Impact of selected *Lactobacillus* and *Bifidobacterium* species on *Listeria monocytogenes* infection and the mucosal immune response

Sinead C. Corr1, Cormac G.M. Gahan1,2 & Colin Hill1

1Department of Microbiology and Alimentary Pharmabiotic Centre, University College Cork, Ireland; and 2School of Pharmacy, University College Cork, Ireland

Correspondence: Cormac G.M. Gahan, Department of Microbiology, University College Cork, College Road, Cork, Ireland. Tel.: +353214901363; fax: +353214903101; e-mail: c.gahan@ucc.ie

Received 18 December 2006; revised 15 March 2007; accepted 30 March 2007. First published online 31 May 2007.
DOI:10.1111/j.1574-695X.2007.00264.x

Editor: Alex van Belkum

Keywords
*Listeria monocytogenes*; probiotic; intestinal cells.

Abstract

The pathogenesis of *Listeria monocytogenes* depends on its ability to attach to and invade the gastrointestinal epithelium and subsequently withstand the host immune response. Despite a thorough understanding of the intracellular phase of infection, relatively little is known about how the pathogen behaves in the gastrointestinal tract and whether it is affected by the presence of host commensal microbiota. *Lactobacillus* and *Bifidobacterium* are two important genera of the human gut microbiota proposed to possess probiotic effects. Here we demonstrate that probiotic bacteria significantly inhibit subsequent listerial infection in an *in vitro* C2Bbe1 epithelial cell model. In the case of *Lactobacilli*, inhibition was due to a combination of acid production and secretion of an as yet unidentified protein. In the case of *Bifidobacterium*, inhibition was attributable to an extracellular proteinaceous secreted compound. In addition, we observed a significant reduction in interleukin-8 and an increase in IL-10 cytokines secreted from epithelial cells following probiotic pretreatment and subsequent infection with *Listeria*. A reduction in the infection of epithelial cells and an altered mucosal immune response suggests that probiotic bacteria could be of therapeutic benefit against listerial infection. This study infers a role for probiotic bacteria as an antagonist of *Li. monocytogenes* infection.

Introduction

The gastrointestinal tract is a complex ecosystem which is likely to contain bacteria with both detrimental and beneficial effects on host physiology. Recently there has been significant research interest in the role of the gut microbiota in influencing the health status of the host. This research work is necessary to inform the deliberate use of bacterial supplements to manipulate the microbiota in a manner that could potentially assist in maintaining health and preventing disease (Holzapfel et al., 1998). Probiotics (defined as any bacterium that when administered to human or animal hosts has health-promoting effects) could offer an alternative to conventional therapies such as antibiotics for the prophylaxis or treatment of intestinal infections (Bourlioux et al., 2003). *Lactobacillus* and *Bifidobacterium*, members of the normal gastrointestinal microbiota, are among the most thoroughly characterized probiotic candidates and have been incorporated into many different foods, including fermented dairy products (Guerin-Danan et al., 1998).

*Lactobacillus* spp. are the most widely used isolates in commercial probiotic products, with strains of *Lactobacillus acidophilus* and *Lactobacillus casei* strain Shirota being utilized due to their associated health benefits. Probiotics are presumed to modulate the indigenous intestinal microbiota and improve health via multiple mechanisms of action, including the direct inhibition of enteric pathogens by decreasing luminal pH, the secretion of bactericidal proteins and the stimulation of defensin production by epithelial cells (Sartor, 2004). They may also block pathogen attachment or invasion of epithelial cells by competing for surface receptors, a process termed colonization resistance (Salminen et al., 1996; Mack et al., 1999; Mattar et al., 2002). In addition it has been shown that *Lactobacillus plantarum* and *Lactobacillus rhamnosus* inhibit adherence of enteropathogenic *Escherichia coli* to HT29 cells by inducing expression of the mucin genes, *muc2* and *muc3* (Mack et al., 2003). Probiotic bacteria also improve epithelial and mucosal barrier function via release of short-chain fatty acids such as butyrate, a by-product of
bacterial fermentation (Cook & Sellin, 1998). Finally, probiotic bacteria have also been shown to alter host immune responses to infection by inducing expression of the anti-inflammatory cytokine interleukin-10 (IL-10), decreasing expression of pro-inflammatory interferon-γ (IFN-γ) and stimulating secretory IgA production (Fukushima et al., 1998; Silva et al., 2004; Di Giacinto et al., 2005).

Listeria monocytogenes is a gram-positive food-borne pathogen that invades through the intestinal epithelium, causing the potentially fatal disease, listeriosis. Listeria monocytogenes is the fourth most common cause of meningal infection, and it can also cause bacteraemia, septicemia or a food-borne febrile gastroenteritis syndrome (Vázquez-Boland et al., 2001). Listeria monocytogenes predominately affects pregnant, newborn and immunocompromised individuals, with a mortality rate of 20% or higher. In addition, Li. monocytogenes has long been used as a model organism for the study of intracellular parasitism and the associated immune response. Although much information is available regarding the infection of intestinal epithelial cells by Li. monocytogenes and subsequent systemic infection, little is known about how the bacterium interacts with the normal gut microbiota.

This study provides evidence that pretreating epithelial cells with strains of Bifidobacterium and Lactobacillus can significantly interfere with subsequent invasion by Li. monocytogenes, a process that is influenced by secreted products of probiotic bacterial cells. In addition, we demonstrate that the interaction between probiotic organisms and mucosal epithelial cells significantly impacts upon the subsequent host immune response.

Materials and methods

Bacterial strains, media and growth conditions

Listeria monocytogenes strains were grown in brain heart infusion broth (Oxoid) aerobically at 37°C. Lactobacillus spp. were grown in MRS broth (Oxoid) for 36 h and then subcultured for a further 24 h. Lactobacillus salivarius strain UCC118 was grown aerobically at 37°C. Lactobacillus acidophilus strain NCDO 1748 and La. casei strain NCDO 1205 were grown anaerobically at 37°C. Lactococcus lactis strain NZ9000 was grown aerobically in GM17 broth at 30°C overnight. Bifidobacterium spp. including Bifidobacterium breve UCC2003, Bifidobacterium infantis CCUG36569 and Bifidobacterium longum JCM7050 were grown in Reinforced Clostridial medium (RCM) for 36 h and then subcultured into MRS broth supplemented with cysteine–HCl for a further 24 h. All Bifidobacteria strains were incubated anaerobically at 37°C.

C2Bbe1 cell culture conditions

C2Bbe1 cells, a clone of the Caco-2 human adenocarcinoma cell line, were used for in vitro assays (ATCC, CRC-2102) (Peterson & Mooseker, 1992). C2Bbe1 cells have brush borders homogeneous to intestinal enterocytes. Cells were maintained in Dulbecco’s modified Eagle’s minimal essential medium (DMEM) with glutamax-1, 4500 mg ml⁻¹ glucose, Gibco) and supplemented with 10% fetal bovine serum (FCS, Gibco), 1% nonessential amino acids (Gibco), 1% penicillin–streptomycin (Gibco) and human transferrin (0.01 mg ml⁻¹, Calbiochem) at 37°C in a 5% CO₂ atmosphere (Forma Scientific). Cells were passaged (1:4 dilution) just before they reached confluency (~5 days) and the medium was changed every 2 days. For in vitro assays, C2Bbe1 cells were trypsinized, centrifuged and the pellet resuspended in 1 mL antibiotic-free DMEM/10% FCS. Cells were seeded onto a flat-bottomed tissue-culture 24-well plate (Sarstedt) at a concentration of 3 × 10⁵ cells well⁻¹ (1 mL total volume per well). Plates were incubated for 72 h at 37°C with 5% CO₂ until confluent.

Pretreatment of monolayers with probiotic bacteria and gentamycin infection assay

Monolayers were incubated with probiotic bacteria in antibiotic-free medium (1 mL total volume per well) at a concentration of 1 × 10⁸ CFU well⁻¹ for 2 h (Fig. 1a). This medium containing the probiotic bacteria was then removed, monolayers washed three times with 1 × PBS and Li. monocytogenes was added at 1 × 10⁸ CFU well⁻¹ in fresh antibiotic-free medium (1 mL total volume per well). Plates were incubated at 37°C with 5% CO₂ for 1 h. Medium containing Li. monocytogenes was then replaced with 1 mL DMEM/10% FCS containing gentamycin at a concentration of 100 μg well⁻¹ for 1 h. Monolayers were then washed three times with 1 mL 1 × phosphate-buffered saline (PBS) and lysed with 100 μL ice-cold sterile dH₂O. Serial dilutions were performed and plated onto brain heart infusion agar plates (Oxoid) to determine CFU per milliliter of invaded bacteria. Agar plates were incubated overnight at 37°C. The gentamycin invasion assay was repeated with La. casei NCDO1205 using differentiated C2Bbe1 monolayers to determine whether a probiotic pretreatment could still protect against listerial infection of differentiated monolayers (Fig. 1b). Differentiated epithelial cells display features of differentiated intestinal cells, including expression of a brush border (Peterson & Mooseker, 1992).

Pretreatment of monolayers with probiotic bacteria contained in impermeable chambers

To determine if an active soluble compound caused the effect on listerial invasion, impermeable millicell chambers...
Pretreatment of monolayers with probiotic supernatants

To determine the nature of the active soluble compound, overnight cultures of bacteria were centrifuged (5000 g for 10 min) and cell-free supernatants obtained. Supernatants were either untreated (~pH 4–5), neutralized to pH 7 with NaOH, or treated with trypsin for 30 min to degrade proteins (Fig. 2b–d). Then, 250-µl volumes of supernatants were added to tissue culture wells (1 mL total volume of media per well) and incubated for 2 h at 37 °C in 5% CO₂. Following incubation, media containing the probiotic supernatants were removed, monolayers were washed three times with 1 mL 1 × PBS and Li. monocytogenes was added at 1 × 10⁸ CFU well⁻¹ in fresh antibiotic-free medium (1 mL total volume per well). Plates were then incubated at 37 °C with 5% CO₂ for 1 h. Following incubation, the gentamycin treatments were performed as described above and CFU per milliliter of Li. monocytogenes which invaded epithelial monolayers was determined. The assay was repeated using pretreatment of monolayers with 250 µL MRS broth, trypsin or culture media at pH 7 to exclude any effect of these treatments on invasion by Listeria (Fig. 2e).

Stimulation of C2Bbe1 monolayers and enzyme-linked immunosorbent assay (ELISA) measurement of cytokine production

To determine if prior exposure of C2Bbe1 monolayers to probiotic bacteria influences the mucosal immune response to Li. monocytogenes, monolayers were incubated with probiotic bacteria at a concentration of 1 × 10⁸ CFU well⁻¹ (1 mL total volume DMEM per well) for 2 h (Fig. 3a and b). Monolayers were then washed three times with 1 mL 1 × PBS and Li. monocytogenes was added at 1 × 10⁸ CFU well⁻¹ in fresh antibiotic-free medium (1 mL total volume per well). Plates were then incubated at 37 °C with 5% CO₂ for 8 h. Following incubation, supernatants were collected and centrifuged at 13000 g for 8 min, transferred to clean microcentrifuge tubes and stored at −80 °C until analysis of cytokines using Duoset ELISA Development kits (R&D Systems). This assay was repeated using initial inoculums of 1 × 10⁷ and 1 × 10⁸ CFU well⁻¹ Li. monocytogenes EGDe so as to control for intracellular numbers present after probiotic pretreatment, due to the observed 1log reduction in Listeria invasion following probiotic pretreatments (Fig. 3c). For human IL-8 and IL-10 measurement, highest standards used were 2000 and 3000 pg mL⁻¹, respectively. Kit protocols were followed and plates were analysed using SOFTPRO-MAX software. Cytokine analysis was also performed on supernatants stimulated with probiotic bacteria alone as a control.

Fig. 1. Invasion of C2Bbe1 epithelial monolayers by Li. monocytogenes EGDe following (a) pretreatment of monolayers with whole Lactobacilli and Bifidobacteria (pretreated monolayers shown by open bars, untreated monolayers shown by solid bars), (b) pretreatment of undifferentiated and differentiated C2Bbe1 monolayers with L. casei NCD01205 (open bars) and effect on subsequent invasion by Li. monocytogenes EGDe. Untreated monolayers are shown by solid bars.

Fig. 2. (a) Effect of probiotic pretreatment of C2Bbe1 monolayers on invasion of Li. monocytogenes EGDe. Untreated monolayers are shown by solid bars. Pretreatment of undifferentiated and differentiated C2Bbe1 monolayers with probiotic bacteria at a concentration of 1 × 10⁸ CFU well⁻¹, (pretreated monolayers shown by open bars, untreated controls, n = 3).

(b) Pretreatment of undifferentiated and differentiated C2Bbe1 monolayers with L. casei NCD01205 (pretreated monolayers shown by open bars, untreated controls, n = 3).

© 2007 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved
Fig. 2. Investigation of the nature of the inhibitory effect. Invasion of C2Bbe1 epithelial monolayers by *L. monocytogenes* EGDe following (a) pretreatment of monolayers with probiotics contained in impermeable millicell chambers, (b) pretreatment with bacterial supernatants at pH 4, (c) pretreatment with neutralized bacterial supernatants, and (d) pretreatment with protein-degraded (trypsin-treated) bacterial supernatants (at ~pH 4). (e) Invasion assay to determine effect of pretreatments with MRS broth (pH 4–5.5), trypsin (used for protein degradation of probiotic supernatants) or media at pH 7 (pH of neutralized probiotic supernatants) on invasion of C2Bbe1 epithelial monolayers. Pretreated monolayers shown by open bars, untreated monolayer shown by solid bar. *P* < 0.05, indicating statistically significant difference between numbers of *Listeria* infecting pretreated monolayers compared with untreated controls, *n* = 3.
Statistical analysis

For in vitro assays, bacterial CFU determinations were expressed as percentage of *Li. monocytogenes* invading untreated monolayers. This permits comparison of assays performed on different days. Numerical results are given as arithmetic means ± SDs of the means. Student’s *t*-test was performed to test statistical significance at *P* < 0.05 for three determinations, unless otherwise stated.

Results

**Probiotic bacteria interfere with invasion of *Li. monocytogenes* EGDe in human epithelial monolayers**

The gentamycin invasion assay was used to assess the invasion efficiency of *Li. monocytogenes* EGDe into the human colon carcinoma Caco-2 epithelial cell clone, C2Bbe1, with and without pretreatment with probiotic bacteria (Fig. 1a). Strains of *Lactobacilli* and *Bifidobacteria* were used, while a strain of *Lactococcus lactis* was also included as a control nonprobiotic strain (insofar as any organism can be said to be unlikely to be a probiotic, lacticocci are not normal members of the human gut microbiota and do not survive well in the gastrointestinal tract). Approximately 3 × 10⁶–3 × 10⁷ CFU mL⁻¹ *Listeria* invaded untreated monolayers. *Listeria monocytogenes* showed significantly decreased infection of epithelial monolayers following pre-exposure of epithelial monolayers to all bacteria, with reductions of between 60% and 90% in relative invasion efficiency (Fig. 1a). While in this assay system *Lactococcus* performed as well as the probiotic strains, it should be noted that this organism is highly unlikely to reach the epithelium in vivo in high numbers or in a viable state. Differentiated epithelial cells display features of differentiated intestinal cells, including expression of a brush border (Peterson & Mooseker, 1992). However, there was no difference observed between undifferentiated and differentiated C2Bbe1 monolayers on inhibition of *Li. monocytogenes* invasion following pretreatment with *L. casei* NCDO1205 (Fig. 1b). For this reason, undifferentiated C2Bbe1 monolayers were used for the remainder of the experiments.

**Involvement of a secreted soluble component**

To investigate the mode of protection further, gentamycin infection assays were performed in which the probiotic bacteria were separated from epithelial monolayers by an impermeable membrane during pretreatment (Fig. 2a). The millicell chambers containing probiotics were then removed, the monolayers washed and *Li. monocytogenes* EGDe added directly to the wells containing epithelial monolayers.

![Graph showing effect of pretreatment of C2Bbe1 epithelial monolayers with probiotic on production of (a) proinflammatory cytokine IL-8 and (b) anti-inflammatory cytokine IL-10, following subsequent infection with *Li. monocytogenes* EGDe.](image-url)
All probiotic bacteria except Lactococcus lactis strain NZ9000 were capable of inhibiting L. monocytogenes invasion (Fig. 2a), essentially to the same extent as in the assays performed without separation (Fig. 1a). Therefore, we concluded that interference with listerial infection does not solely rely on direct physical contact with the epithelial monolayer but involves a secreted soluble component. As Lactococcus lactis strain NZ9000 was unable to cause inhibition when separated from the enterocytes, we concluded that it does not secrete an active component affecting L. monocytogenes invasion.

To determine if the inhibitory effect was due to the reduction in pH associated with the growth of the probiotic bacteria, gentamycin infection assays were performed after pretreatment of epithelial monolayers with cell-free supernatants (~pH 4–5) and neutralized supernatants (Fig. 2b and c). Inhibition of invasion was observed with the bifidobacterial supernatants; however, in the case of Lactobacilli, neutralizing the supernatants eliminated this interference (Fig. 2b and c). This might initially suggest that inhibition by Lactobacilli is due to acid, but proteinase-treated supernatants (at pH 4) also had no effect on invasion (Fig. 2d). As a control for an acid effect on invasion, monolayers were pretreated with culture media at pH 7, but this did not interfere with the ability of L. monocytogenes to invade epithelial cells (Fig. 2e). This suggests that interference with infection by Lactobacilli was due to a secreted proteinaceous molecule active at low pH of the probiotic supernatant. In the case of the Bifidobacteria, neutralized supernatants still provided significant inhibition of listerial invasion, while trypsinized supernatants lost this interference (Fig. 2c and d). Thus, in the case of Bifidobacteria, the observed inhibitory effect appears to be due to a secreted protein factor and not due to low pH. Pretreatment of monolayers with MRS broth (pH 4–5), trypsin or culture media at pH 7 did not affect the ability of L. monocytogenes EGDe to invade epithelial monolayers (Fig. 2e).

Probiotic bacteria alter the host mucosal immune response to listerial infection

Assays were performed to assess the host response to the different probiotic bacteria used in this study (Fig. 3a and b). Treatment of C2Bbe1 monolayers with all probiotic strains for 2 h before stimulation with L. monocytogenes EGDe for a further 8 h significantly reduced proinflammatory IL-8 and increased anti-inflammatory IL-10 production (Fig. 3a and b). There was no detectable IL-8 or IL-10 following stimulation of monolayers with probiotic bacteria only or in monolayers without bacterial treatment (Fig. 3a and b). Infection of monolayers with 1 × 10^8 and 1 × 10^7 CFU well^−1 L. monocytogenes EGDe was performed as a control for intracellular numbers of L. monocytogenes and there was no difference in level of cytokine secreted in either case (Fig. 3c). These results demonstrate that probiotic bacteria can modulate the immune response to listerial infection.

Discussion

This study demonstrates that a variety of probiotic commensal organisms can significantly inhibit L. monocytogenes infection in vitro. Protection of the host gastrointestinal tract from invading pathogens is an important role postulated for probiotic bacteria. Previous studies have shown that probiotic bacteria can inhibit the ability of pathogenic bacteria to interact with intestinal epithelial cells (Bernet et al., 1993; Mack et al., 1999; Resta-Lenert & Barrett, 2003; Sgouras et al., 2004; Tsai et al., 2004). The probiotic E. coli strain Nissle 1917 was shown to interfere with the invasion of intestinal INT407 cells by Salmonella enterica serovar Typhimurium, Yersinia enterocolitica, Shigella flexneri, Legionella pneumophila and L. monocytogenes (Altenhoefer et al., 2004). Lactobacillus casei DN-114 001 inhibits adherence and invasion of intestinal epithelial cells by pathogenic adherent-invasive E. coli (AIEC), a bacterium found to colonize ileal lesions in some Crohn’s patients (Ingrassia et al., 2005). While probiotic bacteria have been found to have beneficial effects on the outcome of certain infections, this has never been specifically investigated in the case of L. monocytogenes.

The present study examined the ability of different probiotic bacteria to inhibit listerial infection of enterocytes, an important step in Listeria pathogenesis (Vázquez-Boland et al., 2001). In this study, we showed that pretreatment of intestinal epithelial cells with probiotic bacteria prior to infection with L. monocytogenes EGDe resulted in a significant decrease in listerial invasion (60–90%). It is important to note that probiotic bacteria or bacterial supernatants were only in contact with the epithelial monolayer and never came into contact with Listeria. Furthermore, when probiotic bacteria were separated from epithelial monolayers by impermeable Millicell chambers, the anti-invasive activity remained. Thus, this anti-invasive activity is independent of direct physical contact with both the intestinal monolayer and L. monocytogenes. Therefore, for all strains, physical blocking of epithelial surface receptors was not the primary mechanism of action. In addition, Lactococcus lactis NZ9000 did not inhibit Listeria invasion when separated from the enterocytes, confirming that not all bacterial strains have this ability. Upon further characterization of this effect, probiotic bacteria were shown to inhibit invasion through secretion of a proteinaceous compound. In the case of the Lactobacilli, this compound (or compounds) was active at low pH. In the case of the

FEMS Immunol Med Microbiol 50 (2007) 380–388 © 2007 Federation of European Microbiological Societies Published by Blackwell Publishing Ltd. All rights reserved
Bifidobacteria, inhibition was not due to an acid effect but rather a secreted proteinaceous factor.

Our work supports previous studies of probiotic inhibition of other pathogens. Strains of Lactobacillus and Bifidobacterium have been shown to impede infection of human intestinal cell lines by E. coli and cell-free culture supernatants from probiotic bacteria significantly reduced numbers of culturable E. coli and the invasiveness of this strain (Gopal et al., 2001). This inhibitory action was proposed to be due to a synergistic action of lactic acid and proteinaceous substances. Furthermore, Lactobacilli have been shown to have multifactorial inhibition against Salmonella in vitro (Fayol-Messaoudi et al., 2005). Similarly, we also observed pH and nonacid-dependent activities of Lactobacilli against Listeria infection of intestinal epithelial cells. Future work in our laboratory is aimed at identification of the exact nature of these secreted proteins and their role in reducing infection.

In this study, the observed probiotic effect is mediated via interaction of the secreted component with the epithelial monolayer, and not via interaction with the invading Listeria. A possible mechanism for this effect may include enhancement of epithelial barrier function through induction of mucin secretion and maintenance of tight junctions and cytoskeletal integrity (O’Hara & Shanahan, 2007). Probiotics have been found to enhance expression of transmembrane and tight junction proteins, resulting in improved barrier function and reduced bacterial translocation (Qin et al., 2005; Shen et al., 2006). Studies have shown that probiotic bacteria can inhibit invasion by pathogenic bacteria through induction of mucin expression, thereby blocking bacterial attachment to intestinal cells (Mack et al., 1999, 2003; Mattar et al., 2002). Further analysis is currently ongoing in our laboratory to determine whether probiotic bacteria inhibit listerial infection in vitro via mucin overexpression or enhanced barrier integrity.

The cell culture model of infection used in this analysis is a robust and tractable in vitro model that provides for initial analysis of host–microbe interactions in a controlled environment. We recognize that the true in vivo environment is clearly more complex. It is apparent that commensal bacteria in situ are located primarily in the lumen of the gastrointestinal tract whilst the mucous overlying epithelial cells, particularly in the villous crypts, contain relatively few microorganisms (Muller et al., 2005). However, both local inflammation and immune sampling of commensal organisms are likely to increase direct contact between the microbiota and host epithelia (Muller et al., 2005; O’Hara & Shanahan, 2007) and would potentially elicit the effects demonstrated in the current study. In addition, by separating commensal bacteria from epithelial cells using Millipore chambers, we clearly demonstrate that direct contact between bacteria and the monolayer is not necessary to influence listerial invasion.

Finally, we have shown that pretreatment of C2Bbe1 monolayers with Lactobacilli or Bifidobacteria significantly reduces the amount of proinflammatory IL-8 produced in response to listerial infection. Pretreatment of monolayers also significantly increased the anti-inflammatory cytokine IL-10 following infection by Li. monocytogenes. This shows that probiotic commensal organisms may regulate the local immune response to Li. monocytogenes infection in the gastrointestinal tract. In the present study, Bifidobacteria and Lactobacilli alone do not induce production of either IL-8 or IL-10 cytokines from epithelial cells. This demonstrates that epithelial cells respond differently to various bacterial species, with the intestinal epithelium remaining immunologically quiescent upon encountering probiotic commensal bacteria (Kelly et al., 2005; O’Hara et al., 2006). Probiotics have been shown to play a role in the development of disease by altering the host mucosal immune response to infection (O’Mahony et al., 2001; Menard et al., 2004). In a previous study, B. infantis and La. salivarius failed to induce IL-8 secretion from epithelial cells but modulated the immune response to S. typhimurium (O’Hara et al., 2006). Bifidobacterium breve and Streptococcus thermophilus secrete metabolites which inhibit lipopolysaccharide-induced TNF-α secretion from peripheral blood mononuclear cell (PBMC) monolayers (Menard et al., 2004). Lactobacillus salivarius ssp. salivarius UCC118 has been shown to reduce tumour development in IL-10-knockout mice (O’Mahony et al., 2001), while the probiotic mixture, VSL#3, when administered to mice with Th-1-mediated colitis, induced anti-inflammatory cytokine IL-10, thus reducing inflammation and mortality (Di Giacinto et al., 2005). This may be an important consideration in the treatment of chronic inflammatory conditions of the gastrointestinal tract that may be influenced by local immune responses (Krammer et al., 2005). Possible mechanisms by which probiotic bacteria alter the host immune response to infection include attenuation of NFκB activation via Toll-like receptor signalling or inhibition of IκB-α ubiquitination, and thus altered expression of target genes (Neish et al., 2000; Kelly et al., 2005; O’Hara et al., 2006; Riedel et al., 2006).

In conclusion, this study demonstrates that probiotic bacteria may have potential for the prophylaxis or treatment of listeriosis. The results of the invasion experiments clearly show the ability of probiotics to impede invasion by Li. monocytogenes significantly. The secreted component (or components) that prevents listerial invasion of intestinal epithelial cells warrants further investigation. Work is in progress to determine whether this anti-invasive activity is due to overexpression of mucin or whether other mechanisms may be involved. Future work using microarray studies examining the global cellular response to Li. monocytogenes infection will consider the influence of the indigenous microbiota upon the host response to infection.
Acknowledgements

This work was supported by a grant from the Higher Education Authority (PRLIT3) and through funding of the Alimentary Pharmabiotic Centre by the Science Foundation of Ireland.

References


