; erythromycin and tetracycline resistant) [38] were grown routinely at 37°C in de Man–Rogosa–Sharpe (MRS) medium (Difco; BD Biosciences, Erembodegem, Belgium) under static conditions. For solid medium, 15 g/l agar was used. If required, antibiotics were used at the following concentrations: 5 μg/ml of erythromycin and 10 μg/ml of tetracycline.

Survival in simulated gastric juice

Simulated gastric juice was prepared as reported previously [39]. The experiments were performed as described previously by Lebeer *et al.* [38].

Survival in the murine gastrointestinal (GIT) tract

To analyse the persistence capacity of the *dltD* mutant *in vivo*, a competition experiment was performed in 6–8-week-old female BALB/c mice, as described previously [38].

DSS-induced colitis model

Moderate to severe colitis was induced in 6–8-week-old female C57/BL6 mice by applying four cycles of 4 days 3% DSS (35–50 000 kDa; MP Biomedicals, Illkirch, France) followed by 3 days of normal drinking water [40]. Mild chronic colitis was induced by applying three cycles of 7 days 1% DSS, followed by 7 days of normal drinking water. In both models, LGG wild-type and *dltD* mutant were administered via the drinking water at a concentration of 10^8 colony-forming units (CFU) per ml throughout the experiment starting 3 days before the first cycle of DSS. Samples were taken from the drinking water throughout the experiment to confirm the concentration of viable cells. Plain phosphate-buffered saline (PBS) was used as a control. The mice given DSS were divided randomly into three treatment groups (PBS, LGG wild-type and *dltD* mutant) and their body weight was monitored daily. Mice were killed by cervical dislocation 29 days (3% DSS model) or 43 days (1% DSS model) after induction of colitis. The entire colon (caecum to anus) was removed and colon length was measured from the ileocaecal junction to the anus. The macroscopic scoring was based on the scoring of Mourelle *et al.* [41], with a maximum score of 9.

Histopathological evaluation of colitis

The colon was divided into segments representing the proximal, mid- and distal colon. From each part of the colon, a

piece was taken, fixed in 6% formalin, embedded in paraffin, cut into slices and stained with haematoxylin and eosin. Stained sections were analysed blindly by a pathologist (G.D.H.) using the scoring of Kojouharoff *et al.* [42] with a maximum of 16.

Quantitative reverse transcriptase–polymerase chain reaction (qRT-PCR)

For qRT-PCR, the remaining part of the colon was snap-frozen in liquid nitrogen and stored at –70°C until total RNA was extracted using the RNeasy Mini Kit (Qiagen, Gaithersburg, MD, USA). First-strand cDNA synthesis was catalysed by SuperScript II RT (Invitrogen, Carlsbad, CA, USA) using 1 μg of total RNA. The enzyme was then inactivated by incubation at 70°C for 15 min. The amount of cDNA was quantified by real-time RT-PCR using specific primers for β-actin, tumour necrosis factor (TNF), interleukin (IL)-10, IL-12p40, transforming growth factor (TGF)-'beta' and interferon (IFN)-γ with the ABI Prism 7700 Sequence Detection System (SDS) from Applied Biosystems (Foster City, CA, USA). The sequences of the primers and *Taq*Man probes for murine TNF, IL-10, IL-12p40, TGF-β, IFN-γ and β-actin have been reported previously [43]. PCR was performed as described by Maerten *et al.* [44] and cytokine expression levels were normalized against the housekeeping gene β-actin. Expression of TLR-1, -2, -4 and -6 was analysed using Power SYBR® Green PCR Master Mix (Applied Biosystems). Data were quantified using the $\Delta\Delta C_t$ method relative to the housekeeping genes β-actin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The sequences of primers for murine β-actin [43], GAPDH [45] and TLR-1, -2, -4 and -6 [46] were reported previously.

Statistics

Values are presented as mean ± standard error of the mean. Macroscopic and histological scores were analysed statistically using the Mann–Whitney *U*-test. Differences in parametric data were evaluated by the unpaired Student's *t*-test. A value of $P \leq 0{\cdot}05$ was considered to be significant.

Results

dltD mutation does not drastically alter the survival capacity of LGG in the GIT

Changing the integrity of the bacterial cell surface can impact highly upon the persistence capacity of probiotic bacteria in the GIT [47]. To exclude the possibility that a difference in probiotic efficacy between LGG wild-type and *dltD* mutant is due merely to a difference in survival, the impact of a *dltD* mutation was first investigated after simulated gastric juice challenge *in vitro* and after transit through the murine GIT, as described in Materials and methods. The *dltD* mutant did not show a reduced survival in simulated gastric juice of pH 4 (Fig. 1a), corresponding to the pH of the murine stomach [48], or *in vivo* in the GIT of healthy mice (Fig. 1b). In addition, both wild-type and the mutant were shown to survive the transit through the DSS-induced inflamed murine GIT in equal numbers (Fig. 1c).

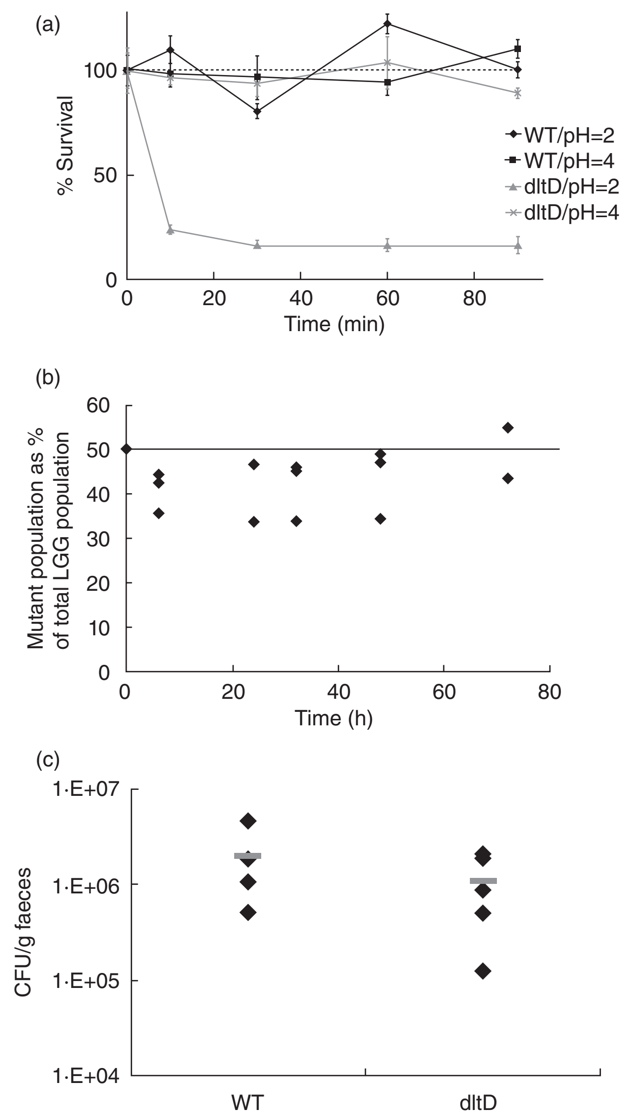

Fig. 1. (a) Comparison of the survival of the *dltD* mutant (grey) and the *Lactobacillus rhamnosus* GG (LGG) wild-type strain (black) in simulated gastric juice at pH 2 and pH 4. The dotted line indicates that the viable cell count at start was taken as 100%. Data are the means of triplicate experiments, and error bars indicate standard error of the mean. (b) Comparison of the persistence of the *dltD* mutant with the wild-type control. A 1 : 1 mixture of wild-type control strain and the *dltD* mutant (ca. 10^9 colony-forming unit (CFU)/mice) was administered to three mice, indicated by the horizontal line. The percentage of mutants in the whole LGG population was determined in the fecal samples, taken at different time-points. Values for individual mice are shown. (c) Persistence of LGG wild-type and *dltD* mutant in 3% moderate to severe dextran sulphate sodium (DSS)-induced colitic mice. Ca. 5×10^8 CFU of each strain were administered daily to five mice. Black diamonds represent individual mice and the grey bars represent the mean value.

Table 1. Effect of probiotic treatment on parameters of colitis in the 3% dextran sulphate sodium (DSS) moderate to severe colitis model.

Treatment	Macroscopic score	Histological score	Colon length (cm)	Weight gain/loss (g)*	Survival rate
PBS (control)	5·9 ± 0·6	12·3 ± 1·1	6·2 ± 0·3	0·8 ± 0·5	8/10
LGG wild-type	6·9 ± 0·3	12·6 ± 0·8	5·4 ± 0·3	–0·5 ± 0·7	8/10
dltD mutant	4·1 ± 0·6†	12·1 ± 0·5	5·9 ± 0·1	1·4 ± 0·3†	10/10

All data represent mean ± standard error of the mean [$n = 8$ for phosphate-buffered saline (PBS) and *Lactobacillus rhamnosus* GG (LGG) wild-type group, $n = 10$ for *dltD* mutant group]. Mice that did not survive were not included in the analysis of the colitic parameters. *Weight gain/loss was determined by calculating the difference in weight between day 28 (end of the experiment) and day 0 (start of the experiment). Comparison between all groups has been calculated; only significant differences are marked. †$P < 0·05$ between *dltD*-mutant and LGG wild-type-treated group.

The *dltD* mutant improves colitic parameters in a moderate to severe colitis model

At the beginning, a number of pilot experiments were performed varying the concentration of DSS (from 1 to 10%), the molecular weight of DSS (35–50 kDa and 500 kDa), the murine strain (BALB/c *versus* C57/Bl6), the sex of the mice, the age of the mice (5–6 weeks *versus* 7–8 weeks) and the number of DSS administration cycles. In C57/Bl6 mice, we could establish moderate to severe colitis by cycles of 3% DSS, as specified in Materials and methods. LGG wild-type and the *dltD* mutant were administered via the drinking water starting 3 days before colitis induction. Daily monitoring of the body weight of the mice showed clear differences between the LGG wild-type and the mutant-treated groups (Fig. 1a). These significant differences were also observed in the macroscopic scoring after the mice were killed at day 29 post-DSS-induction; the administration of LGG wild-type seemed to aggravate the severity of colitic parameters, while the *dltD* mutant appeared to induce some relief (Table 1 and Fig. 2a). Mice in the PBS-treated group and in the wild-type-treated groups, in contrast with the *dltD*-treated group, also showed a decrease in survival, as only eight of 10 mice survived in each of these two groups (Table 1). These four mice were eutha-

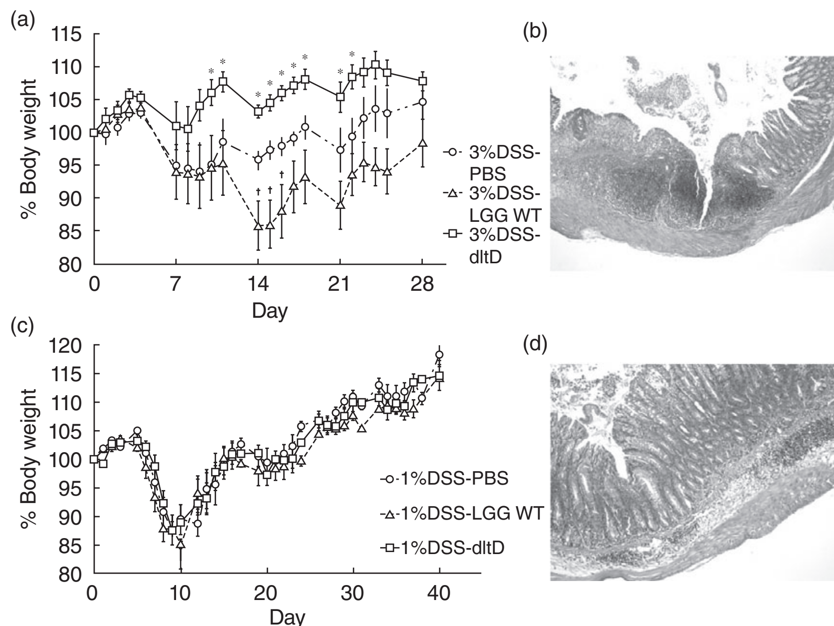

Fig. 2. (a) Body weight curve from the 3% moderate to severe dextran sulphate sodium (DSS)-induced colitis model. Mice were divided into three groups: phosphate-buffered saline (PBS) ($n = 8$), *Lactobacillus rhamnosus* GG (LGG) wild-type ($n = 8$) and *dltD* mutant ($n = 10$). Body weight was monitored daily, starting from the first DSS administration. Body weight is expressed in relative values compared to the body weight at day 0. Data represent mean values ± standard error of the mean (s.e.m.). *$P < 0·05$ between *dltD* mutant and PBS-treated group; †$P < 0·05$ between LGG wild-type and PBS-treated group. (b) Histological photograph of an inflamed part of the colon of a PBS-treated mouse stained with haematoxylin and eosin. Mice displayed mucosal damage with a patchy pattern characterized by a loss of goblet cells and crypts, ulceration and the local influx of inflammatory cells into the lamina propria and submucosa (original magnification ×50). (c) Body weight curve from the 1% DSS-induced colitis model. Mice were divided into three groups (PBS, LGG wild-type and *dltD* mutant). Data represent mean values ± s.e.m. (d) Histopathological section of the colon of a mouse from the PBS-treated group receiving 1% DSS for three cycles (7 days DSS–7 days normal drinking water). Mice showed mucosal damage, restricted mainly to loss of goblet cells and local influx of inflammatory cells into the lamina propria and submucosa (original magnification ×100).

Table 2. Effect of probiotic treatment on colitis severity in the 1% dextran sulphate sodium (DSS) mild colitis model.

Treatment	Macroscopic score	Histological score	Colon length (cm)	Weight gain/loss (g)*	Survival rate
PBS (control) ($n = 10$)	7·5 ± 0·4	11·5 ± 0·5	7·1 ± 0·2	3·2 ± 0·4	10/10
LGG wild-type ($n = 10$)	6·7 ± 0·4	11·1 ± 0·5	6·8 ± 0·1	2·8 ± 0·4	10/10
dltD mutant ($n = 5$)	5·6 ± 0·6†	12·6 ± 0·9	7·0 ± 0·1	2·8 ± 0·5	5/5

All data represent mean ± standard error of the mean. *Weight gain/loss was determined by calculating the difference in weight between day 28 (end of the experiment) and day 0 (start of the experiment). Comparison between all groups has been calculated; only significant differences are marked. †$P < 0{\cdot}05$ between *dltD* mutant and phosphate-buffered saline (PBS)-treated group. LGG: *Lactobacillus rhamnosus* GG.

nized before the end of the experiment for ethical reasons due to severe body weight loss (unintended end-point) and were not included in the analyses of the colitic parameters. The histopathological evaluation of chosen (proximal, mid and distal) colonic segments revealed that the lesions were patchy and were found mainly in the distal part of the colon (Fig. 2b). Sections with severe mucosal damage were characterized by a loss of goblet cells, loss of crypts, epithelial cell necrosis and the local influx of inflammatory cells (mainly neutrophils) in the lamina propria and submucosa (Fig. 2b). The 'patchiness' of the lesions complicated the scoring and, as a consequence, no significant differences in histological scoring could be observed between the treatment groups (Table 1). Similarly, cytokine analysis by qRT-PCR did not reveal significant differences (data not shown).

The *dltD* mutant also improves colitic parameters in a mild chronic colitis model

As described above, in the 3% DSS-induced model the epithelial layer was severely damaged with patchy lesions (Fig. 2b), and the administration of LGG wild-type was shown to be detrimental, in contrast to administration of the *dltD* mutant (Fig. 2a and Table 1). Because Yan *et al.* [25] reported that the intestinal epithelial cells are an important target for certain probiotic actions of LGG, we investigated subsequently whether the detrimental effect of LGG wild-type and the enhanced efficacy of the *dltD* mutant correlated with the integrity of the intestinal barrier. Hereto, C57/BL6 mice received 1% DSS for three cycles of 7 days DSS–7 days normal drinking water. LGG wild-type and *dltD* mutant were then given in the drinking water starting 3 days before colitis induction. In this model, there was no significant difference in body weight between the PBS-, wild-type- and *dltD*-treated groups (Fig. 2c). However, the *dltD*-mutant treated group showed a significantly attenuated colonic inflammation based on the macroscopic score (Table 2). In this milder model, epithelial damage was much less pronounced than in the 3% DSS-induced model, although colitis lesions were still clearly visible (Fig. 2d). Interestingly, the LGG wild-type also showed a trend of ameliorating the severity of the colitic parameters in this mild chronic model, although no significant difference could be observed compared to the PBS-treated group. qRT-PCR results revealed that the administration of the *dltD* mutant reduced mucosal IL-12p40 mRNA expression compared to the PBS-treated ($P = 0{\cdot}0170$) and LGG wild-type-treated groups ($P = 0{\cdot}0363$) (Fig. 3a). IFN-γ expression was also reduced in the *dltD*-treated group and this was significant compared to the PBS-treated group ($P = 0{\cdot}0276$) (Fig. 3b). As these differences in cytokine expression might be downstream effects of a different TLR expression, we subsequently determined TLR-1, TLR-2, TLR-4 and TLR-6 mRNA expression in the three treatment groups. Mice treated with the *dltD* mutant showed a reduced expression of TLR-2 compared to PBS-treated mice ($P = 0{\cdot}0006$) (Fig. 3c). Compared to LGG wild-type-treated mice, we also observed lower expression levels of TLR-1 ($P = 0{\cdot}0179$), TLR-2 ($P = 0{\cdot}0328$) and TLR-4 ($P = 0{\cdot}0443$) in the *dltD* treated group (Fig. 3c–e). No significant differences in cytokines TNF, IL-1β, IL-10 and TGF-β were seen (data not shown). Also no significant changes in expression of TLR-6 (Fig. 3f) were observed between the three treatment groups.

Discussion

Although LGG has potential as an adjuvant in the treatment of IBD, the studies are not always univocal [20–24]. For an optimized and more focused application of LGG – and other probiotics – in IBD, more knowledge about the molecular mechanisms of action is needed. Bacterial cell surface molecules are expected to be key players in determining strain-specific probiotic–host interactions [49]. As LTA is presumed to be a major proinflammatory molecule in Gram-positive bacteria [31], we studied the importance of LGG's LTA structure for its probiotic effects in a murine colitis model by using a mutant that shows a drastic LTA modification. Instead of complete removal of LTA a modification of LTA was introduced, as LTA is an essential part of the cell wall and mutants lacking LTA are not viable [50]. This LGG *dltD* mutant contains LTA molecules that are completely devoid of D-Ala ester substituents, resulting in an altered cell surface charge and altered cell morphology (for details see [37]).

In this work, the performance of LGG wild-type and *dltD* mutant was compared in two experimental set-ups of DSS-induced colitis after confirming that the mutation had no significant effect on survival. In both set-ups, the *dltD* mutant performed better than LGG wild-type, i.e. this mutant appeared to relieve the severity of colitic parameters. LGG wild-type exacerbated the colitic parameters in the

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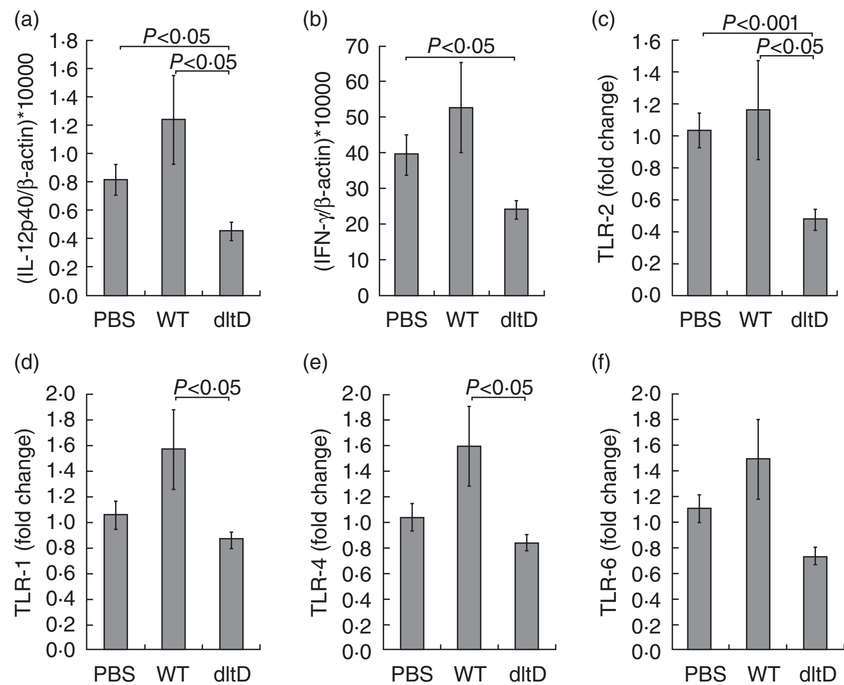

Fig. 3. Cytokine quantification in the colon of dextran sulphate sodium (DSS)-induced colitis mice (1% DSS model). Mice were given phosphate-buffered saline (PBS), *Lactobacillus rhamnosus* GG (LGG) wild-type or *dltD* mutant and killed at day 43 after induction of colitis. Interleukin (IL)-12p40 (a), interferon (IFN)-γ (b), Toll-like receptor (TLR)-2 (c), TLR-1 (d), TLR-4 (e) and TLR-6 (f) were quantified by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) as described in Materials and methods. The expression was normalized against the housekeeping gene β-actin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). IL-12p40 and IFN-γ were analysed with *Taq*Man probes and TLR-expression was measured by SYBR Green ($2^{-\Delta\Delta Ct}$ method). Data represent mean values ± standard error of the mean.

moderate to severe model, but this detrimental effect was not seen in the mild chronic model. We hypothesize that these results could be due to severe disruption of the epithelial barrier by DSS in the moderate to severe colitis model, which was much less pronounced in the mild chronic model. One of the suggested results of this disruption is the increased passage of bacteria (including probiotic LGG) across the epithelial barrier, and subsequent increased internalization and processing by macrophages and dendritic cells in the lamina propria [51]. LTA and other proinflammatory bacterial cell wall components will then become increasingly able to induce a proinflammatory response in these cells.

Dysregulation of TLR expression in IBD could contribute to the proinflammatory response [51]. In the present work, we observed that application of the *dltD* mutant of LGG correlated with a significant down-regulation of TLR-2 expression in the mild chronic 1% DSS-induced colitis model compared to the PBS-treated group. This specific down-regulation of TLR-2 by treatment with the *dltD* mutant could explain the lower expression of the proinflammatory cytokines IL-12 and IFN-γ (as reviewed in [52]). The lower expression of IL-12 suggests that the *dltD* mutant induces fewer proinflammatory cytokines in macrophages and dendritic cells, as IL-12 is a proinflammatory cytokine that is produced mainly by these cell types [53]. DSS-induced colitis also involves the adaptive immune system, especially in more chronic experimental set-ups [54]. IFN-γ, a proinflammatory cytokine typically expressed by T helper 1 cells and known to be up-regulated in chronic DSS-induced colitis [54,55], was also suppressed in the *dltD* mutant-treated group compared to the PBS-treated group. In contrast, treatment with LGG wild-type results in an up-regulation of TLR-1, -2 and -4 compared to the *dltD*-treated group, highlighting the impact of inactivating the *dltD* gene.

It is known that LTA molecules of certain bacteria can induce proinflammatory signalling in macrophages by interaction with TLR-2 [56]. The exact role of D-alanylation in interaction of LTA with specific TLRs (TLR-2, TLR-6) and co-receptors (CD14, CD36) is not yet well established. Based on the crystal structure of TLR-2, the two acyl chains of LTA are suggested to interact with the lipid binding pocket of TLR-2, while the hydrophilic glycerophosphate chain is thought to be exposed to solvent or to interact with TLR-6 or another co-receptor of TLR-2 [57–59]. However, as LTA is a major cell wall compound of lactobacilli, changing the structure of LTA by removing D-alanine residues might as well effect the interactions with other surface molecules and therefore cause pleiotropic effects that can impact indirectly on the anti-inflammatory capacity of the lactobacilli. Nevertheless, our results with the *dltD* mutant compared to the wild-type probiotic strain are in line with those of the study by Grangette *et al.* [36], where a *dltB* mutant of *L. plantarum* NCIMB8826 also showed, compared to the wild-type strain, an enhanced anti-inflammatory capacity *in vitro* in monocytes and in a trinitrobenzene sulphonic acid (TNBS) colitis model [60]. Although both experimental set-ups (probiotic strains and colitis models) differ significantly, the study by Grangette *et al.* [36] and this study both suggest a key role for LTA modification in pro-/anti-inflammatory effects of probiotic lactobacilli.

Finally, the data from our experiments with LGG in the DSS-induced murine colitis model cannot be translated easily to the clinical setting, as introducing bacterial mutants

in humans is not straightforward. However, it is interesting to mention that we also performed a pilot study with LGG in patients with active pouchitis (unpublished). Two patients with acute pouchitis received daily 10^{11} CFU/ml of LGG (Valio, Helsinki, Finland) in capsules for 4 weeks in a randomized cross-over trial (4 weeks probiotics, 4 weeks placebo). In one of the patients, the symptoms of active pouchitis seemed to be exacerbated by the treatment. This study was discontinued and we decided to focus upon animal models, such as presented in this report, to understand more clearly the interaction of LGG with the intestinal mucosa. The data from our experiments, together with reports from other research groups on animal models [28,29] and Crohn's disease patients [61], underline that caution should be taken when applying the wild-type strain of the well-known probiotic LGG in patients with active IBD. This seems to be especially important when the intestinal epithelial barrier function is impaired, as LGG could then show increased proinflammatory activation of macrophages and fewer modulatory signalling effects on intestinal epithelial cells, such as by proteins p40 and p75 [25] and DNA [26].

In conclusion, the difference in therapeutic effect between LGG wild-type and *dltD* mutant *in vivo* suggests a role for the cell surface of the wild-type LGG strain in determining its therapeutic efficacy. Interestingly, these results with the LGG *dltD* mutant show the potential of modifying the cell surface of probiotic strains for better treatment of IBD with probiotics. Combining these modified probiotic strains with the concept of 'designer probiotics' [62] seems to be appealing for the future. One example of such a 'designed' strain is the IL-10-secreting *Lactococcus lactis* strain that shows potential in treatment of IBD [63,64]. Further *in vitro* studies are required to reveal the molecular mechanisms underlying the beneficial effects of this altered cell surface.

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Disclosure

The authors declare no conflicts of interest.

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