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Effects of enteropathogenic bacteria & lactobacilli on chemokine secretion & Toll like receptor gene expression in two human colonic epithelial cell lines

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***Background & objectives*: The intestinal epithelium is part of the innate immune system responding to contact with pathogenic or commensal bacteria. The objective of this study was to compare innate responses of intestinal epithelial cell lines to pathogenic bacteria and to lactobacilli.**

***Methods*: Two human intestinal epithelial cell lines, HT29 (enterocyte-like) and T84 (crypt-like), were exposed to pathogenic bacteria representative of non invasive (*Vibrio cholerae* O1 and O139), adherent (enterohaemorrhagic *Escherichia coli,* EHEC) or invasive (*Salmonella* Typhimurium and *Shigella flexneri*) phenotypes and to non pathogenic *Lactobacillus rhamnosus* GG or *Lactobacillus plantarum*. Interleukin-8 (IL-8) was measured in culture supernatant by ELISA, while mRNA from cells was subjected to quantitative reverse transcriptase PCR for several other chemokines (CXCL1, CCL5 and CXCL5) and for Toll-like receptors (TLR) 2, 4, 5 and 9.**

***Results*: *V. cholerae*, *S.* Typhimurium, *S. flexneri* and EHEC induced IL-8 secretion from epithelial cells into the medium. *Salmonella*, *Shigella* and EHEC, but not *V. cholerae*, significantly increased mRNA expression of CXCL1. None of the pathogens induced CCL5 or CXCL5. *Salmonella* and *Vibrio* significantly increased TLR4 expression, while *Vibrio* and EHEC decreased TLR5 expression. EHEC also decreased TLR9 expression. Lactobacilli attenuated the IL-8 response of the cell lines to *V. cholerae*, *Salmonella*, and EHEC but did not significantly change the IL-8 response to *Shigella*.**

***Interpretation & conclusions*: Distinct patterns of epithelial cell chemokine responses were induced by the bacterial pathogens studied and these were modulated by commensal lactobacilli. Alterations in TLR expression by these pathogens are likely to be important in pathogenesis.**

Key words Chemokine - enteropathogenic bacteria - interleukin-8 - intestinal epithelial cells - *Lactobacillus* - Toll-like receptor

Infectious diarrhoea caused by bacterial enteric pathogens continues to be a major cause of morbidity and childhood mortality in developing countries[1]. While adaptive immunity occurs on exposure to these enteric bacterial pathogens, innate immune responses are increasingly recognized as important to both disease pathogenesis and immunity[2]. Bacterial pathogens cause diarrhoea in various ways. Some such as *Vibrio cholerae* colonize the bowel, produce enterotoxins, and induce fluid secretion and diarrhoea, without invading

the epithelium or mucosa. Others such as *Shigella* species invade the epithelium leading to epithelial cell death and ulceration and causing blood and mucus diarrhoea. Enterohaemorrhagic *Escherichia coli* (EHEC) adhere to epithelial brush border membrane and secrete a cytotoxin. The intestinal epithelial cell is the first cell type that comes into contact with the bacteria recognizing them through pattern recognition receptors, principally the Toll-like receptors (TLRs), located on the cell surface[3]. This contact triggers release of inflammatory mediators, the most prominent of which is interleukin-8 (IL-8) a neutrophil chemotactic peptide[4-6]. Other pro-inflammatory mediators include growth-related oncogene-α (GRO-α, now known as CXCL1) and epithelial cell-derived neutrophil activating factor (ENA-78, also known as CXCL5)[7], both of which are chemotactic to neutrophils. Expression of RANTES (regulated upon activation, normal T-cell expressed and secreted, CCL-5), which is chemotactic to T cells, is increased in rotavirus-infected epithelial cells[8].

Mammalian TLRs play a crucial dual role in host immunity[9,10] and are important for the development of host innate and adaptive immune responses to gastrointestinal infections. TLR2 that recognizes peptidoglycan motifs of bacterial cell walls, and TLR4 that recognizes lipopolysaccharide may be present in epithelial cells, but are more likely to be found in sub-epithelial cells including myofibroblasts[10]. TLR5 that recognizes flagellin, the primary structural component of bacterial flagella, is possibly responsible for pro-inflammatory gene activation in epithelial cells in response to pathogenic bacteria[11]. Interestingly, a commensal *E. coli* strain has also been shown to activate TLR5 in intestinal epithelial cells[12]. TLR9, that recognizes unmethylated CpG DNA, mediates the downregulation of inflammatory gene activation by commensal lactobacilli in an animal model of inflammatory bowel disease[13].

Lactobacilli are normal commensal flora of the gut that are used in the therapy of diarrhoea and intestinal inflammation. *Lactobacillus rhamnosus* GG (LGG), one of the most widely studied probiotic bacteria, is effective in preventing and treating diarrhoea in infancy and childhood[14], and has a cytoprotective effect on intestinal epithelial cells[15]. Other lactobacilli are also used as probiotics, in particular *L. plantarum*, which is widely used in clinical practice[16]. Previous studies have shown that lactobacilli inhibit growth of several pathogens including *V. cholerae*[17,18]. Lactobacilli can also inhibit *Shigella*-induced nuclear factor kappa B (NFκB) activation in epithelial cells[19]. However, the effects of lactobacilli on the interaction between enteric bacterial pathogens and epithelial cells (in particular whether probiotic:pathogen ratio is important, whether these exert a protective effect if administered either prior to or simultaneously with pathogen, and a comparison of probiotic effects against the background of mechanism used to cause diarrhoea) has never been examined. Since epithelial cell interactions occur to different degrees and through different mechanisms with the various bacterial enteric pathogens, it is possible that the innate immune responses induced in epithelial cells vary in type and degree. The present studies were designed to compare the intestinal epithelial cell responses to different enteric bacterial pathogens, to determine the influence of lactobacilli on the responses induced by the enteric pathogens, and to determine whether any of the pathogenic bacteria caused alterations in expression of mRNA for TLRs of interest in colonic epithelial cell lines.

Material & Methods

Bacteria: Clinical isolates of *Salmonella* Typhimurium, *S. flexneri,* enterohaemorrhagic *E. coli* (EHEC), *V. cholerae* O1 and *V. cholerae* O139, cultured from patients with gastroenteritis, were used for these studies. These bacteria were chosen as representative of different types of pathogenesis. *Salmonella* and *Shigella* are invasive pathogens, EHEC adhere to epithelial cell surfaces, and *V. cholerae* are non invasive, non adherent, toxin-producing bacteria. *L. rhamnosus* GG (LGG) was grown from *Culturelle* capsules obtained commercially and *L. plantarum* was obtained from National Chemical Laboratory (NCL), Pune, India. Lactobacilli were grown in de Man Rogosa Sharpe broth (Hi-media, India) under anaerobic conditions for 16 h at 37°C on the day before the experiments.

Cell lines: Two human colon carcinoma cell lines were used in these studies. The HT29 cell line [obtained from National Centre for Cell Science (NCCS), Pune, India] has an enterocyte-like differentiation and is considered to be representative of surface or villus type cells. The T84 cells (ATCC, USA) have a secretory differentiation and are considered representative of the secretory cells that line the bottom of the intestinal or colonic crypts. HT29 cells were grown in 25 cm^2 flasks (Axygen, India) in high glucose Dulbecco's Modified Eagle Medium (DMEM) (GIBCO Invitrogen, India) supplemented with 10 per cent foetal bovine serum (GIBCO, India) containing penicillin (100 U/ml), streptomycin (100 µg/ml) and amphotericin B (10 µg/ml) at 37°C with

5 per cent CO_2[7]. T84 cells were cultured in DMEM and Ham's F12 medium (GIBCO, India) containing penicillin (100 U/ml), streptomycin (100 µg/ml) and amphotericin B (10 µg/ml) at 37°C with 5 per cent CO_2[7].

Bacteria-cell interactions: Bacteria were grown standing overnight at 37°C in Luria–Bertani (L-B) broth (Hi-media, India) to log phase before use, pelleted by centrifugation at 500 *g* for 10 min, and washed three times with sterile phosphate buffered saline (*p*H 7.4). The bacterial concentration in the suspension medium was determined by measurement of absorbance at 540-600 nm and comparison with standard MacFarland tubes. The bacteria were resuspended in antibiotic-free medium and overlaid on epithelial cells to a final concentration of 100 bacteria per epithelial cell for the infection studies[4-7]. HT29 and T84 cell monolayers were cultured in 24-well plates (Falcon Milliwell, BD, USA) and grown until they reached confluence. Prior to infection, cells were washed twice with appropriate medium without serum and antibiotics and maintained in serum free medium for at least 2 h. Epithelial cells were counted from a duplicate well in a Neubauer chamber. The epithelial cells in the wells were overlaid with the test bacteria in suspension at a ratio of 100 bacteria per epithelial cell and incubated at 37^0C for 2 h. The extracellular bacteria were then removed by washing and the cell lines were further incubated for 4 h in the presence of 50 µg/ml gentamicin to kill any remaining extracellular bacteria. At the end, culture supernatant was removed and stored at -20^0C for quantitation of IL-8 secretion, while cells were removed and stored at -80^0C for total RNA isolation. In studies with lactobacilli, the appropriate bacteria were added either alone in order to ascertain whether there was any basal effect, or added either along with or prior to enteropathogenic bacteria. When added prior to the enteropathogen, the lactobacilli were incubated with the cell line for 2 h, washed and removed, and the enteropathogen then added, incubated with the cell line for a further two hours then washed, and the cell line incubated at 37^0C in presence of gentamicin for another 4 h. In addition the effect of lactobacilli concentration was tested using two doses of lactobacilli (ratio of 1:1 or 10:1 lactobacilli to enteropathogen). The latter dose was chosen empirically, since there is no literature to support the use of a specific dose. Experiments were done three times, each time in duplicate wells.

IL-8 assay and quantitative PCR for chemokine and TLR mRNA: The culture supernatants were centrifuged at 12,000 *g* for 10 min and IL-8 was assayed by ELISA (OptEIA Set, Becton Dickinson, USA). The transcriptional profile of several other chemokines, CXCL1, CXCL5 and CCL5, was investigated. Total RNA was isolated from infected cells using Trizol reagent (Sigma, India) as per the manufacturer's instructions. Reverse transcription of RNA was performed as per manufacturer's instructions (*www.finnzymes.fi/pdf/F-572_guidelines.pdf*) in a final volume of 20 µl containing 0.5 mM of each nucleotide triphosphate, 40 units of RNAase inhibitor (Ambion, USA), 50-100 ng of Random Hexamers (Amersham Pharmacia, UK), 200 units of Moloney Murine Leukemia Virus Reverse Transcriptase (MMuLV-RT, Finnzymes, Finland) and 15 µl of the extracted RNA or H_2O, and then incubated at 42°C for 1 h. Subsequently 1 µl of diluted cDNA sample was amplified in a thermal cycler (MJ Research, USA) in 20 µl of 1 x PCR buffer containing 200 µM of each nucleotide triphosphate, 10 picomoles of each primer (Sigma Genosys Bangalore) (Table), and Titanium Taq DNA polymerase (Clontech, Becton Dickinson, USA).

Quantitative real-time PCR[20] was performed with appropriate conditions in a Chromo 4 system (Biorad, USA). 1 µg of RNA was transcribed to cDNA using

Table. Primers used for PCR amplification of chemokines and TLRs

Gene	Forward	Reverse	Product length (bp)
Chemokines:			
CXCL1 (GRO)	5'-ATG GCC CGC GCT GCT CTC T-3'	5'- AGC TTT CCG CCC ATT CTT G-3'	251
CCL5 (RANTES)	5'- TAC CAT GAA GGT CTC CGC -3'	5'- GAC AAA GAC GAC TGC TGG -3'	198
CXCL5 (ENA 78)	5'-GTG TTG AGA GAG CTG CGT TG-3'	5'- TTT TCC TTG TTT CCA CCG CT-3'	215
Toll like receptors:			
TLR 2	5'- CAA TGA TGC TGC CAT TCT CAT -3'	5'- ATT ATC TTC CGC AGC TTG CA -3'	83
TLR 4	5'- AGT TTC CTG CAA TGG ATC AAG G -3'	5'- CTG CTT ATC TGA AGG TGT TGC AC -3'	83
TLR 5	5'-GGC TTA ATC ACA CCA ATG TCA CTA T-3'	5'- GAA ACC CCA GAG AAC GAG TCA G -3'	81
TLR 9	5'-AGT CAA TGG CTC CCA GTT CCT–3'	5'- CGT GAA TGA GTG CTC GTG GTA -3'	93
House keeping gene:			
Beta Actin	5'- TCCCTGGAGAAGAGCTACG – 3'	5'- TAGTTTCGTGGATGCCACA -3'	130

MMuLV-RT. 20 µl of the PCR mix contained 1 x buffer with $MgCl_2$, 0.2 µl of Titanium Taq DNA polymerase, 200 µM of dNTPs (Finnzymes, Finland), 250nM of forward and reverse primers (Sigma Genosys, India), and 1:50,000 diluted SYBR green dye (Amersham, India). Thermal cycling was carried out with initial denaturation at 95˚C for 30 sec, followed by 45 cycles of denaturation at 94˚C for 30 sec, annealing at 58, 67.0, 64 and 69.0˚C respectively for actin, CXCL1, TLR2, TLR4 and TLR9, and extension at 72˚C for 30 sec and then the fluorescence was measured. Product specificity was confirmed by the presence of a single peak in the melting curve analysis. The fold difference of mRNA for each of these chemokines, relative to the house keeping gene beta actin, was calculated by normalizing the threshold cycle (Ct) values of chemokines and TLR with that of the housekeeping gene, actin using the Opticon 3.1 software program (Biorad, USA) on the Chromo 4 instrument.

*Statistical analysis***:** Significance of differences between groups was assessed using one way analysis of variance (ANOVA or Kruskal-Wallis test) with post-hoc tests (Tukey or Dunn's) for differences between individual groups. Two-tailed $P<0.05$ were considered statistically significant.

Results

IL-8 secretion induced by enteropathogenic bacteria: In HT29 cells, basal IL-8 secretion (10±1 pg/ml) was not increased by LGG (9±2 pg/ml). Infection of HT29 cells with enteropathogenic bacteria resulted in secretion of IL-8 into the medium. *V. cholerae* 0139 (3160±324 pg/ml) and 01 (2453±117 pg/ml) both induced IL-8 secretion compared to control or the commensal LGG ($P<0.001$). IL-8 secretion was also noted with *S.* Typhimurium (825±49 pg/ml, $P<0.01$), enterohaemorrhagic *E. coli* (642.14±110 pg/ml, $P<0.05$) and *S. flexneri* (149.6±23 pg/ml) ($P<0.05$ compared to control) (Fig. 1).

Three of the pathogens were also tested in T84 cells. *S.* Typhimurium led to the highest level of IL-8 secretion (665.41±76.9 pg/ml) ($P<0.001$ compared to control) followed by *V. cholerae* 0139 (208±38.1 pg/ml) ($P<0.05$ compared to control). The IL-8 response to *S. flexneri* (50.0±3.43 pg/ml) was quantitatively less than the other pathogens but was significantly ($P<0.01$) higher than control (8±2 pg/ml). LGG did not induce IL-8 secretion (Fig. 2).

*Effects of Lactobacillus on IL-8 secretion induced by enteropathogens***:** Induction of IL-8 secretion by

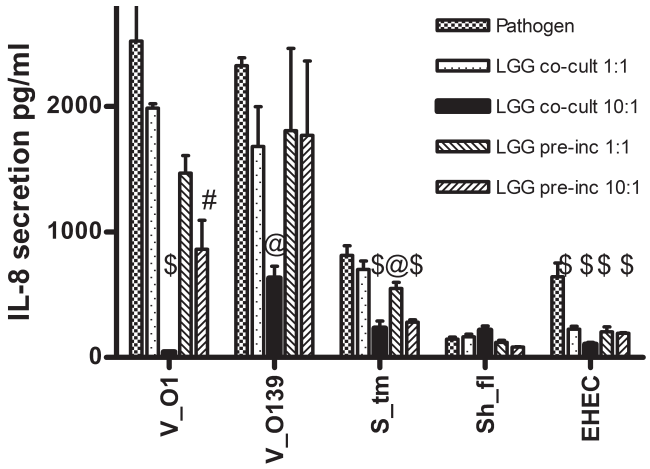

Fig. 1. Secretion of interleukin-8 (IL-8) into the medium by HT29 cell monolayers after 2 h exposure (followed by 4 h culture) to various bacterial pathogens and to Lactobacillus *GG* (LGG). V_O1=*V. cholerae* O1; V_O139=*V. cholerae* O139; S_tm=*S.* Typhimurium; Sh_fl=*Sh. flexneri*; EHEC=enterohaemorrhagic *Escherichia coli*. Co-cult = LGG and pathogen added simultaneously to cell monolayers. Pre-inc = LGG incubated with cell monolayers for 2 h and removed before adding pathogen for 2 h. The ratios 1:1 and 10:1 signify LGG:pathogen ratio. Values are mean ± SEM of at least 3 experiments with duplicate wells. @$P<0.05$ compared to pathogen alone; #$P<0.01$ compared to pathogen alone; and \$$P<0.005$ compared to pathogen alone.

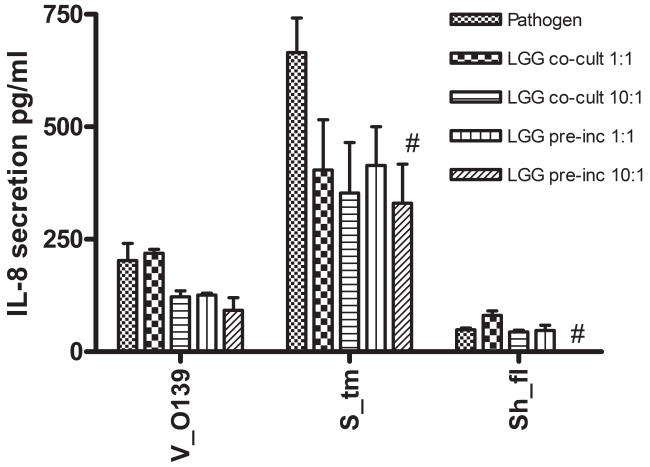

Fig. 2. Secretion of interleukin-8 (IL-8) into the medium by T84 cell monolayers after 2 h exposure (followed by 4 h culture) to various bacterial pathogens and to Lactobacillus GG (LGG). V_O139=*V. cholerae* O139; S_tm=*S.* Typhimurium; Sh_fl=*Sh. flexneri*. Co-cult = LGG and pathogen added simultaneously to cell monolayers. Pre-inc = LGG incubated with cell monolayers for 2 h and removed before adding pathogen for 2 h. The ratios 1:1 and 10:1 signify LGG:pathogen ratio. Values are mean ± SEM of at least 3 experiments with duplicate wells. #$P<0.01$ compared to pathogen alone.

the enteropathogens was modulated by the presence of commensal lactobacilli. LGG inhibited the IL-8 response of HT29 cells to *V. cholerae*, *S.* Typhimurium and EHEC, but not to *S. flexneri*. The effect of LGG was more distinctive when the commensal bacteria were present in higher numbers (10:1 compared to pathogen) and with co-culture more than with pre-incubation (Fig. 1). In T84 cells, LGG significantly inhibited IL-8 secretion caused by *S.* Typhimurium and *S. flexneri*, when pre-incubated with cells in high concentration (Fig. 2).

Comparison of LGG and L. plantarum: LGG and *L. plantarum* both produced similar changes in enteropathogen-induced IL-8 secretion, and these differences between the two *Lactobacillus* species were not significantly different (Fig. 3). Similar results (not shown) were obtained using T84 cells.

*Bacteria effect on gene expression of CXCL1, CCL5 and CXCL5***:** Significant upregulation of gene expression for CXCL1 was noted in response to *S.* Typhimurium, *S. flexneri* and EHEC (Fig. 4). Interestingly neither of the two strains of *V. cholerae* upregulated CXCL1 expression. The commensal bacterium LGG did not alter CXCL1 expression. None of the pathogens altered CCL5 expression in HT29 cells (Fig. 5). CXCL5 was not detectable by PCR in any of the cell cultures with or without pathogen incubation.

Gene expression of TLR 2, 4, 5 and 9 in HT29 and T84 cells: *S.* Typhimurium and *V. cholerae* O139 upregulated TLR4 mRNA expression in HT29 cells (Fig. 6). The increase in gene expression was attenuated by simultaneous exposure to LGG. Expression of TLR2, TLR5 and TLR9 did not significantly increase after exposure of HT29 cells to *S.* Typhimurium, but *V.*

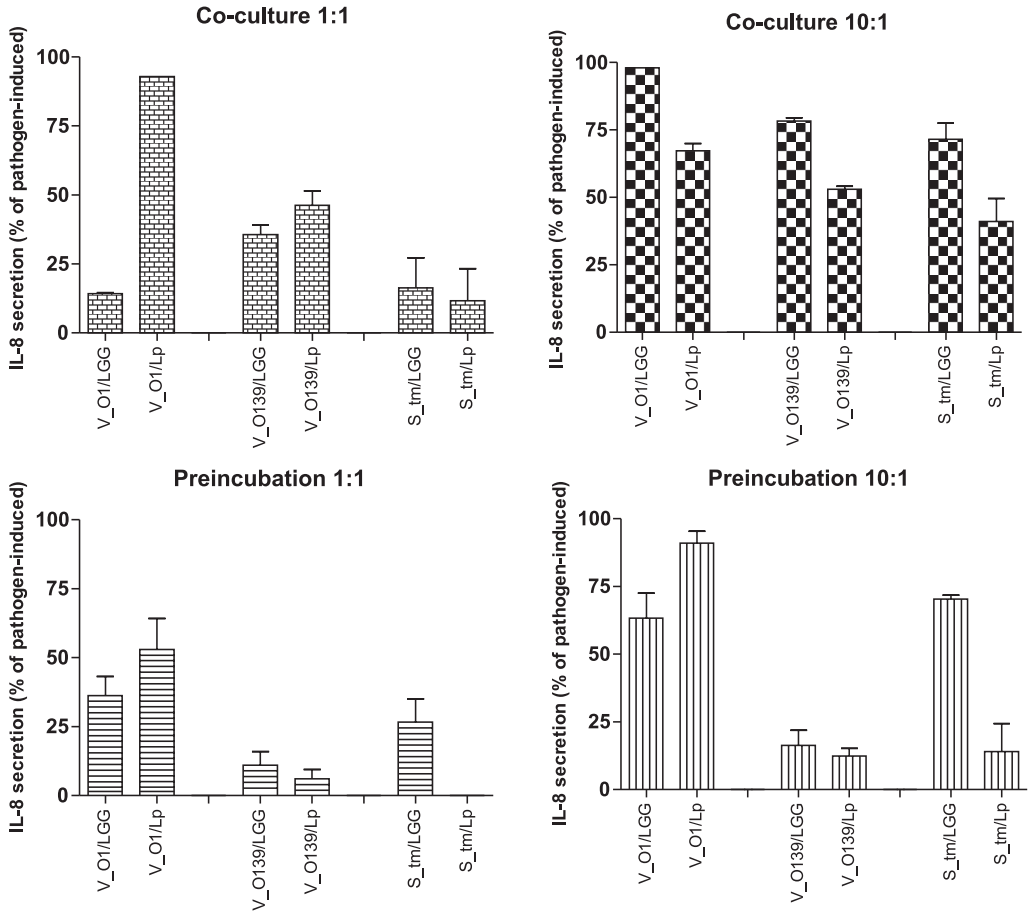

Fig. 3. Comparison of effect of *L. rhamnosus* GG (LGG) and *L. plantarum* (Lp) on interleukin-8 secretion from HT29 cells in response to enteropathogenic bacteria. HT29 cell monolayers were exposed to pathogen and lactobacilli either simultaneously (co-culture) for 2 h followed by 4 h culture after removal of bacteria, or first exposed to lactobacilli for 2 h and then to pathogen for 2 h followed by 4 h culture. V_O1=*V. cholerae* O1; V_O139=*V. cholerae* O139; S_tm=*S.* Typhimurium; The ratios 1:1 and 10:1 signify LGG: pathogen ratio. Values are mean ± SEM of at least 3 experiments with duplicate wells. #$P<0.01$ compared to pathogen alone. None of the differences was statistically significant, *i.e.*, both lactobacilli were equally effective in inhibiting pathogen-induced IL-8 secretion.

cholerae O139 reduced TLR5 mRNA levels (Fig. 6). *S. flexneri* did not induce any significant change in TLR expression while EHEC reduced expression of both TLR5 and TLR9 (Fig. 6).

Discussion

The present studies show that several bacterial pathogens induced IL-8 secretion and activation of CXCL1 in two intestinal epithelial cell lines representative of surface and crypt cell types.

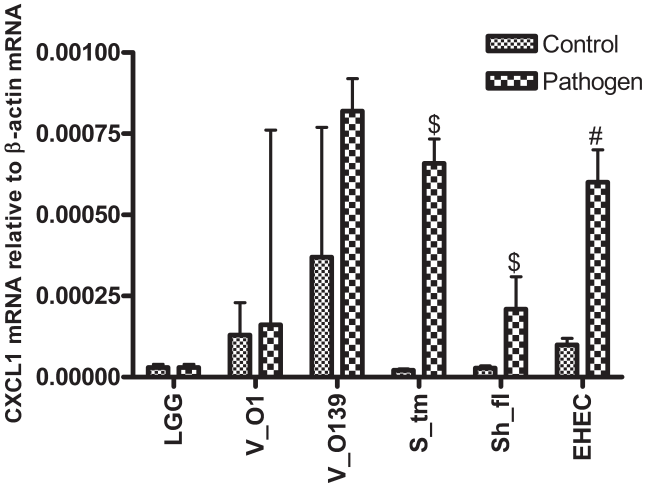

Fig. 4. Effect of pathogens on mRNA expression of CXCL1 in HT29 cells. LGG = *Lactobacillus* GG; V_O1=*V. cholerae* O1; V_O139=*V. cholerae* O139; S_tm=*S.* Typhimurium; Sh_fl=*Sh. flexneri*; EHEC=enterohaemorrhagic *Escherichia coli*. Values are mean ± SEM of 3 experiments in duplicate. #P<0.01 compared to corresponding control; \$$P$<0.005 compared to corresponding control.

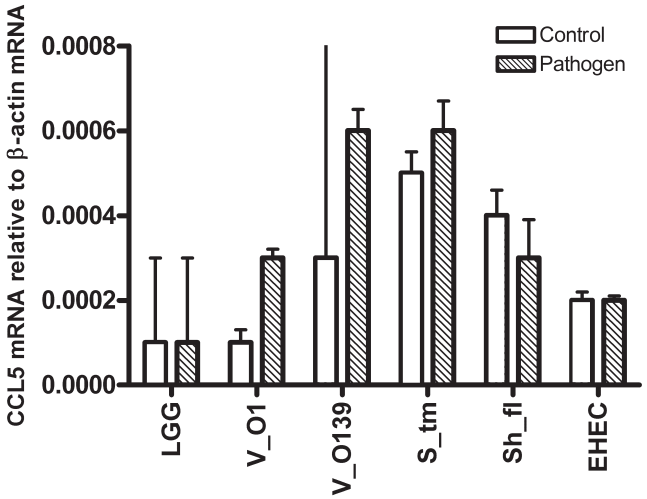

Fig. 5. Effect of pathogens on mRNA expression of CCL5 in HT29 cells. LGG = *Lactobacillus* GG; V_O1=*V. cholerae* O1; V_O139=*V. cholerae* O139; S_tm=*S.* Typhimurium; Sh_fl=*Sh. flexneri*; EHEC=enterohaemorrhagic *Escherichia coli*. Values are mean ± SEM of 3 experiments in duplicate. None of the differences was statistically significant.

Lactobacilli were shown to variably modulate the IL-8 response. The epithelium is the first line of defense against entry of enteropathogenic bacteria from the lumen of the intestine and colon, and releases mediators as part of the innate defenses of the host[21]. At the same time the commensal bacteria resident in the colon have a protective effect in maintaining mucosal immune tolerance[22].

Bacteria cause intestinal disease through a variety of different mechanisms. The rationale for these studies was to directly compare the ability of organisms using these different pathogenetic mechanisms to induce chemokine responses in the intestinal epithelium, using two cell lines as surrogates of the normal epithelium. While all the pathogenic bacteria tested induced secretion of IL-8 from HT29 and T84 cells, *V. cholerae* induced extremely high levels of IL-8 secretion from HT29 cells, but only a tenth as much from T84 cells. *Salmonella* induced equivalent levels of IL-8 secretion from both HT29 and T84 cells. The non invasive *V. cholera* induced a much more marked inflammatory mediator response in villus-like cells than in crypt-like cells. On the other hand, the invasive *Salmonella* stimulated IL-8 secretion from crypt-like cells in equal measure as from villus-like cells. This provides support to the suggestion that epithelial cells might recognize pathogenic microorganisms via their capability to access defined anatomical localizations, one such restricted site being the crypt[23]. *Vibrio* and *Salmonella* are both flagellated organisms and thus likely to elaborate flagellin that will be recognized by the innate immune system via TLR5[5,24]. In the present study, *V. cholerae* downregulated TLR5 expression in epithelial cells, while upregulating TLR4. The significance of this finding remains unclear. Studies in intestinal epithelial cell lines suggest that prolonged exposure to lipopolysaccharide (LPS) can result in tolerance to flagellin[25]. In monocytes and monocyte-macrophage cell lines, LPS induced a state of cross-tolerance to flagellin by blocking downstream signaling from TLR5. Thus, bacterial pathogens have evolved a variety of mechanisms to elude the immune system and favour their residence in the human host. The present demonstration that TLR5 expression was downregulated by *V. cholerae* is another facet of this survival ability of pathogens. The role of TLR4 in the chemokine response induced by *V. cholerae* remains to be elucidated. EHEC increased IL-8 secretion from the HT29 cell line. Although the effect of EHEC was not examined in T84 cells, induction of IL-8 secretion

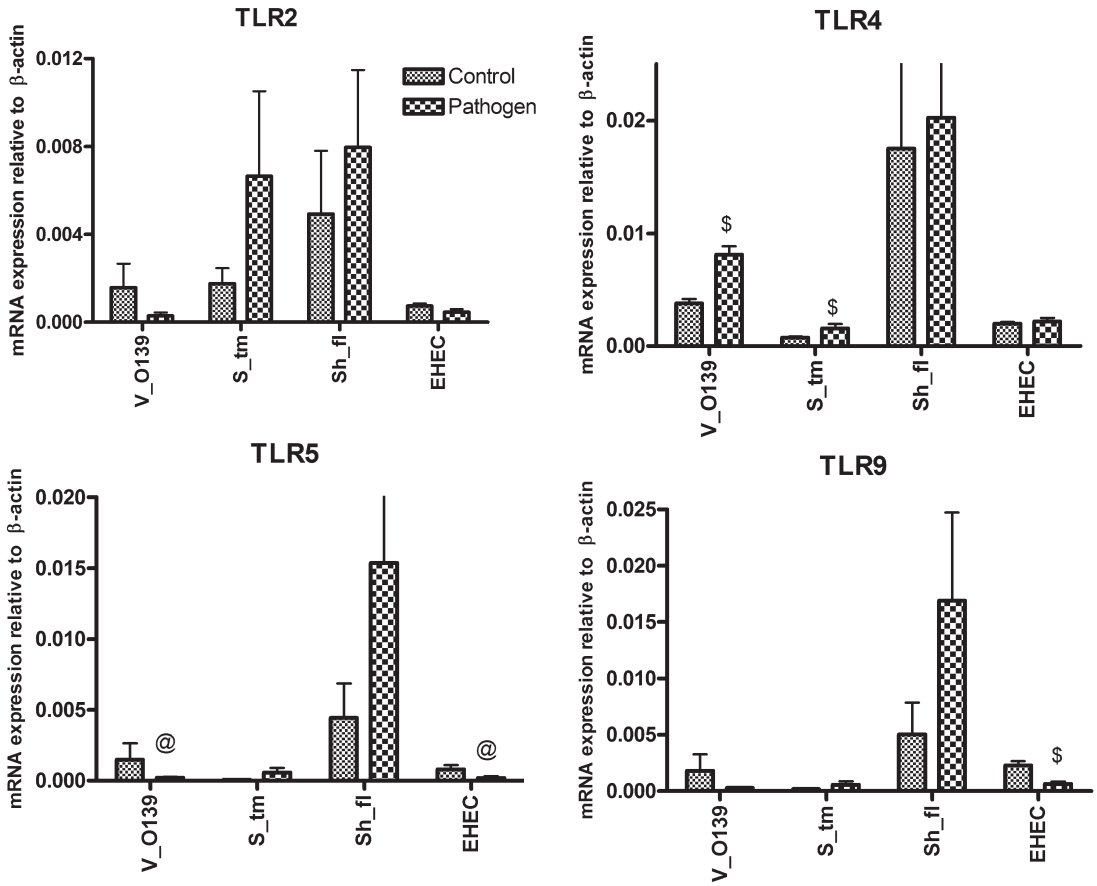

Fig. 6. Gene expression of the Toll-like receptors (TLR) 2, 4, 5 and 9 in HT29 cells in the basal state and after exposure to pathogenic bacteria. V_O139=*V. cholerae* O139; S_tm=*S.* Typhimurium; Sh_fl=*Sh. flexneri*; EHEC=enterohaemorrhagic *Escherichia coli*. All values shown are mean ± SEM of 3 experiments in duplicate, normalized to expression of beta-actin. @$P<0.05$ compared to control and \$$P<0.005$ compared to control.

by EHEC has been reported earlier in T84 cells[26]. *S. flexneri*, a non flagellated but invasive enteropathogen, induced IL-8 secretion in smaller amounts compared to the other enteropathogens. Since these bacteria lack flagellin, the predominant signal for IL-8 secretion comes via lipopolysaccharide[27] and this could be the reason for lower IL-8 secretion compared to the other bacteria. Also, in monocyte-macrophages, *Shigella* has been shown to induce apoptosis in the cells that then leads to reduction in pro-inflammatory cytokine production[28].

In this study, gene expression of CXCL1 was increased in response to the invasive and adherent bacteria but not to *V. cholerae*. The expression of CCL5, a chemoattractant of T lymphocytes, was not increased in response to any of the bacteria. Surprisingly, expression of epithelial neutrophil activating peptide (CXCL5) was not detected by PCR in either cell line before or after contact with bacteria. Enteropathogen-induced IL-8 secretion from epithelial cells was modulated by LGG and *L. plantarum*. These commensal probiotic bacteria inhibited the IL-8 response to all the pathogens studied with the exception of *Shigella*. Increasing the ratio of commensal to pathogenic bacteria resulted in a numerically (not statistically) greater inhibition. Both co-culture and preincubation with the commensal were effective in inhibiting IL-8 secretion. *L. reuterii* has been reported to reduce IL-8 secretion from HT29 and T84 cells in response to *S.* Typhimurium[29]. In this study both LGG and *L. plantarum* induced this anti-inflammatory response.

Chemokine gene expression induced by enteropathogenic bacteria in the epithelial cells was modulated by LGG. The modulatory effects on IL-8 gene expression paralleled the IL-8 secretory response. Attenuation of CXCL1 expression was mostly noted with higher concentrations of *Lactobacillus*. As the frontline of the mucosal immune system, the intestinal

epithelium is constantly exposed to large amounts of a variety of TLR ligands. The present study suggests that enteric pathogens upregulate or downregulate specific TLRs in epithelial cells and this is an additional mechanism that impacts on host immunity and disease development. These effects on TLR expression are likely to explain the differential effects of these bacteria on IL-8 secretion by intestinal epithelial cells.

In conclusion, bacterial enteric pathogens induced pro-inflammatory gene expression in intestinal epithelial cell lines leading to secretion of IL-8, a potent chemoattractant of neutrophils. Differences in their ability to elicit this response may depend on both epithelial cell type and the specific pathogen. Alteration of TLR expression in epithelial cells by enteric pathogens may be of importance in the genesis of the specific chemokine response. Lactobacilli prevented the activation of pro-inflammatory genes and secretion of cytokines. Although the effects of lactobacilli in diarrhoea are most clearly documented for LGG and in the case of rotavirus diarrhoea, it is likely that probiotic lactobacilli will have roles in preventing and ameliorating other diarrhoeal illnesses. The effect of lactobacilli in antagonizing epithelial cell chemokine expression by pathogenic bacteria may be of relevance to their therapeutic efficacy in preventing or ameliorating infective diarrhoea.

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