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Evaluation of a *Streptococcus thermophilus* strain with innate anti-inflammatory properties as a vehicle for IL-10 cDNA delivery in an acute colitis model

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ABSTRACT

The aim of this work was to develop a *Streptococcus* (*S.*) *thermophilus* strain with improved anti-inflammatory properties due to the incorporation of the therapeutic cDNA delivery plasmid pValac::il-10. To achieve this purpose, cells of *S. thermophilus* CRL807, previously selected as being an important anti-inflammatory strain, were electroporated with pValac::il-10 plasmid. In order to confirm the functionality of the developed strain, it was co-cultured with human epithelial cells Caco-2 and the production of IL-10 was evaluated by ELISA. Bacterial suspensions of *S. thermophilus* CRL807 containing pValac::il-10 plasmid or of the wild-type (WT) strain were administered *in vivo* using a murine model of intestinal inflammation. The animals treated with *S. thermophilus* CRL807 pValac::il-10 showed a lower body weight loss, microbial translocation to liver and damage scores in their intestines at macroscopical and microscopic levels. Furthermore, a significant increase was observed in the concentration of IL-10 in the intestinal contents of these mice compared to the rest of the experimental groups, accompanied by decreased levels of pro-inflammatory cytokines. The insertion of the therapeutic pValac::il-10 plasmid increased the intrinsic anti-inflammatory activity (synergetic effect) of *S. thermophilus* CRL807 which could be included in novel treatment protocols for inflammatory bowel diseases.

1. Introduction

The term inflammatory bowel diseases (IBD) comprises a group of disorders of the gastrointestinal tract characterized by recurrent inflammation, which require lifelong treatments and can cause significant morbidity [1]. Even though the exact etiology of these diseases remains unknown, cytokines have been directly involved in the pathogenesis of IBD and play a crucial role in the control of intestinal inflammation and in the clinical symptoms associated with IBD [2].

Interleukin-10 (IL-10) is one of the most important anti-inflammatory cytokines involved in the maintaining intestinal homeostasis. Its ability to regulate inflammatory pathways through the suppression of pro-inflammatory cytokines allows IL-10 to be a therapeutic candidate for the treatment of IBD [3]. However, subcutaneous treatment with human IL-10 is limited because in low concentrations it does not induce remission of the disease and in high concentrations it causes undesirable secondary effects [4,5]. Also, oral administration is not the best route for IL-10 due to its extreme sensitivity to the gastrointestinal tract environment [6]. However, the administration of IL-10 can become a viable treatment option with the development of new technologies for the its delivery at the mucosal level [7]. Many research groups have thus tried to develop new anti-inflammatory therapies for the treatment of IBD using IL-10 as the active compound, using for example microencapsulation techniques and viral vectors [8–11]. However, many of these methods are expensive, methodologically complicated and even risky.

Lactic acid bacteria (LAB) have shown to be effective vehicles for the delivery of heterologous proteins of technological, therapeutic or prophylactic interest [12,13]. Therefore, the use of genetically modified lactic acid bacteria (GM-LAB) is an attractive alternative for the delivery of IL-10 as an active molecule at the mucosal level [14]. The first evidence of the potential use of a recombinant LAB as

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a therapeutical vehicle was published in the year 2000, when it was shown that a strain of *Lactococcus* (*L.*) *lactis* secreting IL-10 prevented the development of colitis in IL-10 knockout mice [15], and reduced inflammation in a chronic colitis model [16]. An important step forward in the safety use of GM-LAB for therapeutic purposes was the construction of a biological containing system for the *L. lactis* strain producing human IL-10 [17]. This containing system was evaluated in Crohn's disease patients without causing any adverse effects [18]. These results show the importance of evaluating new administration methods to accomplish a more effective local delivery of IL-10 at intestinal mucosal level using therapeutic LAB.

In the present study a DNA delivery system using lactococci that allows the gene of interest (in this case coding for IL-10) is delivery by the LAB but expressed by the host cells [19]. In this manner a *L. lactis* strain expressing Fibronectin Binding Protein A (FnBPA+) from *Staphylococcus aureus* on its surface and containing pValac::il10 plasmid was found to be effective in the prevention of inflammation in an acute TNBS murine model of colitis [20]. The same strain without FnBPA was also shown to be effective for the prevention of colitis in a Dextran Sulfate Sodium (DSS)-induced murine model [21] and in a chronic TNBS-induced colitis model [7]. These results led us to hypothesize that using *Streptococcus* (*S.*) *thermophilus* CRL807, a strain that was previously shown to have an important anti-inflammatory potential [22], as a delivery vehicle for pValac::*il-10* plasmid could provide a synergic effect in the intrinsic anti-inflammatory potential of this new recombinant strain.

Therefore, the aim of this study was to assess the anti-inflammatory activity of a *S. thermophilus* CRL807 with innate immunomodulatory properties containing the therapeutic pValac::*il-10* plasmid using an acute colitis model in mice.

2. Materials and methods

2.1. Construction of Streptococcus thermophilus carrying the plasmid pValac::il-10

S. thermophilus CRL807 wild-type (WT) strain from CERELA Culture Collection (Tucumán, Argentina) was grown for 16 h at 37 °C without agitation in LAPTg (1% glucose, 1.5% peptone, 1% tryptone, 1% yeast extract, and 0.1% Tween 80) medium.

Electrocompetent cells of *S. thermophilus* CRL807 were prepared and transformed with the plasmid pValac::*il-10* as previously described [22]. Aliquots of 100 μL and the remaining volume of the electroporated cells (500 μL centrifuged and resuspended in 100 μL) were seeded in plates of agar LAPTg containing 3.5 μg/mL of chloramphenicol, which were incubated at 37 °C during 72 h. The colonies were transferred to liquid LAPTg media containing 10 μg/mL of chloramphenicol in order to confirm the acquired resistance in the recombinant strains. The presence of the pValac::*il-10* plasmid in the putative recombinant colonies of *S. thermophilus* CRL807 was confirmed by PCR. The strain harboring this plasmid was named *S. thermophilus* CRL807 pValac::*il-10*.

2.2. In vitro evaluation of the functionality of S. thermophilus CRL 807 pValac::il-10 strain

The ability of the transformed strain to deliver IL-10 cDNA was evaluated using co-cultures with Caco-2 cells grown in RPMI1640 media (Sigma, St. Louis, USA) supplemented with 10% fetal bovine serum (FBS) in a 24-well plates. These cells were growth until 80% confluence and then incubated during 3 h without bacteria (negative control), with *S. thermophilus* CRL807 WT strain, with *S. thermophilus* CRL807 pValac::*il-10* strain or with *L. lactis* MG1363 pValac::*il-10* (positive control), in a cell:bacteria ratio of $1:10^3$ (cells were re-suspended from one well with trypsin treatment and counted in a Neubauer chamber to know the approximated cell concentration in each well). After this period of incubation, complete medium supplemented with gentamicina (20 mg/L) was added and the plates were incubated for 2 h. Then, cells were washed and new media, without antibiotic, was added. The following day the cell culture media was discarded, cells were trypsinized, re-suspended in PBS with anti-protease (Complete EDTA-free Protease inhibitor cocktail, Roche Diagnostics, Mannheim, Germany) and sonicated with 3 cycles of 30 s alternated with 30 s of incubation on ice. The lysed cells were centrifuged at 1000*g* during 10 min at 4 °C and the supernatant was stored at −20 °C until the determination of IL-10 concentrations using an ELISA set (BD Bioscience, San Diego, California, USA). The concentration of proteins was determined and the concentration of IL-10 was expressed as pg/mg of protein.

2.3. Evaluation of the anti-inflammatory activity of S. thermophilus CRL807 pValac::il-10 strain in an acute colitis mouse model

Once the biological activity of the *S. thermophilus* CRL807 pValac::*il-10* strain was confirmed *in vitro*, the anti-inflammatory potential was assessed *in vivo* in an acute intestinal inflammation model induced with trinitrobenzenesulfonic acid (TNBS) as previously described [23].

Briefly, BALB/c mice (female, 5 weeks old) were fully anesthetized with an intraperitoneal injection of ketamine hydrochloride (Holliday-Scott S.A., Buenos Aires, Argentina; 100 μg/g body weight) mixed with xylazine hydrochloride (Rompun; Bayer, Division Sanidad Animal, Buenos Aires, Argentina; 5 μg/g body weight). Intestinal inflammation was then induced by intrarrectal instillation with a TNBS solution (Sigma, St. Louis, MO, USA; 2 mg/mouse) dissolved in 0.01 M phosphate-buffered saline (PBS; pH 7.4) and mixed with an equal volume of ethanol (50% ethanol), using a 4 cm length catheter.

Mice from the **control group** received PBS mixed with ethanol (without TNBS), using the same technique. The day before TNBS instillation, mice were divided in 3 experimental groups ($n = 8$): **TNBS group** (Inflammation **control group** which did not receive LAB); **TNBS-CRL 807 WT group** which received *S. thermophilus* CRL807 WT strain, and **TNBS-CRL 807 pVALAC:IL-10 group**, which received *S. thermophilus* CRL807 pValac::*il-10* strain.

Oral bacterial administration to mice was performed with a gavage syringe. Each mouse received daily 100 μL of the bacterial suspension containing 1×10^9 cfu/mL, beginning one day before inflammation induction and until the end of the experience (3 days post-TNBS). Mice from the control and TNBS groups received daily the same volume of saline solution.

Animal groups were fed *ad libitum* with balanced rodent diet and water and maintained in a room with a 12-h light/dark cycle at 18 ± 2 °C. Body weight was controlled daily. All animal protocols were approved by the Animal Protection Committee of CERELA (CRL-BIOT-LT-2010/1A), and all experiments comply with the current laws of Argentina.

Mice were euthanized three days after TNBS instillation. Liver was dissected, weighed and homogenized in 5 mL 0.1% (*w*/*v*) peptone solution under sterile conditions. Serial dilutions of the homogenate were plated in triplicate in the following media: Mann–Rogosa–Sharp (MRS; Britania, Buenos Aires, Argentina), MacConkey (Britania, Buenos Aires, Argentina) and LAPTg. Bacterial growth was evaluated after 48–72 h incubation at 37 °C.

Large intestines were dissected, macroscopically examined and then processed for histological examination using standard methods. Serial paraffin sections of 4 μm were made and stained with hematoxylin-eosin for light microscopy examination.

Macroscopic lesions and extent of colonic damage and inflammation were assessed using previously described grading systems [20]. The blind analyses were performed by two different scientists. High macroscopic or histological damage scores indicate increased damage in the intestines.

The intestinal contents were collected washing the large intestines with 500 μL of PBS containing anti-protease. After homogenization in a vortex, they were centrifuged (8000*g*, 10 min, 4 °C) and supernatants were stored at −20 °C until the determination of the following cytokines: IL-6, IFNγ, TNF, IL-17 and IL-10 using Cytometric Bead Array kit, CBA, mouse Th1/Th2/Th17 (BD Bioscience, San Diego, EE.UU).

2.4. Statistical analysis

Results were expressed as the mean values of independent results ± the standard deviation (DS). For animal experiments, 5 mice of each group were sacrificed and samples were collected. The experimental protocols were repeated 3 times. Considering that no interactions were observed between these 3 independent assays, results were analyzed together.

Statistical analysis were performed using one-way ANOVA followed either by Tukey's multiple comparisons test or by Dunnett's multiple comparisons test using GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com). Unless otherwise stated, significant differences between values are considered when $p \leqslant 0.05$.

3. Results

3.1. Construction of a functional S. thermophilus CRL807 strain for IL-10 cDNA delivery

S. thermophilus CRL807 pValac::*il-10* strain was constructed and the presence of the plasmid was confirmed by PCR (data not shown). The functionality of the putative strain was verified *in vitro* evaluating the IL-10 production by eukaryotic cells using human epithelial cells (Caco-2). As it can be observed in Fig. 1 epithelial cells co-cultured with *S. thermophilus* CRL807 pValac::*il-10* strain showed a significant increase in the levels of IL-10 reaching similar values to those observed for the cells co-cultured with *L. lactis* MG1363 strain containing pValac::*il-10* plasmid (positive control). IL-10 production was very low or not detected in those cells co-cultured with the WT strain, or in those co-cultured with media without bacteria (control).

3.2. Effect of S. thermophilus CRL807 pValac::il-10 on animal live weight in a murine model of colitis

The anti-inflammatory effect of the new developed strain was evaluated in an acute intestinal inflammation model induced by TNBS. Even though mice that received the *S. thermophilus* CRL807 WT strain showed lower weigh loss than inflamed mice from the TNBS group, this effect was enhanced in mice that received the recombinant strain *S. thermophilus* CRL807 pValac::*il-10*, which lost weight only in the first day after TNBS inoculation and then maintained their body weight to levels between 90 and 95% of the start of the experiment (Fig. 2).

3.3. Effect of S. thermophilus CRL807 pValac::il-10 on intestinal damage and microbial traslocation in a murine model of colitis

The large intestinal damage observed macroscopically was significantly reduced in mice treated with the *S. thermophilus* CRL807 WT strain, in comparison to the mice from TNBS group. However,

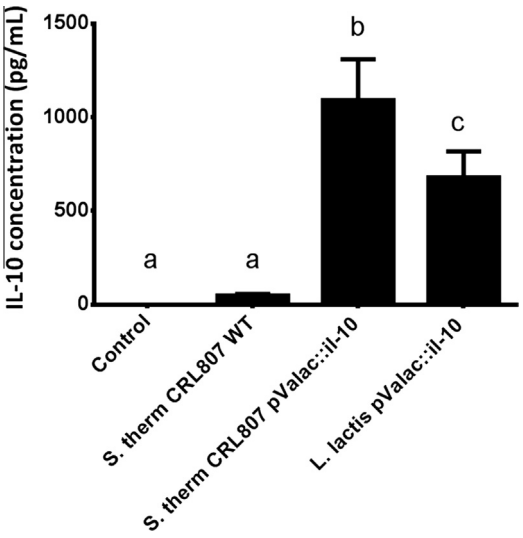

Fig. 1. IL-10 production in Caco-2. The Caco-2 cells were co-incubated with media without bacterial supplementation (control), with *S. thermophilus* CRL807 WT strain, with *S. thermophilus* containing the plasmid pValac::*il-10*, or with *L. lactis* MG1363 containing the plasmid pValac::*il-10*. Each value represents the mean of $n = 5 \pm$ SD. Means with a different letter differs significantly with a $p \leqslant 0.05$.

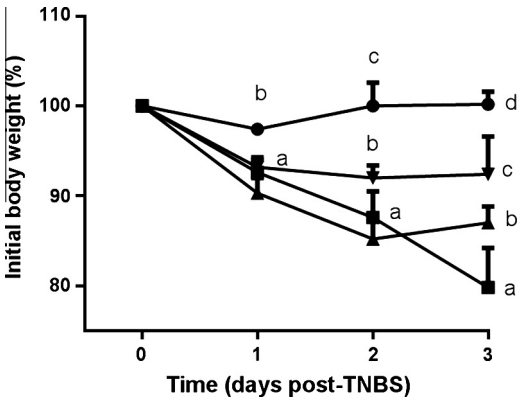

Fig. 2. Body weight loss. Live body weight was measured in mice from the control (circles) and TNBS (squares) group, and TNBS groups that received treatment with *S. thermophilus* CRL807 WT strain (triangles) or with *S. thermophilus* CRL807 strain containing the pValac::*il-10* plasmid (lozenge). Results were expressed as the percentage of the live body weights of animals at a given time point with respect to the initial body weight. For each data point, each value represents the mean of $n = 15 \pm$ SD. Means at each time with a different letter differs significantly with a $p \leqslant 0.05$.

this damage was lower for those mice that received the recombinant strain containing the therapeutic plasmid pValac::*il-10*, which showed damage score values that were no significantly different from those observed in mice from the control (non-inflamed) group (Fig. 3a and c).

At the histological level, mice receiving *S. thermophilus* CRL807 WT strain as well as those receiving the strain containing pValac::*il-10* showed a decrease in their intestinal damage scores without significant differences compared to the control group (Fig. 3b) However, there were more animals with lower values of damage score in the TNBS-CRL807 pVALAC:IL-10 group than in the CRL807 WT group. Most of the animals that received the recombinant-LAB presented in their large intestinal slides, low number of infiltrating cells, intact crypt architecture and less thickening of the muscle layer (Fig. 3d).

The observed intestinal damage was associated with the results obtained from the analysis of the microbial translocation to liver.

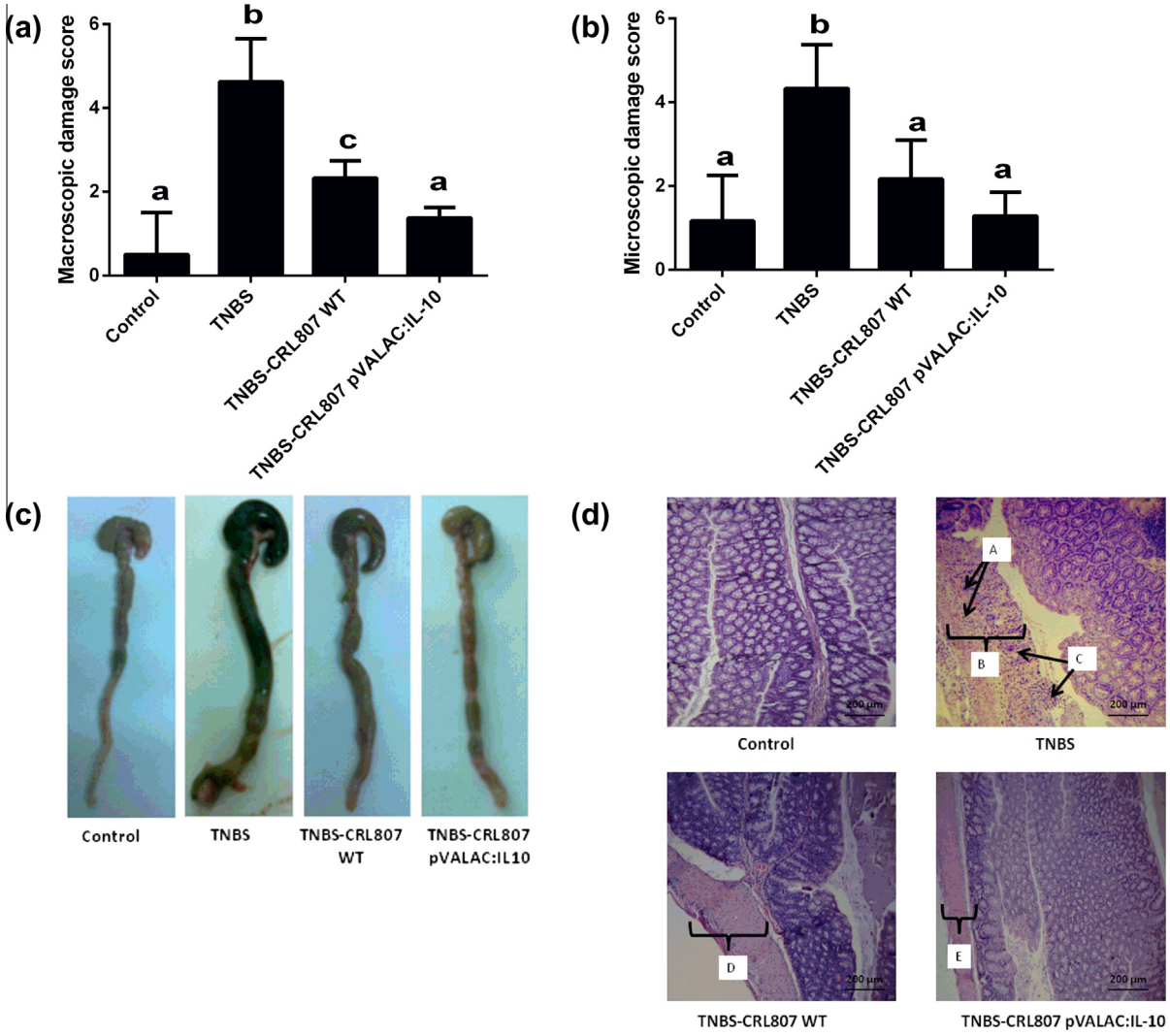

Fig. 3. Grade of damage in the large intestines. Macroscopic (a) and microscopic (b) damage scores of large intestines of mice belonging to the control and TNBS groups and TNBS groups of mice that also received suspensions of *S. thermophilus* CRL807 WT (TNBS-CRL807 WT) or *S. thermophilus* CRL807 containing the pValac::*il-10* plasmid (TNBS-CRL807 pVALAC:IL-10). Each value represents the mean of $n = 15 \pm$ SD and those with a different letters differ significantly with a $p \leqslant 0.05$. Representative photographs of the large intestines (c) and histological slides observed at a magnification of 100× (d) of mice from each group are provided. Note that the intestine of the mouse belonging to the TNBS group shows higher damage, with blood vessel proliferation prominent inflammatory infiltrates that alter the normal architecture of the crypts; this damage is lowered in the intestine of the mouse from the TNBS-CRL807 WT group, however the lower damage score is observed in the intestine of the mouse from the TNBS-CRL807 pVALAC:IL-10 group where there is no thickening of the mucosa muscular layer and there is a higher number of globet cells (A: blood vessel proliferation, B: loss of the crypt architecture; C: prominent inflammatory infiltrates; D: thickening of the external muscular layer; E: normal external muscular layer; F: globet cells).

Mice that received *S. thermophilus* CRL807 strain that contained or not the pValac::*il-10* plasmid reduced microbial translocation to liver compared to TNBS group; however mice from TNBS-CRL807 pVALAC:IL-10, showed the lowest microbial growth in liver in all the evaluated media (Fig. 4).

3.4. Effect of *S. thermophilus* CRL807 pValac::il-10 on intestinal cytokine profiles

All these results did not only confirm the intrinsic anti-inflammatory effect conferred by the WT strain, but also showed that the addition of pValac::il-10 plasmid enhanced its protective properties. Since the synergic effect found in the genetically modified strain was related with the presence of pValac::*il-10* plasmid, the immunological response in the intestines of mice was evaluated through a cytokine profile analysis (Fig. 5). The assessment of the concentrations of IL-10 (Fig. 5a) in the intestinal fluids showed that all the experimental groups inoculated with TNBS significantly increased the levels of this anti-inflammatory cytokine, compared to the control group. The administration of the strain containing the therapeutic plasmid pValac::*il*-10 significantly increased the levels of IL-10, compared to mice from TNBS and TNBS-CRL 807 WT groups, confirming the synergetic effect of the cDNA plasmid.

The induction of inflammation also led to an increase in the levels of different pro-inflammatory cytokines in the intestinal fluids of mice from the TNBS group. Oral treatment with *S. thermophilus* CRL807 WT strain as well as with the recombinant *Streptococcus* strain containing the therapeutic pValac::*il*-10 plasmid was able to significantly decrease the levels of the pro-inflammatory cytokines IFNγ, TNFα and IL-6 (Fig. 5b–d respectively). It is important to note that the treatment with the strain containing pValac::il-10 diminished more significantly the levels of all these cytokines.

Finally, considering that the concentration of these cytokines can be affected by the intestinal transit that is altered during an inflammatory process, the ratios between the levels of IL-10 and

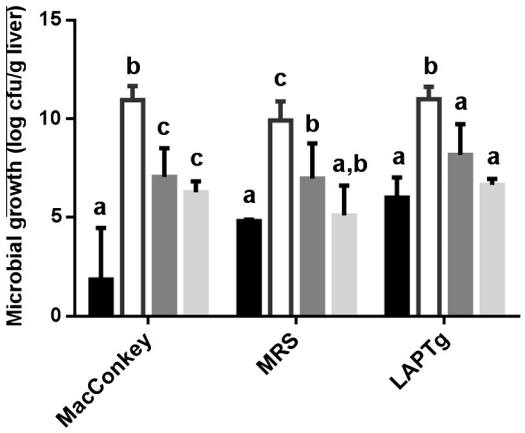

Fig. 4. Microbial translocation to liver. Microbial growth in MacConkey, MRS and LAPTg culture media resulting of the plating of the macerated livers from the mice of the control (black) and TNBS (white) groups, and TNBS groups that received treatment with *S. thermophilus* CRL807 WT strain (dark grey) or with *S. thermophilus* CRL807 strain containing the pValac::*il*-10 plasmid (light grey). Each value represents the mean of $n = 15 \pm SD$ and those from a same growth media with a different letters differ significantly with a $p \leqslant 0.05$.

the levels of the pro-inflammatory cytokines were calculated for each mouse (Fig. 5e).

After analyzing the ratio of each animal, it was observed that only the TNBS-CRL807 pVALAC:IL-10 group increased significantly the IL-10/IFNγ ratio in comparison to the rest of the groups (Fig. 5e). Mice that received either the WT strain or the GM-LAB strain showed significant increases in the IL-10/TNFα and IL-10/IL-6 cytokine ratios in their intestinal fluids compared to the mice from TNBS group. Furthermore, IL-10/TNFα and IL-10/IL-6 cytokine ratios obtained in mice from the TNBS-CRL807 pVALAC:IL-10 group were not significant different from those obtained in the control group.

4. Discussion

Most of the studies that involve the use of GM-LAB for the delivery of IL-10 are based on bacterial cells delivering this cytokine [6,16,24,25]. In contrast, the delivery of DNA at the mucosal level would allow the eucaryotic host cells to express the cytokyine directly at the site of interest [26,27]. This novel strategy for IBD treatment was previously studied in IBD animal models, where

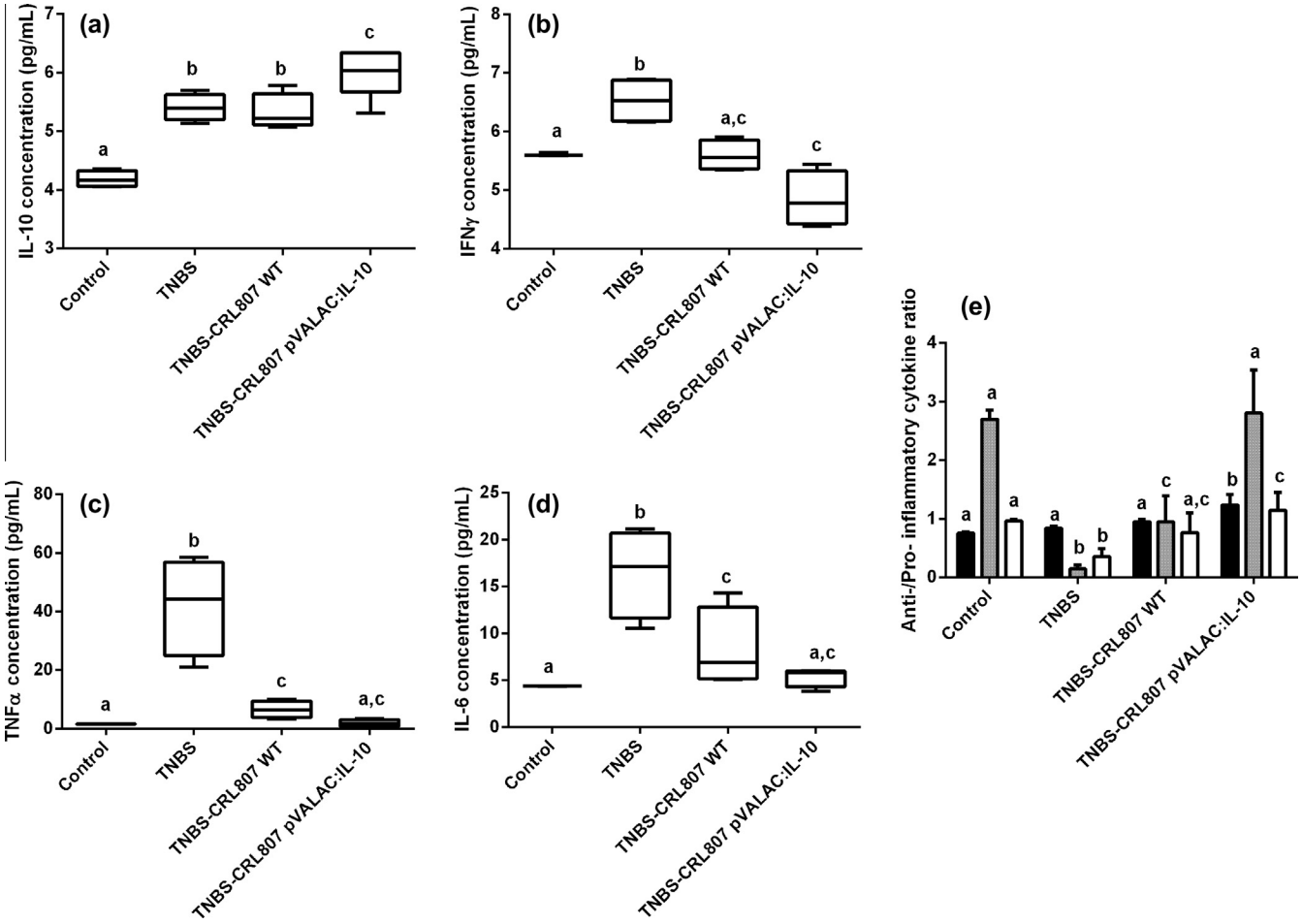

Fig. 5. Cytokine concentrations in large intestinal fluids. The large intestinal fluids of mice belonging to the control and TNBS groups, and of mice that received TNBS and were supplemented with *S. thermophilus* CRL807 WT (TNBS-CRL807 WT) or *S. thermophilus* CRL807 containing the pValac::*il-10* plasmid (TNBS-CRL807 pVALAC:IL-10), were used to evaluate the concentrations of IL-10 (a), IFNγ (b) TNFα (c) and IL-6 (d) by flow cytometry. Each box shows the values for the group (25th and 75th percentiles) with the outliers and the median line, obtained from $n = 15$ mice (from 3 independent trials). Boxes with a different letters differ significantly with a $p \leqslant 0.05$. The ratios (*e*) between the levels of IL-10 and the levels of pro-inflammatory cytokines: IFNγ (black bars), TNFα (grey bars) and IL-6 (white bars) in the intestinal fluids of mice represent the mean of $n = 15 \pm SD$ and those from a same cytokine ratio with a different letters differ significantly with a $p \leqslant 0.05$.

the expression vector named pValac (Vaccination using LAB) for the delivery of the il-10 gene [19] was delivered by a non-immune stimulating strain of *L. lactis* expressing FnBPA [20] and also using a non-invasive *L. lactis* that did not express this surface protein [21]. The IL-10 cDNA delivery by the non-invasive and invasive strains of *L. lactis* MG1363 were compared in a chronic TNBS colitis model where it was shown that both strains effectively prevented the onset of flare-up episodes of colitis, with a higher increase in the levels of IL-10 in the intestinal tissues of those animals receiving *L. lactis* MG1363 containing pValac::*il*-10 [7].

L. lactis is a model bacterium that was used for delivery of different molecules; however, it was demonstrated that the selection of other LAB with intrinsic anti-inflammatory properties can increase the potential of strategies based in the use of GM-LAB. *S. thermophilus* CRL807 was selected for its anti-inflammatory properties evaluated *in vitro* and *in vivo* (del Carmen et al., 2014). In that work was also showed that genetically modification of *S. thermophilus* CRL807 to produce the anti-oxidant enzymes catalase and superoxide dismutase increased its beneficial effect in an acute TNBS colitis mouse model.

Considering these previous results, in this particular study, *S. thermophilus* CRL807 was used as a vehicle for the therapeutic plasmid pValac::*il*-10. The results in this current study, using a different IBD model, confirmed once again the innate anti-inflammatory properties of the *S. thermophilus* CRL807 WT strain. Mice that received this strain decreased the severity of the inflammation with less body weight loss, less macroscopic and microscopic intestinal damages and also changes in the cytokine profiles with diminution of pro-inflammatory cytokines compared to the inflammation control group (TNBS group). However, similar to the results obtained previously for genetically modified strains that produced anti-oxidant enzymes, the genetic modification of *S. thermophilus* CRL807 for the delivery of IL-10 cDNA increased its anti-inflammatory effect. Mice that received *S. thermophilus* CRL807 pValac::*il*-10 were those that showed the best anti-inflammatory properties. This effect was accompanied by a significantly increased production of IL-10 in intestinal fluids of mice compared to the TNBS group, increasing the normal anti-inflammatory response of mice probably as a consequence of the incorporation of the pValac::*il*-10 plasmid into the host cells which maintains a continual production of IL-10 in mice preventing inflammation more effectively. These did not occur using other strains delivering IL-10 protein, where the cytokine or the LAB producing the cytokine reached the intestinal light exerting their effect, but there was no increase in the endogenous levels of IL-10 [6]. Considering that epithelial turnover occurs approximately between 1 and 2 days and that this could be increased during inflammation, the constant production of IL-10 by these host cells could be induced by the daily administration of the strain. More importantly, this sustained production of IL-10 maintained a significant increase in the anti-inflammatory to pro-inflammatory cytokine ratio observed in the mice intestinal fluids. This also correlates with recent studies where it was confirmed that the treatment with a recombinant *L. lactis* strain producing IL-27 [28], was capable of inducing an endogenous IL-10 increase which resulted more efficient than the treatment with the recombinant *L. lactis* strain producing IL-10 [16]. In recent studies, it has been shown that IL-10 and IL-30 is able to co-ordinate between different immune cells (such as natural killer cells) and thus providing the fundamentals to the positive effects of the induction of IL-10 locally in the gastrointestinal tract and giving rise to other potential applications of IL-10 therapies for other pathologies such inflammation, cancer, autoimmune diseases and liver fibrosis [29,30]. It is also important to note that even when the anti-inflammatory/pro-inflammatory cytokine ratios in the intestinal fluids were reported increased in mice that received *L. lactis* expressing FnBPA and containing pValac::il-10, the increase of IL-10 was not observed (del carmen et al., 2013), showing the importance of LAB selection to enhance the anti-inflammatory potential of the delivery system. In our study, considering the potential of the WT strain to modulate the host immune response, these results can be the addition of innate and acquired properties in the new GM-LAB.

Even though the most popular vehicle for the delivery of molecules of interest has been *L. lactis*, the use of other LAB strain with intrinsic anti-inflammatory properties (such as *S. thermophilus* CRL807) is possible to construct new GM-LAB with enhanced anti-inflammatory effects for delivery of molecules of interest, such as IL-10 cDNA. The current study serves as proof of concept validating the functionality of these strains *in vivo*; however, expression vectors should be optimized for the functionality of these particular strains, and antibiotic resistance genes should be eliminated. The GM-LAB for the delivery of IL-10 represents an attractive alternative to traditional therapies for IBD. Different strategies for the delivery of the anti-inflammatory cytokines have been studied being the pValac::*il*-10 system one of the most interesting for its future use as a gene therapy approach.

5. Conclusion

We can conclude that the genetic modification of LAB with intrinsic anti-inflammatory properties, for the delivery of genes of interest at intestinal mucosal level causes a synergic increase of the potential benefits associated to its use and should be tested further in therapeutic formulations as alternative or complement to the traditional treatments used in IBD patients.

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