Extracellular MUC3 mucin secretion follows adherence of Lactobacillus strains to intestinal epithelial cells in vitro

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SMALL INTESTINE

Background: Mucins are large complex glycoproteins that protect intestinal mucosal surfaces by limiting access of environmental matter to their epithelial cells. Several mucin genes have been described, including MUC3 that is a membrane associated mucin of the small intestine. Increased MUC3 mRNA transcription is induced by incubation of intestinal epithelial cells with a Lactobacillus strain known to be adherent to them.

Aims: To determine whether increased epithelial cell MUC3 mucin expression in response to Lactobacillus strains results in increased extracellular secretion of MUC3 mucins and the importance of epithelial cell adherence in modulation of MUC3 mucin expression.

Methods: HT29 cells grown to enhance expression of MUC3 mucins were incubated with selected Lactobacillus strains. Spent cell culture medium was collected for detection of secreted MUC3 mucins using dot blot immunosassay with a generated MUC3 antibody. Post-incubation HT29 cell RNA was collected for analysis of MUC3 expression by northern blot using a MUC3 cDNA probe. In vitro binding studies using Lactobacillus strains incubated alone or coincubated with enteropathogenic Escherichia coli strain E2348/69 were used for adherence and inhibition of adherence studies, respectively.

Results: Lactobacillus strains with minimal ability to adhere to HT29 cells failed to induce upregulation of mucin gene expression. There was a direct correlation between upregulation of MUC3 mucin mRNA expression and extracellular secretion of MUC3 mucin. The same Lactobacillus strains that increased extracellular secretion of MUC3 mucin led to reduced adherence of enteropathogen E coli E2348/69 during coincubation experiments.

Conclusion: Probiotic microbes induce MUC3 mucin transcription and translation with extracellular secretion of the MUC3 mucins. Epithelial cell adherence enhances the effects of probiotics on eukaryotic mucin expression.

We have previously shown that coincubation of Lactobacillus plantarum strain 299v (Lp299v) and L rhamnosus strain GG (LrGG) with an enteropathogenic Escherichia coli (EPEC) strain E2348/69 or enterohaemorrhagic E coli O157:H7 strain inhibited the adherence of either of the E coli strains to mucin producing intestinal epithelial cells. We also showed that incubation of Lp299v with HT29 cells could upregulate expression of MUC3 mRNA. One of the basic properties used to identify Lp299v and LrGG as potentially useful probiotics is their ability to adhere to intestinal epithelial cells.

To determine whether adherence is important to the process of mucin gene upregulation, we studied adherence of different strains of Lactobacillus, including a mutant strain lacking an adhesin, and measured alteration in the expression of mucin gene mRNA expression. In addition, we evaluated whether the MUC3 mucin produced was a secreted form by antibody analysis of spent cell culture medium using an antibody generated for this purpose. Finally, to show that the mucin product secreted into the cell culture medium was biologically active, we employed a bioassay to study inhibition of adherence of an enteric pathogen to the mucin producing epithelial cells.
**MATERIALS AND METHODS**

**Bacteria and growth conditions**

A stock culture of EPEC strain E2348/69 (serotype O127: H6) was maintained at 4°C on trypticase soy agar slants (Becton-Dickinson Microbiology Systems, Cockeysville, Maryland, USA). Stocks of *Lactobacillus* strains were maintained at 4°C on MRS agar (Difco Laboratories, Detroit, Michigan, USA). Bacterial strains were kindly provided by Dr James Kaper (EPEC strain E2348/69, Center for Vaccine Development, Baltimore, Maryland, USA), and Dr Khem Shahani (*L. acidophilus* strain DDS-1 (LaDDS); University of Nebraska, Lincoln, Nebraska, USA).

EPEC strain E2348/69 is a member of the family of non-invasive, non-enterotoxin, diarrhoeagenic producing pathogens that produce a characteristic attachment/effacement lesion with epithelial cells. LGG (American Type Culture Collection 53103, Rockville, Maryland, USA) was originally isolated in vitro from stool specimens of healthy humans. The LaDDS strain is from a dairy source and has been reported to stimulate murine macrophage production of interleukin 1 and tumour necrosis factor α. Lp299v is a member of a genetically well defined subgroup of *L. plantarum* isolated from intestinal mucosa. Strains of this subgroup agglutinate *Saccharomyces cerevisiae* in a mannose sensitive manner and carry a mannose specific adhesin. The *L. plantarum* strain adh− (*Lp adh−*) is a spontaneous mutant of Lp299v that no longer agglutinates *S cerevisiae* in a mannose sensitive manner. Restriction fragment length polymorphism analysis did not detect differences between Lp299v and its derivative Lp adh− (Siv Ahrne, personal communication).

**Cell growth conditions**

HT29 cells (American Type Culture Collection) were grown in McCoy’s 5a culture medium (Life Technologies, Gaithersburg, Maryland, USA) for enhanced MUC2 expression. Cells were progressively transferred from the usual glucose containing medium to a glucose free 5 mM galactose containing McCoy’s 5a culture medium to increase MUC3 mRNA expression and reduce MUC2 mRNA expression. Culture medium was supplemented with 10% heat inactivated qualified fetal bovine serum (Life Technologies). For bacteria free studies, an antibiotic/antimycotic mixture (100 U/ml penicillin G, 100 mg/ml streptomycin sulphate, and 0.25 mg/ml amphotericin B; Life Technologies) was added to cell culture medium. Cell cultures were grown at 37°C in a humidified atmosphere with 5% CO₂, and were passed after washing with Earle’s balanced salt solution (Life Technologies) using trypsin-EDTA (Life Technologies).

**Microbial adherence assays**

To evaluate binding of *Lactobacillus* strains to HT29 cells, 10³ *Lactobacillus* CFU/well (5x10⁵ CFU/ml) in 0.1 ml phosphate buffered saline (PBS), at pH 7.4, 25°C, were added to HT29 cells. After four hours of incubation at 37°C, cell culture medium was aspirated off the cells and cells were washed four times with Dulbecco’s PBS (pH 7.4, 37°C) to remove non-bound bacteria. Cells were released from polystyrene wells by adding 0.25 ml of trypsin-EDTA for 10 minutes. Then ice cold sterile PBS was added to each well followed by agitation to dissociate well contents. Serial dilutions of bacteria were plated on MRS agar and incubated overnight at 37°C for subsequent CFU quantification. All experiments were run in triplicate and results are based on at least five separate experiments. Viability of the microbes in cell culture medium without cells for the same incubation period and during the washing procedures was also determined.

The in vitro inhibition of EPEC adherence assay was similar to that previously described. Briefly, 10³ *Lactobacillus* CFU/well in 0.1 ml PBS, pH 7.4, 25°C, were added to 1.9 ml of antibiotic free cell growth medium (5x10⁵ CFU/ml) for an hour prior to addition of EPEC to maximise the effect. EPEC strain E2348/69 (10⁶ CFU in 0.1 ml PBS, pH 7.4, 25°C) was added to wells (5x10⁴ CFU/ml) of a 12 well polystyrene tray (Fisher) and incubated with the cells for three hours at 37°C. The inoculum of EPEC is a minimal infective dose in humans and does not increase mucin gene transcription. Quantification of adherent EPEC was performed by serial dilutions and enumeration of CFU on MacConkey agar. All experiments were run in triplicate.

**Alteration in MUC2 and MUC3 mRNA expression**

HT29 cells were grown to confluence in culture flasks (Falcon 3028; Becton-Dickinson, Franklin Lakes, New Jersey, USA). The same relative number of bacteria to cell contact area were added to culture flasks as were added to wells of 12 well plates used for adherence assays. Specifically, 4.5x10⁵ CFU/flask (1.5x10⁶ CFU/ml) were added as the cell contact area of the culture flask is 45x greater than the cell contact area of a well of 12 well plates.

Total RNA was isolated from HT29 cells using the guanidine isothiocyanate-cesium chloride cushion ultracentrifugation technique with modifications to minimise mRNA shearing, as previously described. RNA was stored in 0.3 M sodium acetate with 2.5 volumes of ethanol at −70°C. Northern blots were formed and underwent probe hybridisation using either a cDNA probe to the tandem repeat region of MUC2 (clone SMUC41) or MUC3 (clone SIB 124; kindly provided by Drs James Gum and Young Kim), as previously described. Analysis of mucin gene signals using Phosphor screen autoradiography (Molecular Dynamics, Sunnyvale, California, USA) and area integration using ImageQuant software (version 3.3; Molecular Dynamics) relative to levels of 28S mRNA were as previously described.

**Antibody to MUC3 peptide**

To compare transcription levels of MUC3 mRNA to translation expression, an antibody to detect protein product was required. Polyclonal antiserum was produced by repeated intraperitoneal injections of Balb/C mice with a KLH conjugated peptide sequence (TSR RTT RIT). This 9 mer peptide sequence was deduced from the cDNA sequence of the tandem repeat region. A 1:5000 dilution of peroxidase conjugated goat antimouse (Jackson ImmunoResearch, West Grove, Pennsylvania, USA) was added to culture flasks as were added to wells of 12 well plates for the same incubation period and during the washing procedures. The viability of the microbes in cell culture medium without cells for the same incubation period and during the washing procedures was also determined.

The reactivity and specificity of the antibody in mouse immune serum was determined by dot blot immunobassay, as described by Towbin and Gordon. Briefly, spent culture media overlaying HT29 cells grown to enhance MUC2 or MUC3 expression were collected separately and to purify the mucins, cell culture medium was subjected to density gradient ultracentrifugation, as described previously. Twenty µg (Lowry protein) from a mucin enriched fraction was applied as a spot onto nitrocellulose paper strips and allowed to air dry for one hour. Nitrocellulose strips were incubated with 3% bovine serum albumin (Sigma, St Louis, Missouri, USA) in 10 mM Trizma base (Sigma) with 0.9% saline at pH 7.4 for 30 minutes. Strips were then washed with Tris saline. Mouse immune serum was added in serial dilutions for incubation overnight at 4°C. Afterwards, the strips were washed with Tris saline at room temperature to remove the unbound antibody.

A 1:5000 dilution of peroxidase conjugated goat antimouse IgG (Fc fragment specific; Jackson ImmunoResearch, West Grove, Pennsylvania, USA) was added to the nitrocellulose
Mucin mRNA expression induced by proteolytic degradation of native mucins
culture medium was collected separately, and to minimise changed to serum free medium prior to the addition of different strains. After six hours of incubation, each cell culture medium was collected separately, and to minimise proteolytic degradation of native mucins' protease inhibitors were added. The inhibitors included 5 mM N-ethylmaleimide (Sigma), 2 mM phenylmethylsulphonyl fluoride (Sigma), and 0.01% sodium azide (Sigma) in a 5 mM EDTA solution (Sigma). Spent culture medium was centrifuged to remove cellular and particulate debris and then passed through a 0.02 µm filter for sterilisation. The volume of soluble supernatant was reduced by vacuum centrifugation (SpeedVac SC200; Savant Instruments, Farmingdale, New York, USA). Equivalent amounts from the spent culture supernatants from cells incubating with different Lactobacillus strains (20 µg protein) were applied as a spot onto nitrocellulose paper and air dried, and the experimentation proceeded as above. For these experiments, mouse immune serum was added at a dilution of 1:100.

Indirect immunofluorescence
To further validate that the antibody generated was to MUC3 mucin, we performed indirect immunofluorescence on normal

Figure 1 (A) Lactobacillus adherence to HT29 cells. Lactobacillus strains 10^5 colony forming unit (CFU)/well were incubated for four hours with HT29 cells grown in glucose free galactose containing cell culture medium to enhance MUC3 expression. Non-adherent bacteria were washed off (×4) and adherent bacteria were quantified by CFU determination on MRS agar plates. Lp299v, Lactobacillus plantarum strain 299v; LrGG, L rhamnosus strain GG; LaDDS, L acidophilus strain DDS-1. (B) Similar experiments were performed using the parent Lp299v strain and its adhesin negative genetic mutant Lp adh-- strain. In both, five experiments were run in triplicate and results are expressed as mean (SEM).

Figure 2 Mucin mRNA expression induced by Lactobacillus strains. Lactobacilli (4.5×10^6 colony forming units) were added to flasks in an equivalent amount as cell surface contact area of 12 well plates. After one hour of incubation, northern blots were hybridised using random-primed 32P labelled cDNA probe to the MUC3 tandem repeat. Mucin mRNA levels were quantified by area integration of Phosphor strips and incubated for one hour at room temperature. Horseradish peroxidase colour development reagent containing 4- chloro-1-naphthol (Bio-Rad, Richmond, California, USA) was added to develop any positive reaction. Dot blot immunassay was performed in a similar fashion for spent cell culture medium from HT29 cells grown in galactose containing medium incubated with Lp299v, LrGG, and LaDDS to quantify whether increased secreted MUC3 mucins were present. For this study, the cell culture media were changed to serum free medium prior to the addition of different Lactobacillus strains. After six hours of incubation, each cell culture medium was collected separately, and to minimise proteolytic degradation of native mucins' protease inhibitors were added. The inhibitors included 5 mM N-ethylmaleimide (Sigma), 2 mM phenylmethylsulphonyl fluoride (Sigma), and 0.01% sodium azide (Sigma) in a 5 mM EDTA solution (Sigma). Spent culture medium was centrifuged to remove cellular and particulate debris and then passed through a 0.02 µm filter for sterilisation. The volume of soluble supernatant was reduced by vacuum centrifugation (SpeedVac SC200; Savant Instruments, Farmingdale, New York, USA). Equivalent amounts from the spent culture supernatants from cells incubating with different Lactobacillus strains (20 µg protein) were applied as a spot onto nitrocellulose paper and air dried, and the experimentation proceeded as above. For these experiments, mouse immune serum was added at a dilution of 1:100.

Indirect immunofluorescence
To further validate that the antibody generated was to MUC3 mucin, we performed indirect immunofluorescence on normal
human tissue as tissue localisation in the small intestine is known. Biopsies of normal human duodenum were quick frozen in liquid nitrogen, fixed with OCT fixative, and stored at −70°C. Sections were cut 5 μm thick with a cold microtome and fixed onto microscope slides using acetate. After being warmed to room temperature and washed with PBS, the microscope slides were incubated for 30 minutes at room temperature in a 1:20 dilution of mouse anti-MUC3 mucin serum. The microscope slides were again washed with PBS, and a 1:20 dilution of fluorescein isothiocyanate conjugated to goat IgG/Fc fragment specific (Jackson ImmunoResearch) was added. The microscope slides were then incubated in darkness for 30 minutes at room temperature, washed with PBS, and examined by fluorescence microscopy. Control slides were stained with equal dilutions of PBS, fluorescein isothiocyanate alone, or preimmune mouse serum. The study protocol for obtaining a biopsy received prior approval from the Institutional Review Board of Children's Hospital in Omaha.

**Statistical analysis**

Group data are expressed as means (SEM). Analyses between multiple groups were determined using one factor ANOVA followed by Fisher's protected least significant difference test with 95% confidence intervals. Post hoc ANOVA analyses were determined using the Statview software program (version 5.0.1; SAS Institute Inc., Cary, North Carolina, USA). Analysis between groups were determined using the two tailed unpaired Student t test.

**RESULTS**

**Adherence of Lactobacillus strains**

As shown in fig 1A, there were differences in adherence of the Lactobacillus strains to HT29 cells grown to enhance MUC3 expression. Adherence of LrGG (1.1×10^4 (1.1×10^4) CFU/well) was about 10x greater than Lp299v (1.6×10^3 (1.6×10^3) CFU/well) that was in turn about 10 times greater than LaDDS (1.3×10^3 (86) CFU/well). There was no loss of viability of any of these Lactobacillus strains in the galactose containing cell culture medium over a similar four hour incubation period (data not shown) or during the washing procedures. Similar results were determined for binding of the Lactobacillus strains to HT29 cells grown in glucose containing cell culture medium. That is, LrGG adherence to HT29 cells (1.1×10^4 (0.1×10^4) CFU/well; mean (SEM)) was greater than either Lp299v (1.6×10^3 (0.2×10^3) CFU/well; p<0.05) or LaDDS (1.3×10^4 (0.1×10^4) CFU/well; p<0.05).

In separate experiments, we compared adherence of Lp299v with the mutant Lp adh− strain which has lost the ability to agglutinate Saccharomyces due to loss of production of the mannose sensitive binding ligand required for HT29 cell attachment. As shown in fig 1B, adherence of Lp299v (1.7×10^4 (6.9×10^4) CFU/well) to MUC3 expressing HT29 cells was about 10 times greater than the non-agglutinating Lp adh− strain (1.9×10^4 (3.8×10^4)) which showed minimal binding similar to the LaDDS strain in the previous set of experiments (fig 1A).

**Mucin mRNA expression**

Levels of MUC3 mRNA expression of HT29 cells coincubated with Lactobacillus strains are shown in fig 2. Expression levels of MUC3 mRNA increased about threefold with addition of either Lp299v or LrGG compared with control cells. Both Lp299v and LrGG increased mucin gene expression levels to the same magnitude over control cells that were grown in sterile media. However, there was no difference in MUC3 mRNA expression for cells incubated with LrGG compared with controls (p>0.05). For comparison, we also evaluated MUC2 mRNA expression as it is known to exist only as a secreted form. Similar to MUC3 mRNA expression levels, ANOVA analysis revealed that MUC2 mRNA expression levels of incubation of HT29 cells grown to express MUC2 for LrGG (304 (52)% of control) and Lp299v (227 (49)% of control) were similar and both greater than MUC2 mRNA expression by control cells incubated without bacteria or HT29 cells incubated with LaDDS (153 (27)% of control) (data not shown).

**Figure 3** Immunassay of HT29 spent cell culture medium. Immune serum generated against a 9 mer peptide (TSSRRTRRT) of deduced MUC3 sequence was incubated with MUC2 (A) and MUC3 mucins (B) isolated from HT29 cell culture medium. Mucin protein (20 µg) was blotted onto nitrocellulose. A 1:100 dilution of mouse antisera was added. After washings, bound antibody was detected by a horseradish peroxidase colourisation reaction. A black dot in each frame is pencil marking used to indicate where medium was blotted initially. High reactivity was observed for spent culture medium of cells grown in galactose containing medium to enhance MUC3 expression. In contrast, there was less reactivity to material from spent culture medium of HT29 cells grown in a glucose containing medium that is associated with high MUC2 mucin mRNA expression and low MUC3 mucin mRNA expression.
results are similar to those previously published by Chang and colleagues using an antibody to the MUC3 tandem repeat region. Taken together, these results demonstrate the expected location for antigenic material derived from mucin and that the polyclonal antiserum reacts strongly with material enriched with MUC3 mucins.

**MUC3 mucin extracellular expression**

Anti-MUC3 serum was then used in a dot blot immunosassay using spent cell culture medium derived from HT29 cells grown in galactose containing medium incubated with different *Lactobacillus* strains. As shown in fig 5, a central black dot indicating the application site of the spent cell culture medium is visible in each panel. Surrounding the application spot of Lp299 and LrGG there was far greater reactivity than the blot of spent cell culture medium from HT29 cells without a probiotic added (none) or with LaDDS added.

**Inhibition of enteropathogenic *Escherichia coli* epithelial cell adherence**

We correlated the biological significance of increased MUC3 gene transcription and secreted expression of MUC3 mucins in the presence of *Lactobacillus* strains in their ability to adhere to the HT29 cells by performing an in vitro inhibition of the adherence assay. For these experiments, controls were EPEC strain E2348/69 incubated with HT29 cells without *Lactobacillus* bacteria. As shown in fig 6A, recovery of EPEC bound to HT29 cells grown in galactose containing medium (for enhanced MUC3 expression) coincubated in the presence of Lp299v and LrGG was less than controls with EPEC alone. There was no difference in inhibition of EPEC coincubated with LaDDS compared with adherence of controls with EPEC alone for MUC3 expressing HT29 cells.

In fig 6B, the non-adherent Lp adh− strain was unable to impact the adherence of EPEC E2348/69 (8.8 × 10⁵ CFU/well) to MUC3 producing HT29 cells. In contrast, Lp299v reduced the EPEC adherence (4.8 × 10⁵ (3.8 × 10⁴) CFU/well) that is similar to the effect elicited by the adhering *Lactobacillus* strains depicted in fig 6A.

For comparison, we also evaluated inhibition of EPEC adherence to MUC2 producing HT29 cells coincubated with different *Lactobacillus* strains as MUC2 is the major mucin of the colon and exists only as a secreted mucin. For HT29 cells grown in glucose containing medium (for enhanced MUC2 expression), similar results to fig 6A were determined. That is, there was decreased adherence of EPEC to HT29 cells with Lp299v (6.3 × 10⁴ (0.7 × 10⁵) EPEC CFU/well; p<0.005) and LrGG (1.00 × 10⁴ (0.20 × 10⁵) EPEC CFU/well; p<0.005) but not LaDDS (1.40 × 10⁴ (2.50 × 10⁵) EPEC CFU/well; p>0.05) compared with controls (1.60 × 10⁴ (3.00 × 10⁵) EPEC CFU/well (mean (SEM)).

**DISCUSSION**

In the current study we confirmed that incubation of Lp299v with HT29 cells upregulated expression of MUC3 mRNA. We
also showed that other Lactobacillus strains may possess this same capability but it is not a universal property of probiotic strains (fig 2). To detect MUC3 mRNA expression, a cDNA probe to the central tandem repeat region was utilised. Recently, the existence of a second MUC3 gene has been proposed. Both human MUC3 genes, now called MUC3A and MUC3B, show such significant similarity that the cDNA probe that was used in these studies would recognise transcripts from both genes.

MUC3 is a member of the membrane bound mucins along with MUC1, MUC4, and MUC12, and these various mucins can be expressed as membrane anchored, soluble, or secreted forms. Both membrane bound variants and splice variants coding for secreted forms have been described for MUC3 mucins. With upregulation of expression of MUC3 mRNA, the question then arises whether the mucins are cell anchored or secreted. This has biological consequences. If the upregulated mucin gene products were membrane bound, then in order for Lactobacillus strains to limit EPEC binding to intestinal epithelial cells all potential mucin binding sites for EPEC would have to be occupied by the Lactobacillus organisms. This would suggest there were common mucin binding sites for the gram positive Lactobacillus strains and the gram negative E. coli. Alternatively, if the translated product of MUC3 mucin gene upregulation was a secreted mucin, then mucin EPEC interactions could occur away from the intestinal epithelial cell membrane surface and the trapped microbes could then be swept away with the peristaltic motions of the intestinal tract. As we demonstrated increased MUC3 mucins in the spent cell culture medium following probiotic incubation, the latter explanation is favoured. The rapidity of the response, which is within one hour, would match a biologically relevant time period and alteration of expression of other intestinal epithelial cell genes by non-virulent intestinal microbes has been demonstrated within a similar time frame. Rapidity of response would be expected to be an essential element for a member of the inducible innate protective response of the intestinal epithelial cell.

Traditionally, antibiotics have been administered for microbial related diseases of the intestinal tract. Their usage adds to the increasing concern regarding bacterial resistance or induction of other pathological processes such as haemolytic uremic syndrome, and this has led to a search for other interventions. Many enteric pathogens must first adhere to intestinal epithelial cells to initiate intestinal disease. Limiting access of pathogens to intestinal epithelial cells is one strategy to prevent disease that has been investigated previously. For example, the competitive inhibition of bacterial adherence by mimicry of receptors on the apical surface of enterocytes using oral administration of sialylated glycoproteins has been shown to protect newborn calves from an enterotoxigenic E coli strain K99. However, carbohydrate analogues or their synthetic imitators can be difficult and expensive to produce. Furthermore, identification of the causative organism for a given diarrhoeagenic to know which ligand should be administered takes time.

In contrast to specific glycomimetics, mucins are high molecular weight glycoproteins that express an array of glycan structures. There is a high content of carbohydrates with N-glycan oligosaccharides in the N and C terminal unique regions and O-glycosidic bonds between N- acetyl galactosamine and both serine and threonine in the central tandem repeat region of the mucin peptide backbone. In addition to N-acetyl galactosamine, other sugars found in O- glycans are fucose, N-acetyl glucosamine, galactose, and sialic acid. Terminal sugars that are attached to the core structures include galactose, N-acetyl glucosamine, fucose, and sialic acid. Thus this complex array of carbohydrates provides a broad range of potential sites for bacterial carbohydrate binding protein ligands of microbes. For EPEC, the adherence process is characterised by an initial relatively distant low affinity interaction mediated by the bundle forming pilus, followed by an essentially irreversible adherence to epithelial cells that is mediated via the protein intimin. We previously showed that exogously added MUC3 mucins into an in vitro adherence assay system with EPEC incubating with non-mucin producing epithelial cells quantitatively inhibits EPEC adherence. Up-regulation of intestinal mucins may provide both a rapid response and the potential to provide receptor mimicry against a broad range of organisms associated with diseases of the intestinal tract. Upregulation of mucin expression may explain the prevention of flare ups of chronic pouchitis following oral administration of probiotics reported by Gionchetti and colleagues as anaerobic bacterial concentrations correlate with the number of mucosal chronic inflammatory cells in...
pouchitis. In animal models, administration of *Lactobacillus* reduced the severity of mucosal inflammation of colitis in interleukin 10 gene deficient mice and methotrexate induced enterocolitis in rats. The diminished inflammation correlated with reduced adherent and translocated aerobic bacteria, and *Enterobacteriaceae* and gram negative anaerobes, respectively. In the current study, we have confirmed that preincubation of some probiotic strains of *Lactobacillus* with EPEC inhibit the adherence of the enteropathogen to intestinal epithelial cells. Not all *Lactobacillus* strains have this capacity as the mutant strain of Lp adh and LaDDS failed to diminish the binding of EPEC E2348/69 compared with cells grown in a sterile cell culture medium. A difference between Lp adh and LaDDS and the other two strains that we demonstrated was the ability to adhere to an intestinal epithelial cell. While LrGG had the greatest ability to bind to HT29 cells, Lp299v was demonstrated to have about 10 times greater ability to adhere to the cells compared with Lp adh and LaDDS in our assay. The difference between LrGG and Lp299v may be a result of the vigour in comparison with Lp adh– compared with Lp adh.

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