Probiotic *Lactobacillus Rhamnosus GG* Protects Against *P. Gingivalis* And *F. Nucleatum* Gut Dysbiosis

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Abstract

Objectives: This study investigated changes induced by *Porphyromonas gingivalis* and on gastrointestinal histology and gut microbiome in a mouse model of experimental periodontitis. The effect of probiotic *Lactobacillus rhamnosus* GG (LGG) in altering these changes was also investigated.

Methods: Thirty-six mice were allocated into six groups. Experimental alveolar bone loss was induced by oral inoculation with *P. gingivalis* and *F. nucleatum*. LGG was orally inoculated or orally gavaged. Gastrointestinal tissue changes were assessed using histological analysis and immunohistochemistry. Caecal microbiome was analysed by sequencing 16S rRNA genes of caecal content.

Results: Inoculation with *P. gingivalis* and *F. nucleatum* induced inflammation throughout gastrointestinal tract ($p < 0.05$), increased expression of IL-6 in ileum ($p = 0.052$) and altered composition of caecal microbiome ($p < 0.05$) in experimental mice compared to controls. Mice treated with LGG had reduced tissue inflammation in duodenum ($p = 0.044$) and lowered levels of IL-6 in ileum ($p = 0.048$) when compared with disease. LGG therapy influenced gut microbiome changes.

Conclusions: *P. gingivalis* and *F. nucleatum* inoculation induced significant changes in intestinal inflammation and caecal microbiome. Oral gavage with LGG exerted a protective effect against intestinal inflammation and limited gut microbiome changes associated with *P. gingivalis* and *F. nucleatum*.

Keywords: Probiotics, periodontal disease, periodontal treatment, gut microbiome

Introduction

Periodontitis is a chronic inflammatory condition affecting both the supporting soft and hard tissues of the teeth (Kinane *et al*., 2017). The condition is influenced by elevated numbers of specific bacteria which may become pathogenic as a result of changes in the local environment controlled by the host (Darveau, 2010; Marsh and Devine, 2011).

Porphyromonas gingivalis and *Fusobacterium nucleatum* are two bacterial species of the human oral microbiome associated with the pathogenesis of periodontal disease (Kilian *et al*., 2016). Previous research has demonstrated that oral administration of *P. gingivalis* (10^{10} CFU/ml) in mice twice a week for five weeks induces insulin

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resistance, systemic inflammation and endotoxemia associated with changes in the gut microbiota of the ileum (Arimatsu *et al.*, 2014). Additionally, a single oral administration of 10^9 CFU/ml of *P. gingivalis* (strain W83) induced dysbiosis of the gut microbiota, significantly increasing the proportion of Bacteroidetes, decreasing the proportion of Firmicutes and increasing serum endotoxin levels (Nakajima *et al.*, 2015). Dual infection with *F. nucleatum* and *P. gingivalis* in mice aggravated alveolar bone loss and inflammation when compared with animals treated with either bacterium alone (Polak *et al.*, 2009). However, the implications of repeated oral inoculations with *F. nucleatum* and *P. gingivalis* on the gut microbiome have not been studied in depth. As such, further studies are required to elucidate the effects of these bacteria on the gut histology and microbiome (Blasco-Baque *et al.*, 2016).

The administration of probiotics to modulate inflammation is one of several contemporary approaches considered as an option to address bacterial imbalances and prevent bone loss in periodontitis (Gatej *et al.*, 2017). Probiotics have been traditionally used as therapeutic and prophylactic strategies for conditions such as inflammatory bowel disease, colitis, chemotherapy induced mucositis and diarrhoea (Varankovich *et al.*, 2015). A limited number of clinical trials have investigated the effects of probiotics in the management of periodontal disease (Morales *et al.*, 2016, Shimauchi *et al.*, 2008). *Lactobacillus rhamnosus GG* (LGG) is a probiotic that has been extensively and safely used in gastrointestinal clinical applications (Ciorba and Stenson, 2009). More recently, LGG was shown to effectively supress bone loss in a mouse model of *P. gingivalis* and *F. nucleatum* induced bone loss, irrespective of the mode of administration (Gatej *et al.*, 2019).

The primary aim of the current study was to determine if any changes occurred in intestinal inflammation and in the structure and diversity of the intestinal microbiome when mice were orally inoculated with *P. gingivalis* and *F. nucleatum* in an accepted mouse model of periodontal disease. The secondary aim was to determine if LGG administration influenced these changes.

Materials and methods

This project was approved by the University of Adelaide Animal Ethics Committee (M-2015-116) and complied with National Health and Research Council (Australia) Code of Practice for Animal Care in Research and Training (2014).

Preparation of bacterial inocula

P. gingivalis (strain W50) and *F. nucleatum* (ATCC 25586) bacterial inocula were prepared as previously described (Gatej *et al.*, 2019).

Murine periodontitis model

Thirty-six 6-8 week old BALB/c female mice were obtained from the Laboratory Animal Services of the University of Adelaide and housed in a PC2 animal holding facility (OGTR certification No 2067/2008). Mice were randomly assigned to six groups (n = six mice/group) (Figure 1). Animals from groups PD, PD + LGG Gav and PD + LGG Oral were inoculated as previously described (Gatej *et al.*, 2019) over two sessions with an inoculum containing *P. gingivalis* and *F. nucleatum* suspended in 2% (v/v) carboxymethyl cellulose (CMC). Animals from group LGG Gav, PD + LGG Gav, LGG Oral, and PD + LGG Oral received a daily dose of 200 µl of 2-9 x10^9 CFU/ml of LGG in sterile 2% CMC in phosphate buffered saline (PBS). Groups LGG Oral and PD + LGG Oral received oral inoculation of LGG in which bacteria were directly swabbed around the molars using a small brush. Groups LGG Gav and PD + LGG Gav received oral gavage of LGG in which bacteria were administered directly into the stomach using a 24-gauge ball-tipped gavage plastic needle attached to a syringe. Probiotic treatment started three days prior to the induction of periodontitis and continued daily for the whole duration of the experiment (Figure 1). Probiotic inoculation took place in the morning, after which mice had immediate, unrestricted access to food and water. At the completion of the study (day 44), animals were killed by cervical dislocation under anaesthesia with a final solution of xylazine (20 mg/kg of body weight) and ketamine (100 mg/kg of body weight).

Gastrointestinal Histopathological Analysis

Tissue samples of the duodenum, jejunum, ileum and colon were collected, fixed in neutral buffered formalin, before being processed and embedded in paraffin wax. Sections of tissue were cut using a rotary microtome and stained with haematoxylin and eosin. A total tissue injury score was generated based on the occurrence of eight histological criteria in the duodenum, jejunum and ileum and six criteria in the colon (Howarth *et al.*, 1996, Wardill *et al.*, 2016). Two blinded operators scored the following parameters: villous fusion and villous atrophy (for duodenum, jejunum and ileum only), disruption of brush border, crypt loss, disruption of crypt cells, infiltration of neutrophils and lymphocytes, dilation of lymphatics or capillaries and oedema. Each parameter was scored as present = 1 or absent = 0.

16S rRNA Metagenomics Gene Sequencing of Caecal Samples

A QIAmp® Fast DNA Tissue Kit (Qiagen, Hilden, Germany) was utilised for DNA extraction in accordance to the manufacturer's protocol. Briefly, aliquots from a master mix containing buffers and enzymes were placed in tissue disruption tubes (Pathogen Lysis tubes, Qiagen,

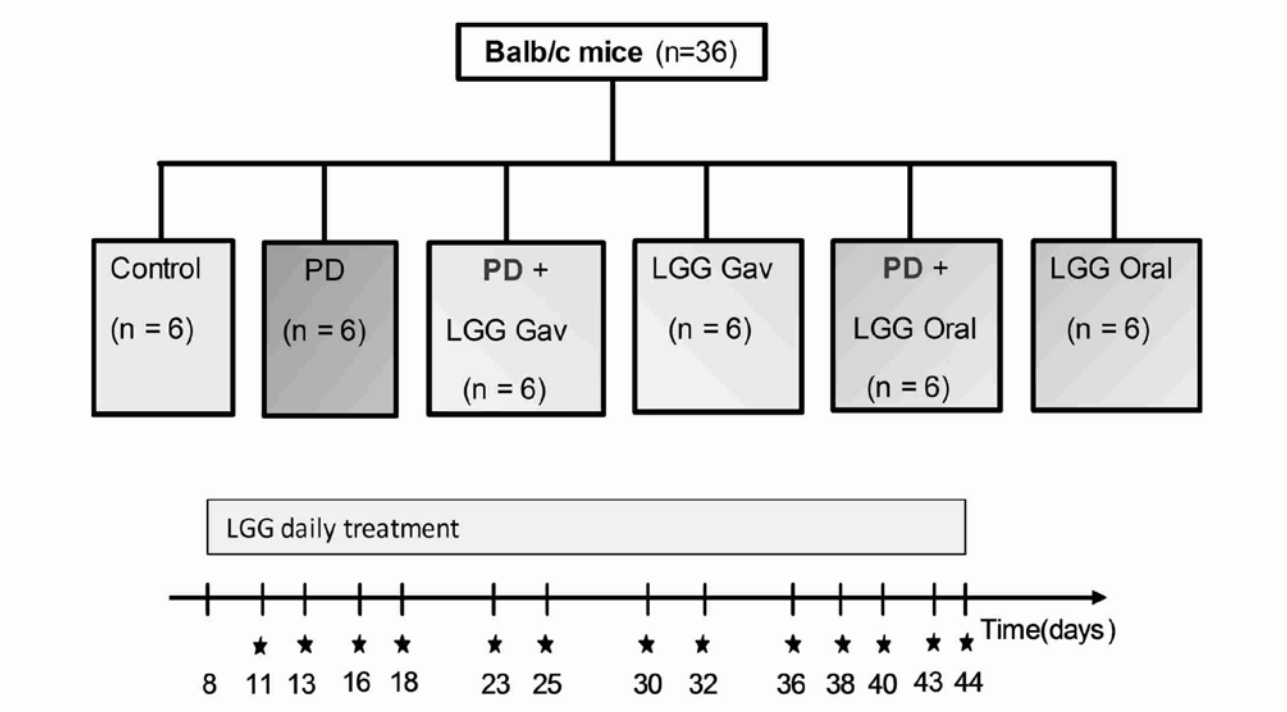

Figure 1*: Study design and timeline of treatment. Control, n=6 (no periodontitis, no treatment), PD, n=6 (periodontitis, no treatment), PD + LGG Gav, n=6 (treatment with LGG via oral gavage (Gav) and subsequent periodontitis), LGG Gav, n=6 (treatment with LGG via oral gavage), PD + LGG Oral, n=6 (treatment with LGG via oral inoculation and subsequent periodontitis), LGG Oral, n=6 (treatment with LGG via oral inoculation); LGG treatment started three days prior to disease induction and it continued daily for the whole duration of the experiment (day 44); PD = Periodontitis, Gav = Gavage, Oral = Oral inoculation, LGG = Lactobacillus rhamnosus GG; * = Inoculation with P. gingivalis and F. nucleatum*

Hilden, Germany). A small caecal sample (5-25 mg) from each animal was added into each tissue disruption tube and processed. Samples were homogenised using the VortexGenie®2 (Scientific Industries Inc., Bohemia, New York, USA) and incubated for ten minutes at 56°C, followed by centrifugation for 10 minutes at 10000 g. At this point, 5.3 mg lysozyme was added to each tube to improve the disruption of the Gram-positive bacterial cell wall and samples were incubated for one hour at 37°C, followed by buffer addition. A spin column was used to separate DNA from the rest of the solution. The concentration and quality of the DNA were assessed using a NanoDrop 2000c spectrophotometer (Thermo Scientific, Waltham, MA, USA). Aliquots of 30 µl from each sample were placed into 0.6 ml sterile PCR tubes and sent to Flinders Genomics Facility (Flinders University, Adelaide, SA, Australia) where all samples underwent Illumina sequencing library preparation using an Illumina MiSeq (Illumina, San Diego, CA, USA). All further analysis of the amplicon datasets was conducted within the QIIME2 package (QIIME 2 2017.12). Core diversity analyses were completed by sub-sampling each sample to 9,500 sequences and examining Shannon's Diversity index for alpha-diversity and a Bray Curtis metric for beta-diversity.

Immunohistochemistry of ileum samples

Immunohistochemical analysis using the avidin-biotin peroxidase method was carried out for pro inflammatory cytokine Interleukin 6 (IL-6) on formalin fixed, paraffin embedded sections of the ileum. Briefly, samples were dewaxed in histolene, rehydrated through graded ethanol and rinsed in phosphate buffered saline (PBS). Sections were blocked for 30 minutes with 3% normal horse serum. Subsequently they were incubated with the respective primary polyclonal goat antibody (1:2000, R&D Systems, Inc.) diluted in normal horse serum (Thermo Fisher Scientific, Waltham, MA, USA). This was followed by biotinylated secondary anti-goat antibody (Vector Laboratories Inc., Burlingame, CA, USA) for 30 minutes. Streptavidin biotin complex was added onto the sections for one hour then developed with 20 mM 3,3'diaminobenzidine (DAB) (Sigma, St Louis, MO, USA). Slides were counter stained with hematoxylin (Thermo Fisher Scientific, Waltham, MA, USA), dehydrated and mounted. IL-6 stained tissue sections were scored by two independent, blinded operators based on the presence and intensity of staining in the epithelial cells, lamina propria and submucosa. Each section was scored as 0 for no staining, 1 for mild staining, 2 for moderate staining and 3 for intense staining based on the method as described (Warren *et al.*, 2012).

Statistics

The power of this study was 85% for a sample size of six per group and a significance level of 0.05 based on the expected difference regarding the primary outcome. GraphPad Prism 6 (GraphPad Software Inc., La Jolla, California, USA) was used for statistical analysis of IL-6 and histology and differences between the six groups were analysed using the Kruskal–Wallis test, followed by Dunn's multiple comparisons test. All values shown are mean ± standard error of the mean (SEM). For metagenomics sequencing data analysis, statistical differences between groups were identified using a PERMANOVA test with pseudo F for beta diversity, pairwise Kruskal-Wallis for alpha diversity, and analysis of composition of microbes (ANCOM) with 999 permutations for detection of specific taxa associated with different treatments. A p-value of <0.05 was considered statistically significant.

Results

The mice did not lose weight across the duration of the experiment and there were no other adverse events observed.

P. gingivalis and F. nucleatum administration lead to increased IL-6 protein expression in the ileum

Histopathological analysis demonstrated a significant increase in the degree of inflammation present in all parts of the gastrointestinal tract of disease mice (PD group) when compared with controls (duodenum $p = 0.0143$, jejunum $p = 0.0009$, colon $p = 0.0442$, ileum $p = 0.0017$) (Figure 2 a, b, c, d). This was characterised by an increased infiltration of macrophages, neutrophils, lymphocytes and plasma cells in the lamina propria (Figures 3A (c and d), 3B (j and k), 3C (f and h), 3D (m and n)). Inflammatory marker IL-6 expression was elevated in the ileum of the disease mice when compared with controls ($p = 0.052$) (Figure 4).

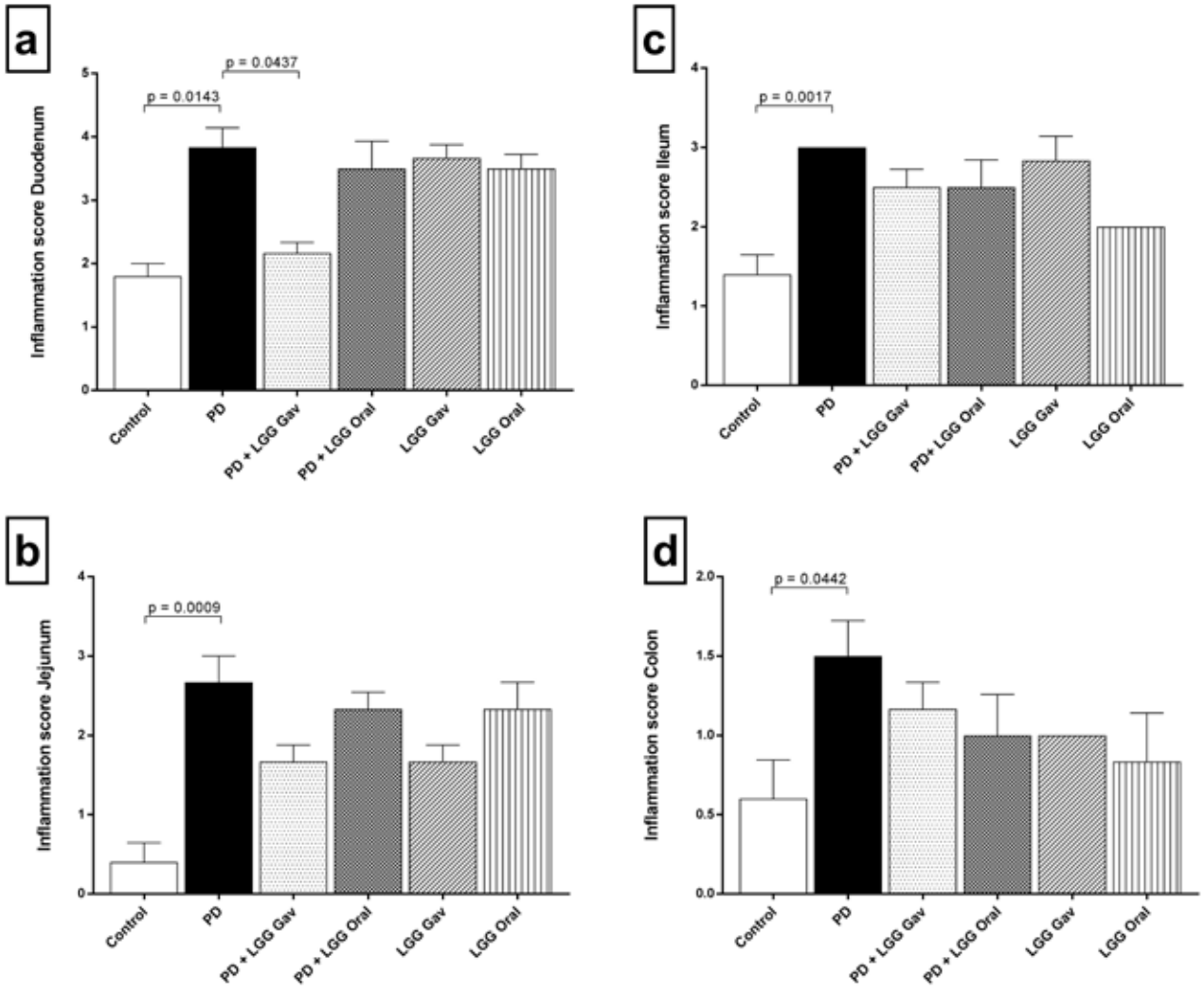

Figure 2: ***Disease mice (PD) presented significant inflammatory changes in the a. duodenum (p = 0.0143), b. jejunum (p = 0.0009), c. ileum (p = 0.0017), and d. colon (p = 0.0442) when compared with Control. Disease mice (PD) also presented significant inflammatory changes in the a. duodenum when compared with LGG gavage treated group (p = 0.0437). Bars represent mean inflammatory score ± SEM. PD = Periodontitis, Gav = Gavage***

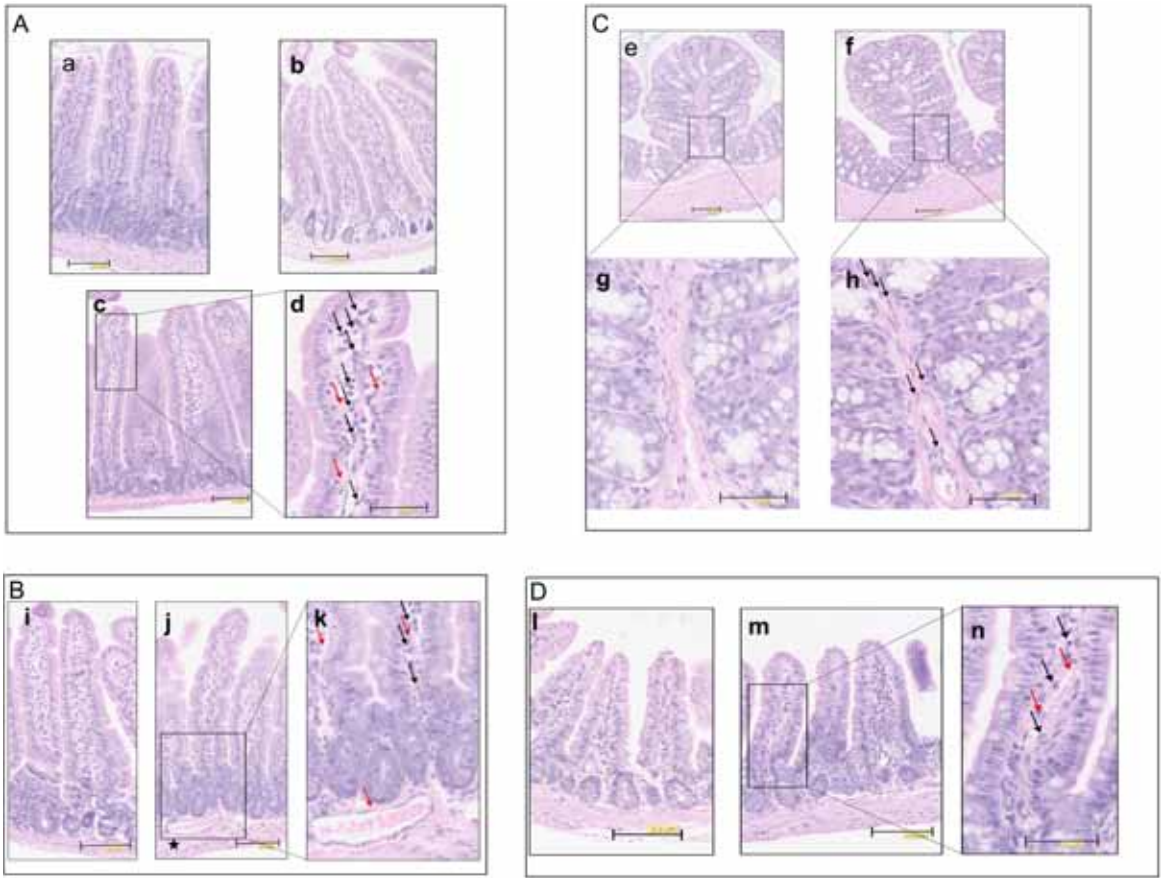

Figure 3: ***Representative images of standard haematoxylin and eosin staining of the: A. Duodenum in group: Control (a), PD + LGG Gav (b), PD (c, d); PD group presented an increase in inflammatory infiltrate (polymorphonuclear cells and lymphocytes) (black arrows), and dilation of lymphatics and capillaries; (red arrows); B. Jejunum in group: Control (i), PD (j, k); PD group presented an increase in inflammatory infiltrate of polymorphonuclear cells and lymphocytes (black arrows), dilation of lymphatics and capillaries (red arrows) and thickening of the mucosa (black star); C. Colon in group: Control (e, g) and PD (f, h). PD group presented an increase in inflammatory infiltrate (polymorphonuclear cells and lymphocytes) (black arrows); D. Ileum in group: Control (l), PD (m, n); PD group presented an increase in inflammatory infiltrate of polymorphonuclear cells and lymphocytes (black arrows) and dilation of lymphatics and capillaries (red arrows); Scale bars represent 100 µm (a, b, c) and 50 µm (d, g, h, k, n); Abbreviations: PD = Periodontitis, Gav = Gavage***

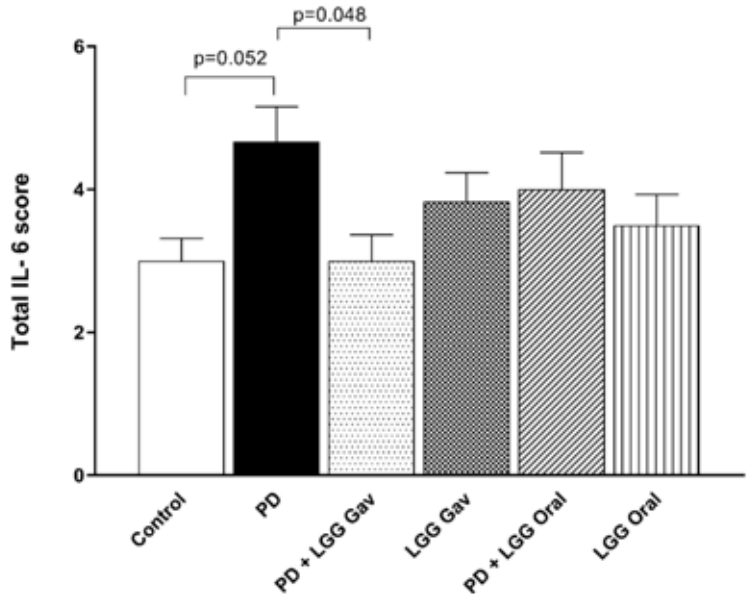

Figure 4: ***A. Total IL-6 immunohistochemistry ileum score; There was a significant difference between LGG Gav + PD and PD (p = 0.048), Control and PD (p = 0.052); Scale bars represent 100 µm; Abbreviations: PD = Periodontitis, Gav = Gavage***

P. gingivalis and F. nucleatum administration altered the caecal microbiome

There was a significant change in the caecal microbiome of mice inoculated with *P. gingivalis* and *F. nucleatum* (PD group) when compared to controls (p = 0.01, Figure 5a). This was associated with a change in alpha-diversity between the treated and untreated groups (p = 0.068, Figure 5b), suggesting that the lack or introduction of species rather than the abundances was responsible for changes in composition. ANCOM analysis identified a single unclassified Clostridiales taxa present in the Control group and absent in disease mice, suggesting that this taxa may be reduced during disease in this model.

Prior use of LGG prevents intestinal inflammatory changes induced by *P. gingivalis* and *F. nucleatum*

Mice treated with LGG via oral gavage (PD + LGG Gav) had significantly lower inflammatory scores in the duodenum than disease mice (PD) (p = 0.0437) (Figures 2a and 3Ab). Based on histological analysis, no significant inflammatory differences in the jejunum, ileum or colon were detected for any of the treatment groups PD + LGG Gav and PD + LGG Oral when compared with disease (Figures 2 b, c, and d). Expression of IL-6 in the ileum was significantly decreased in animals treated with LGG via gavage (PD + LGG Gav) when compared with disease (p = 0.048) (Figure 4).

Treatment with LGG prevents gut microbiota changes associated with *P. gingivalis* and *F. nucleatum* inoculation

The Bray Curtis distances between mice treated with LGG using both methods and those treated with the two oral pathogens were compared. A PERMANOVA test found no significant differences in the diversity of the caecal microbiota between groups of mice treated with LGG (PD + LGG Oral and PD + Gav LGG), and Control (p >0.05, Figure 5b). Similarly, the use of LGG prior to *P. gingivalis* and *F. nucleatum* inoculation did not change the abundance or diversity of the caecal microbiome of treated mice when compared with

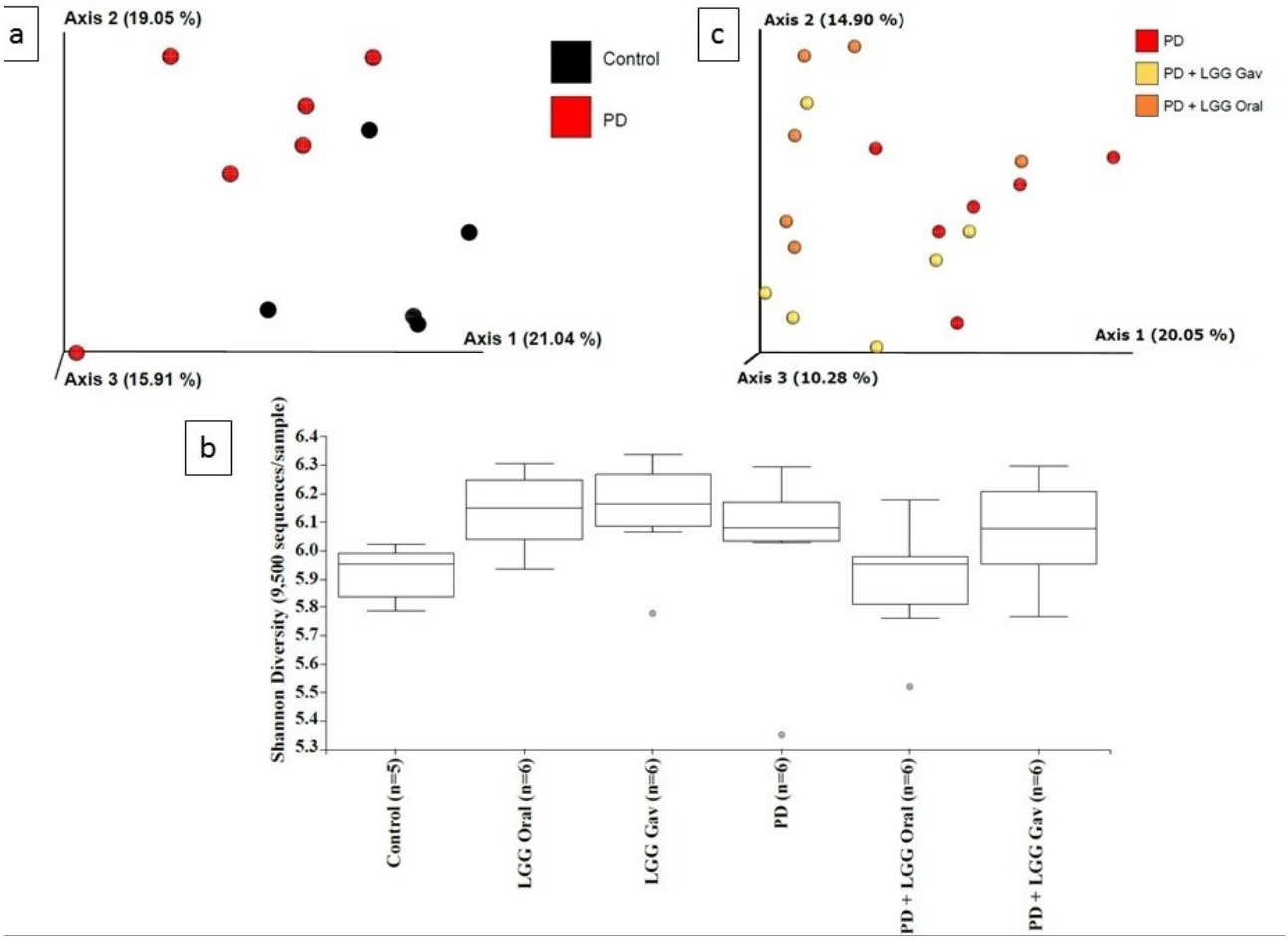

Figure 5: ***a: Shannon's diversity index (H) for all groups (9,500 sequences per sample for rarefaction, H = 3.33; p = 0.068); Principal coordinates analysis plot comparing caecal microbial community composition. Each point represent s a subject. Beta diversity index showing statistically significant differences between b. Control and PD (PERMANOVA pseudo F test statistic: 2.08592; p = 0.01); c. PD and PD + LGG Oral (PERMANOVA pseudo-F; test stat 2.08; p = 0.015) and PD and PD + LGG Gav (PERMANOVA pseudo-F; test stat 1.82; p = 0.024); d. Control and LGG Oral (PERMANOVA pseudo-F; test stat 1.8719; p = 0.012) and Control and LGG Gav (PERMANOVA pseudo-F; test stat 2.4596; p = 0.009). Abbreviations: PD = Periodontitis, Gav = Gavage***

disease (PD and PD + LGG Oral, H = 1.64; p = 0.22; PD and PD + LGG Gav, H = 1.0; p = 1, Figure 5b). However, the bacterial composition of caecal microbiota was significantly altered in LGG treated mice when compared with disease. The differences related to how the LGG was administered, as orally administered LGG had a greater influence than the gavage treatment (PD and PD + LGG Oral, p = 0.015 (PERMNOVA; pseudo F = 2.08), PD and PD + LGG Gav p = 0.024 (PERMNOVA; pseudo F = 1.82, Figure 5c). ANCOM analysis identified a Lachnospiraceae taxa, again within the Clostridiales order, present in PD + LGG Gav mice and absent in mice with disease, suggesting once more that a Clostridiales taxa was absent during disease. Comparing PD + LGG Oral with PD, ANCOM analysis identified the presence and absence of separate sequences of Clostridiales taxa between the two groups as well as Cyanobacterial taxa present in PD and absent in PD + LGG Oral.

Mice treated exclusively with LGG, either via oral inoculation (LGG Oral) or via oral gavage (LGG Gav), presented no differences in inflammatory scores for the duodenum, jejunum, ileum or colon when compared with controls ($p > 0.05$) (Figure 2). No change was seen in IL-6 expression in the ileum of treated animals when compared with controls (Figure 4).

Discussion

The gut microbiome plays an important role in health and disease (Quigley, 2013). An alteration of the composition of the gut microbiome has been associated with gastrointestinal conditions such as inflammatory bowel disease and irritable bowel syndrome (Swidsinski *et al.*, 2005) and systemic conditions including type 2 diabetes (Qin *et al.*, 2012) and obesity (Payne *et al.*, 2011). Current research suggests the gut microbiome may also play an important role in regulating bone health (McCabe *et al.*, 2015). However, the mechanisms of the interactions between gut inflammation and bone loss are yet to be determined (McCabe *et al.*, 2015).

An important finding of this study was the gastrointestinal changes induced in mice following inoculation by *P. gingivalis* and *F. nucleatum*. We have previously reported significant mean alveolar bone loss, increased presence of osteoclastic (TRAP) cells and inflammatory infiltrates in the PD group for this model (Gatej *et al.*, 2019, Cantley *et al.*, 2011){Cantley, 2009 #276}. In the current study, PD mice had significant inflammatory changes in the gastrointestinal tract, represented by increased numbers of inflammatory cells in the jejunum, ileum, duodenum and colon. Messora *et al* (2013) first reported changes in the gastrointestinal structure of animals with induced bone loss in a 44 days study (Messora *et al.*, 2013). Eight rats with ligature-induced periodontitis presented notable alteration of the intestinal morphology with significantly lower mean values in the jejunum villous height and crypt depth when compared with probiotic treated mice. (Messora *et al.*, 2013). The authors suggested these changes were due to an increase in pathogenic bacterial counts in the gastrointestinal tract contributing to increased inflammation (Messora *et al.*, 2013).

In the present study, mice inoculated with *P. gingivalis* and *F. nucleatum* had a significant increase in the expression of the pro-inflammatory cytokine IL-6 in the ileum when compared with controls. This is in agreement with a previous study which found significantly elevated expression of IL-6 in the small intestine in mice 48 hours after a single *P. gingivalis* oral administration (Nakajima *et al.*, 2015). IL-6 is an important mediator secreted by T cells and macrophages (Tanaka *et al.*, 2014). Although identified in the acute phase response of the inflammatory process or infection, persistent IL-6 production can lead to the development of immune-mediated diseases including diabetes (Kristiansen and Mandrup-Poulsen, 2005) and rheumatoid arthritis (Nishimoto, 2006). IL-6 regulates T cell differentiation, activation and resistance against apoptosis with roles in maintaining chronic intestinal inflammation in inflammatory bowel diseases such as Crohn's disease and ulcerative colitis thus being considered a 'master regulator of intestinal disease' (Waldner and Neurath, 2014). Studies using *in vitro* and *in vivo* models identified IL-6 as a potential new target for the therapy of gastrointestinal inflammation (Markus, 2014). In periodontitis, IL-6 is a mediator of bone resorption, stimulating osteoclast formation and thus being associated with the pathogenesis of this condition (Shao *et al.*, 2009). In the present study, oral inoculation with *P. gingivalis* and *F. nucleatum* led to a significant change in the bacterial composition of the caecum microbiome. Unclassified Clostridiales taxa were present in the caecum of control mice but absent in mice administered with *P. gingivalis* and *F. nucleatum*. There is growing evidence for these two bacterial species to alter the microbial balance towards dysbiosis thus supporting systemic inflammation. Studies have shown oral administration of *P. gingivalis* (10^{10} CFU/ml) twice a week for five weeks in mice resulted in altered proportion between Bacteroidetes and Firmicutes in the ileal microbiome with significant increase in the order of Bacteroidales (Arimatsu *et al.*, 2014). These changes coincided with increases in IL-6 serum levels and insulin resistance, which further led to inflammatory changes in adipose tissue and liver (Arimatsu *et al.*, 2014). Findings from the current study related to the absence of Clostridiales bacteria from the caecum of disease mice are consistent with a previous mouse study demonstrating that a single oral administration of 10^9 CFU/ml of *P. gingivalis* (strain W83) significantly decreased the proportion of Clostridiales in the gut of *P. gingivalis* infected mice when compared

with controls (Nakajima *et al.*, 2015). Reduction in the abundance of intestinal Clostridiales bacteria has been previously associated with development of Clostridium difficile infections (Vincent *et al.*, 2013) and increased risk of colorectal cancer (Ahn *et al.*, 2013, Zackular *et al.*, 2014). Nakajima *et al.* (2015) also demonstrated that mice infected with *P. gingivalis* presented with higher quantities of bacterial DNA in their liver (Nakajima *et al.*, 2015). Additionally, intestinal gene expression of proteins involved in intestinal permeability, such as Tjp1 and Ocln, were down-regulated in the small intestine of infected mice (Nakajima *et al.*, 2015). Gut microbiota changes, induced by oral administration of *P. gingivalis* and represented by increased proportion of the phylum Bacteroidetes and decreased proportion of the phylum Firmicutes, preceded systemic inflammatory changes and may provide a mechanistic link in the associations between periodontitis and systemic disease (Nakajima *et al.*, 2015). In the current study, bacterial DNA from *P. gingivalis* or *F. nucleatum* was not detected in caecum or faecal samples, suggesting these bacteria did not colonise the gastrointestinal tract. This finding suggests that changes induced by inoculation with these bacteria may be attributed to the ability of *P. gingivalis* and *F. nucleatum* to alter the composition and structure of the gut microbiome by eliciting systemic inflammation rather than interspecies bacterial competition between these pathogens and gut microbiota (Hotamisligil and Erbay, 2008).

Dysbiosis of the gut microbiome has been previously associated with changes in epithelial barrier function with increases in intestinal permeability regulated by tight junction proteins such as occludin and claudins (König *et al.*, 2016). Downregulated expression of tight junction proteins in the ileum has been reported in mice orally inoculated with *P. gingivalis* (10^9 CFU/ml) twice per week for five weeks (Arimatsu *et al.*, 2014). The results of the current investigation may be based on mechanisms involving two factors. The first factor involves loosening of tight-junctions of the epithelial barrier by inflammatory cytokine IL-6 produced by T cells which may allow more antigens to cross the barrier and result in inflammation in the gut. The second and a more critical role is played by the disruption of the gut microbiota that can influence the systemic production of pro-inflammatory cytokines and may be the cause for a disrupted epithelial barrier function.

A key finding of the current study relates to mice pre-treated with LGG and subsequently inoculated with *P. gingivalis* and *F. nucleatum*. We have previously demonstrated that pre-treatment with LGG in a mouse model of experimental periodontitis significantly reduced bone loss ($p < 0.0001$) and gingival inflammation ($p < 0.0001$) for the treated groups when compared with the disease group (Gatej *et al.*, 2019). These results were seen irrespective of the mode of administration (oral gavage or oral inoculation) suggesting LGG colonisation in the mouth was not a prerequisite for the inhibition of bone loss. Further, we did not find evidence of LGG colonization in the gut in any treatment group, again suggesting that the protection mediated by LGG may be via immune modulation, rather than microbial competition. In the current study, treatment with LGG administered via oral gavage prior to and during inoculation with *P. gingivalis* and *F. nucleatum* demonstrated a significant protective effect on the ileum, preventing inflammatory changes, such as increased inflammatory infiltrate in the lamina propria, induced by the two pathogenic bacteria. Previous animal studies demonstrated that oral administration of Bacillus subtilis (1.5×10^8 CFU/ml) to Wistar rats significantly protects the small intestine from changes induced by ligature-induced periodontitis, probiotic treated rats presenting lesser or no defects of the villi, basal lamina degeneration and infiltration of neutrophils in the jejunum when compared with disease (Messora *et al.*, 2016). In the current study, the underlying mechanism for these changes may be attributed to the protective ability of LGG to maintain Clostridiales taxa within the caecal microbiome during disease. It is clear that immune mechanisms of probiotic action leading to prevention of gut inflammation and dysbiosis are complex (Schmidt *et al.*, 2014) and future investigations are needed.

Conclusion

The present study shows that oral administration of *P. gingivalis* and *F. nucleatum* in a mouse model of induced periodontitis resulted in intestinal inflammation and associated changes in the composition of the gut microbiome. Pre-treatment with LGG via oral gavage prior to and during *P. gingivalis* and *F. nucleatum* inoculation significantly reduced intestinal inflammation for the probiotic treated groups compared with disease. Additionally, pre-treatment with LGG via oral gavage prevented gut microbiome changes associated with *P. gingivalis* and *F. nucleatum* inoculation, restoring the composition of the caecal microbiome. Further studies are required to provide more insights into the mechanisms driving these observed changes.

Acknowledgements and conflict of interest

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