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International Journal of Food Microbiology 110 (2006) 62–68
INTERNATIONAL JOURNAL OF Food Microbiology
www.elsevier.com/locate/ijfoodmicro

Interaction of bifidobacteria with Caco-2 cells—adhesion and impact on expression profiles

Christian U. Riedel [a,b,*], Francis Foata [b], Darlene R. Goldstein [c], Stephanie Blum [b], Bernhard J. Eikmanns [a]

[a] *Department of Microbiology and Biotechnology, University of Ulm, 89068 Ulm, Germany*
[b] *Immunology Group, Nutrition and Health Department, Nestlé Research Center, PO Box 44, CH-1006 Lausanne, Switzerland*
[c] *Institut de Mathématiques, École Polytechnique Fédérale de Lausanne, Bâtiment MA, Station 8, 1015 Lausanne, Switzerland*

Received 13 July 2005; received in revised form 2 December 2005; accepted 15 January 2006

Abstract

The aim of the present study was to study different strains of bifidobacteria for adhesion to Caco-2 intestinal epithelial cells (IECs) and to test for the mRNA response of these cells following interaction with bifidobacteria. Adhesion was tested at different pH conditions using model epithelia consisting of transwell cultures of fully differentiated Caco-2 cells. Microarrays were used to characterize changes in global expression profiles of Caco-2 cells co-cultured with peripheral blood mononuclear cells (PBMCs) and challenged with non-pathogenic Escherichia coli D2241 or four different strains of bifidobacteria. Furthermore, cytokine mRNA of IECs in responses to challenge with *Bifidobacterium bifidum* S17 or *E. coli* D2241 was tested in PBMC-sensitised Caco-2 cells using RT-PCR. Bifidobacteria showed strain-specific adhesion to Caco-2. Shift of apical pH from 7 to 4.5 resulted in strain-specific changes of adhesion. Global expression profiles of PBMC-sensitised Caco-2 cells revealed differential expression of a significant number of genes only after challenge with *E. coli* D2241 while cells were essentially unresponsive to challenge with four strains of bifidobacteria showing different adhesion properties. Using a RT-PCR approach, in the same system a similar differential expression after challenge with *E. coli* D2241 or *B. bifidum* S17 was observed for various immune markers. The presented results suggest that Caco-2 cells might be specifically unresponsive to challenge with bifidobacteria irrespective of the level of adhesion.

Keywords: Bifidobacteria; Adhesion; intestinal epithelial cells

1. Introduction

Bifidobacteria are Gram-positive, anaerobic microorganisms that inhabit mainly the colon of healthy infants and adults. Depending on the methods used for detection, their portion of the total microbial count varies but they undoubtedly constitute one of the predominant species of the human colonic and faecal microflora (Guarner and Malagelada, 2003). Using fluorescent in situ hybridisation, up to 90% of all bacteria in faecal samples of breast fed infants and 3–5% of the adult faecal microflora were found to represent bifidobacteria (Harmsen et al., 2000; Harmsen et al., 2002).

Generally, adhesion to IECs is regarded as one of the main criteria for the selection of probiotic lactobacilli and bifidobacteria as it might contribute to the inhibitory action of probiotics towards colonization of pathogenic bacteria and is thought to help the probiotic to persist in the intestine for a prolonged period of time (Simmering and Blaut, 2001; Tuomola et al., 2001). Caco-2 intestinal epithelial cells grow to fully differentiated monolayers (Pinto et al., 1983) and are frequently used to study bacterial adhesion properties and inflammatory reactions of IECs. In a recent publication (Haller et al., 2000) it was demonstrated that in Caco-2/PBMC co-cultures species from the genus *Lactobacillus* as well as pathogenic and non-pathogenic *Escherichia coli* elicited differential cytokine responses in the IEC line. Basolateral PBMCs were required for this differential response. Moreover, the probiotic strain (*Lactobacillus johnsonii* La1) did not induce secretion of proinflammatory cytokines. Adhesion of lactobacilli to cultured

* Corresponding author. Alimentary Pharmabiotic Centre and Microbiology Department, University College Cork, Cork, Ireland. Tel.: +353 21 490 1388; fax: +353 21 490 3101.
E-mail address: c.riedel@ucc.ie (C.U. Riedel).

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doi:10.1016/j.ijfoodmicro.2006.01.040

intestinal epithelial cells has been studied extensively (Jacobsen et al., 1999; Tuomola and Salminen, 1998) and some of the mechanisms of adhesion of lactobacilli have been studied in detail (Adlerberth et al., 1996; Coconnier et al., 1992; Granato et al., 1999; Granato et al., 2004; Greene and Klaenhammer, 1994). However, adhesion of bifidobacteria to IECs has been investigated to a lesser extent and there is a significant lack in mechanistic studies.

Several beneficial health effects have been claimed to be based on the presence of bifidobacteria in the colon (Asahara et al., 2004; Lievin et al., 2000; McCarthy et al., 2003; O'Mahony et al., 2005; Reddy, 1999; Resta-Lenert and Barrett, 2003; Saavedra et al., 1994). As a consequence, bifidobacteria become increasingly interesting for probiotic applications in pharmaceutical and dairy products. One of these potential applications is their use in the treatment of inflammatory bowel disease and there is increasing evidence for anti-inflammatory effects of probiotics containing bifidobacteria on chronic intestinal inflammation (Furrie et al., 2005; Gionchetti et al., 2000; Gionchetti et al., 2003; Madsen et al., 2001; McCarthy et al., 2003). However, given their predominance in the intestinal flora in the early stages and throughout life there is a substantial lack in the understanding of their effects on IECs under homeostatic conditions.

The aim of this study was to investigate adhesion of bifidobacteria to the model intestinal epithelia consisting of Caco-2 cells in combination with PBMCs under different conditions and changes evoked in the cellular response upon bacterial challenge.

2. Materials and methods

2.1. Bacterial strains and cultivation

The bifidobacterial strains used in this study were Bifidobacterium lactis NCC 362, *Bifidobacterium longum* NCC 490, *Bifidobacterium adolescentis* NCC 251, *Bifidobacterium*bifidum NCC 189 (all from the Nestlé Culture Collection, NCC), *Bifidobacterium breve* MB226, and human isolates *B. bifidum* S16, *B. bifidum* S17 and Bifidobacterium infantis/*longum* E18 (all from strain collection C. Staudt and B. Eikmanns, University of Ulm). Non-pathogenic *E. coli* D2241 (NCC) served as Gram-negative control. Bifidobacteria were cultured in de Man-Rogosa Sharpe medium (Difco) supplemented with 0.5 g cysteine l^{-1} at 37 °C under anaerobic conditions (GasPack, Oxoid). *E. coli* D2241 was grown aerobically at 37 °C in brain heart infusion (Difco). For experiments bacteria were used in early stationary phase.

2.2. Cell culture

Caco-2 cells were maintained in DMEM (4.5 g l^{-1} glucose; Amimed, Basel, Switzerland) with 20% (v/v) foetal calf serum (FCS), 1% (v/v) non-essential amino acids (NEAA, Sigma, Basel, Switzerland), and 1% (v/v) penicillin–streptomycin (Sigma). Medium was changed every 2–3 days. Cells were grown transwell cultures on 6-well format tissue culture inserts (BD Falcon®, Milian, Switzerland) for 18–21 days post-confluence to achieve fully differentiated monolayers. Complete differentiation was confirmed by measurement of transepithelial electrical resistance (TEER) using a Millicell-ERS voltohmmeter (Millipore, MA, USA) and cells were used when TEER was >1000 Ω cm^2. PBMCs were freshly isolated from human blood of healthy male donors (age<60 years) by Ficoll-Hypaque centrifugation and maintained in RPMI 1640 medium (Sigma), supplemented with 20% (v/v) human AB serum (Amimed), 1% (v/v) NEAA and 10 μg gentamicin ml^{-1} (Sigma).

2.3. Adhesion of bacteria to intestinal epithelial cells in vitro

To test the adhesion of bacteria to Caco-2 cells, bacteria were radio-labelled during growth in the presence of 10 μCi of ^{3}H-adenine ml^{-1} (1 Ci = 3.7×10^{10} Bq), washed three times with cold PBS (Sigma) and adjusted to 10^8 cells/ml (total bacterial counts, tbc) in RPMI medium (Sigma) with 1% (v/v) NEAA and 10 μg gentamicin ml^{-1}. No serum was used to prevent bacterial clumping due to complement. For adhesion assays at different pH, bacteria were resuspended the medium adjusted to pH 7.0 or 4.5 with acetic acid (Fluka, Basel, Switzerland). Just before the adhesion assay, Caco-2 monolayers were washed twice with RPMI. Incubation with bacteria was performed with 1 ml of normal medium in the basolateral compartment of the transwell cultures. In the apical compartment, 1 ml of the bacterial suspension in medium at different pH was added. After incubation for 1 h at 37 °C, non-adherent bacteria were removed by washing the cells three times with PBS. In the case of apical pH 4.5, maintenance of the pH gradient across the confluent IEC monolayers was confirmed by pH measurement in the basolateral medium at the end of incubation. Adherent bacteria were removed from the cell culture insert together with eukaryotic cells by lysis in 1 ml of 1 M NaOH (Fluka) and incubation for 30 min at room temperature. The suspension was then transferred to a scintillation vial. 1 ml of benzethonium hydroxide (1 M in methanol, Sigma) was added and after incubation for 1 h at 60 °C, radioactivity was counted in a β-counter (1219 Rackbeta, Perkin Elmer, MA, USA). Triplicates of 100% values were prepared for each bacterial culture by spinning down 1 ml of the original labelled and washed bacterial suspension for 10 min at 13000 rpm (Hereaus Biofuge Stratos), lysis in 1 ml 1 M NaOH, and further treatment as for the cell/bacterial lysate. Adhesion of the bacteria to the eukaryotic cells was calculated as percentage relative to the 100% values.

2.4. Statistical analysis of adhesion experiments

All adhesion experiments were performed in three or more independent runs of triplicates. For statistical analysis the insert effect will be seen as a simple repetition, while from run to run more factors (e.g. differences in bacterial cultures, labelling efficiency etc.) can have an impact on adhesion. To adjust the adhesion values for the run effect, an analysis of variance (ANOVA) was carried out using a model, where a run is seen as a random outcome of a distribution of runs. So the run will be handled as a random effect. This has the advantage not to fit

every run separately but to fit the variance of the distribution of runs. This will gain degrees of freedom and result in a more reliable statistical inference. An impact of different conditions (pH 4.5 or 7) will be shown by testing the null hypothesis that there is no difference ($X_1=X_2$; X_1=adhesion at pH 7 and X_2=adhesion at pH 4.5) by estimating the probability (p-value) of the null-hypothesis. If this p-value is smaller than 0.001 we will reject the null hypothesis and accept the alternative hypothesis: $X_1<>X_2$. This will be regarded as statistically significant. Statistical analysis was performed using SAS 8.02 software.

2.5. Co-culture experiments

For co-culture experiments IECs were grown as described above. After differentiation of the eukaryotic cells, inserts were washed and transferred to new wells with 4×10^6 freshly isolated PBMCs in 2 ml RPMI supplemented with 20% (v/v) human AB serum 1% (v/v) NEAA and 10 μg gentamicin ml^{-1}. These co-cultures were incubated with 2×10^7 bacteria in 2 ml of RPMI supplemented with 1% (v/v) NEAA and 10 μg gentamicin ml^{-1} in the apical compartment of the transwell cultures. Any access of bacteria to the basolateral PBMCs across differentiated Caco-2 monolayers was excluded by TEER measurement as described above confirming complete integrity of the monolayers. After 16 h incubation under cell culture conditions, total RNA from washed epithelial cells was isolated using NucleoSpin® RNAII (Macherey-Nagel, Düren, Germany). RNA was quantified using Ribogreen® RNA quantification kit (Molecular Probes, Basel, Switzerland). Quality of the RNA was tested using the RNA 6000 Nano Assay (Agilent Technologies, CA, USA). After reverse transcription using random hexamers (GeneAmp® RNA PCR, Applied Biosystems), cDNA of the genes for TNF-α, IL-1α, and IL-6 were amplified using the following primers: 5′-CAGAGGGAAGAGTTCCCCAG-3′ and 5′-CCTTGGTCTGGTAGGAGACG-3′ for TNF-α, 5′-GATCATCTGTCTCTGAATCA-3′ and 5′-TCCAGATTATGTAATGCAGC-3′ for IL-1α, and 5′-CCAGTACCCCCAGGAGAAGA-3′ and 5′-CCATCTTTGGAAGGTTCAGG-3′ for IL-6. PCR on β-actin cDNA served as control for RNA quantity (primers: 5′-GGCGACGAGGCCCAGAGCAAGAGAGGCAT-3′ and 5′-CGATTTCCCGCTCGGCCGTGGTGGTGAAGC-3′. 30 cycles of amplification were performed except for the β-actin gene (25 cycles).

2.6. Microarrrays

For expression profiling of PBMC-sensitised Caco-2 cells bacterial challenge was conducted as described above. RNA of Caco-2 cells was isolated after 6 h of bacterial challenge. Experiments were performed on three consecutive passages of Caco-2 cells using PBMCs from three different donors. Within a single experiment RNA of at least 12 inserts were pooled for each treatment. The Human Genome Oligo Set Version 2.0 (Operon, AL, USA), representing 21,329 genes in the form of optimised 70-mer oligonucleotides, was spotted onto GAPSII Slides (Corning, MA, USA) using a OmniGrid Microarrayer (GeneMachines, San Carlos, USA), equipped with Steath SMP3 Micro Spotting Pins (Telechem, CA, USA) at the IZKF Chip-Facility, University of Ulm. Printing concentration of the oligonucleotides was 40 μM in 3× saline sodium citrate (SSC) buffer containing 1.5 M betaine (Sigma). DNA adhesion to the surface of the GAPSII slides was achieved by 15 min incubation at 80 °C followed by irradiation with UV light at 254 nm with an energy output of 120 mJ cm^{-2} in a Stratalinker Model 2400 UV illuminator. 12 μg total target RNA (Caco-2) or 12 μg of universal standard RNA (Stratagene, CA, US) were transcribed to cDNA and subsequently labelled with Cy3 or Cy5 (Amersham Biosciences, NJ, USA; Cy3: target RNA; Cy5: standard RNA) using the Amino Allyl cDNA Labeling Kit (Ambion, Austin, USA). Labelled target and standard RNAs were mixed and, after precipitation, resolved at 80 °C in 130 μl ULTRAhyb® buffer (Ambion). After hybridisation for 16–18 h at 37 °C using a GeneTac® hybridisation chamber (Genomic Solutions, Cambridgeshire, UK), slides were washed three times in 2× SSC with 50% formamide (Sigma) and 0.1% Tween 20 (Sigma), pH 7.0 at 45 °C using a flow time of 30 s and a hold time of 3 min. Slides were then washed for 2 min in 1× PBS, 0.05% Tween 20, pH 7.0 at 25 °C. Slides were dried in a centrifuge by spinning for 5 min at 1250×g. Hybridisation signals were visualised using an Axon 4000B dual laser scanner (Axon Instruments, CA, USA). For each spotted oligonucleotide, background and foreground intensities for both channels were obtained by measuring fluorescence at 532 nm (Cy3) and 635 nm (Cy5) and gene expression was quantified with GenePix Pro 4.0 imaging software (GenePix 4000A). Spots flagged for insufficient quality on any of the microarrays were excluded from further analysis. To reduce the variability in gene expression ratios, no background subtraction was performed. Gene expression ratios $M=\log_2(R/G)$ (with R=red fluorescence intensity, G = green fluorescence intensity) were normalised by print-tip loess, using the average expression $A=\log_2(R*G)$ in the Cy3 and Cy5 channels as input (Yang et al., 2002). Only spots with an average intensity of $A>10$ and no flags on any array were retained for further analysis. For identifying differential expression we use a modification of the ordinary t-statistic, or M divided by its standard error (S.E.). An empirical Bayes procedure was used to adjust the t-statistic in a way that decreases the number of false positives due to low sample size (Lonnstedt and Speed, 2002; Smyth, 2004). In this procedure, the S.E. in the t-statistic denominator is adjusted by moving (or 'shrinking') the individual gene S.D. closer to the overall gene S.E. The resulting moderated t-statistic was used for statistical analysis of the microarray data resulting in a probability (p-value) for differential expression for each gene.

Where indicated, the resulting list of differentially expressed genes were further studied for their biological function using the annotation of their gene product (protein) in the gene ontology clustering (http://www.geneontology.org/) according to the EntrezGene database at http://www.ncbi.nlm.nih.gov/.

3. Results and discussion

3.1. Adhesion of bifidobacteria to Caco-2 cells

For adhesion experiments bifidobacteria were used after 16 h of growth in a batch culture. At this stage, cultures had a pH 4–5

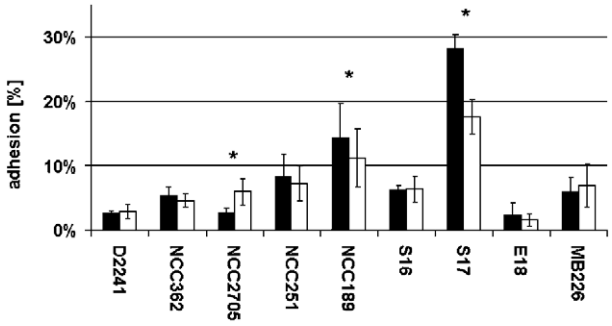

Fig. 1. Adhesion of different bacteria to Caco-2 at pH 7 (■) or pH 4.5 (□). Radiolabelled bacteria were incubated with Caco-2 cells for 1 h. Non-adherent bacteria were removed and the remaining radioactivity was quantified. Data represent means ± standard deviation of three independent experiments conducted in triplicate. *Indicates statistical significance ($p<0.001$).

due to the acetic and lactic acid produced as end products of fermentation. Therefore, a possible influence of the pH on adhesion was investigated by performing adhesion experiments to Caco-2 cells at pH 7.0 or 4.5. Results from three independent experiments performed in triplicate are shown in Fig. 1. In general, the bacteria showed strain-dependent adhesion to Caco-2 cells with some strains showing high adhesion (*B. bifidum* strains NCC 189 and S17), others showing very weak adhesion (*B. infantis longum* E18) and the majority being intermediately adhesive. Almost identical results for adhesion were obtained when fully differentiated monolayers of T84 cells were used (data not shown). When apical pH was shifted for 7 to 4.5, strain-specific changes in adhesion were observed. A statistically significant effect of pH was seen for *B. longum* NCC 2705 and *B. bifidum* strains NCC 189 and S17. While *B. longum* NCC 2705 showed increased adhesion at pH 4.5 for the two strains of *B. bifidum* (NCC2705 and S17) adhesion was higher at neutral pH. Strain- and species-specific adhesion of bifidobacteria to Caco-2 and other IEC lines is not a new phenomenon and has been described previously (Bernet et al., 1993; Crociani et al., 1995; Del Re et al., 2000). However, of importance for the following experiments, the strain-specific changes in adhesion induced by acidic pH suggest different mechanisms of adhesion amongst the tested bifidobacteria. For further experiments we thus selected four strains of bifidobacteria showing different adhesion characteristics. These strains were *B. lactis* NCC362 (intermediate adhesion, no effect of pH), *B. longum* NCC2705 (intermediate adhesion, increased at pH 4.5), *B. bifidum* S17 (high adhesion, decreased at pH 4.5), and *B. longum infantis* E18 (low adhesion, no effect of pH).

3.2. Microarrray experiments

To investigate the impact of bifidobacteria on IEC, global expression profiles were compared using RNA of PBMC-sensitised Caco-2 cells after challenge with *B. lactis* NCC362, *B. longum* NCC2705, *B. bifidum* S17, *B. longum*/*infantis* E18 or *E. coli* D2241. As shown above the four strains of bifidobacteria differ markedly in their adhesion to Caco-2 both in terms of absolute adhesion and changes due to acidic pH. After normalisation and statistical analysis for each bacterium tested genes were ranked by their probability (p-value) to be differentially regulated in PBMC-sensitised Caco-2 after bacterial challenge compared to untreated cells. Interestingly, at any level of statistical significance ($p<0.05$, 0.01, or 0.001), only a few genes were expressed differentially after stimulation with any of the four bifidobacteria while challenge with *E. coli* D2214 led to differential expression of markedly more genes (Table 1). When the most stringent cut-off p-value ($p<0.001$) was applied, *E. coli* D2241 induced differential expression of 51 genes, while only very few genes changed expression significantly with *B. lactis* NCC362 (7 genes), *B. longum* NCC2705 (1), *B. bifidum* S17 (7), *and B. longum*/*infantis* E18 (5). This indicates that in this model of the intestinal epithelium Caco-2 cells were rather unresponsive to bifidobacteria while challenge with *E. coli* led to differential expression of a number of genes. The genes differentially expressed when *E. coli* D2241 was used to challenge the cells were studied in greater detail. A known biological function according to the gene ontology could be assigned to 30 of the 51 genes. These genes, their change in expression, and their biological function are shown in Table 2. The majority of these could be classified into three major groups of biological function. 6 out of 30 genes (20%) are directly involved in signal transduction and another 6 genes (20%) are involved in transcription. 13% (4 out of 30) are involved in cellular defence and immune response. This suggests that, after challenge with non-pathogenic *E. coli*, PBMC-sensitised Caco-2 cells become activated and a cellular response to the challenge is generated on several levels.

In a recent in vivo study, it was shown that colonisation of mice with different commensal bacteria including *B. infantis* ATCC15697 led to differential changes in expression for various genes in ileal tissue samples, depending on the bacterium used for colonisation (Hooper et al., 2001). The discrepancy of differential expression of genes in vivo but not in vitro upon challenge with bifidobacteria could arise from the fact that in the in vivo studies changes in expression of whole tissues were monitored. In these tissues multiple cell types, including epithelial cells, mesenchymal cells, intraepithelial lymphocytes, and immune cells of the lamina propria, are present. Additionally, in monocolonised mice all these cell types are exposed for the first time to a non-self antigen. A secondary regulation of genes in the intestinal mucosa could possibly occur through sampling of antigen by professional antigen presenting cells in the Peyer's Patches (Man et al., 2004) or via direct sampling of antigen by

Table 1
Number of genes showing differential expression at the indicated levels of statistical significance for each bacterial challenge

Cut-off p-value	Number of genes differentially expressed					
	E. coli D2241	*L. johnsonii* La1	*B. lactis* NCC362	*B. longum* NCC2705	*B. bifidum* S17	*B. longum*/*infantis* E18
$p<0.05$	1024	1040	357	176	480	321
$p<0.01$	363	457	71	15	90	40
$p<0.001$	51	185	7	1	7	5

PBMC-sensitised Caco-2 cells were challenged with different bacterial stimuli for 6 h and isolated RNA of Caco-2 cells were used for microarray experiments.

dendritic cells (Rescigno et al., 2001) and subsequent activation of immune cells. As a consequence, the observed changes in expression profiles upon colonisation with bifidobacteria might stem from immune cells and cells from mesenchymal tissues rather than from the epithelial cells or epithelial cells are activated by immune cells. The in vitro data of the presented study originates solely from the IEC line. An influence of immune cells that have been in contact with bacteria, on gene expression in IECs is ruled out as there is no direct access of bacteria to PBMCs across the confluent and differentiated monolayers of Caco-2 cells.

To check whether immune cells potentially influence on adhesion of the bacteria, adhesion experiments were performed with all strains as described above at pH 7. Additionally, 4×10^6 PBMCs were added to the basolateral compartment of the transwell cultures. For none of the tested bacteria presence of basolateral PBMCs had an effect on adhesion (data not shown).

Table 2
Genes corresponding to the oligonucleotides on the microarrays showing statistically significant changes in expression in PBMC-sensitised Caco-2 cells after 6 h of challenge with *E. coli* D2241 compared to unstimulated controls

GeneBank accession	*M*-value	Biological process
NM_012285	0.55	Two-component signal transduction system
AF162668	0.84	Signal transduction
AK054766	0.99	Signal transduction
AL162032	0.95	Signal transduction
NM_000193	1.32	Cell–cell signalling
NM_002351	−0.76	Cell–cell signalling, cellular defence response
NM_052945	1.16	Immune response
X58529	1.22	Immune response
NM_006737	0.96	Cellular defence response
NM_024939	0.61	Nuclear mRNA splicing
NM_012482	1.27	Negative regulation from RNA polymerase II promotor
AL050353	0.79	RNA processing
AK055710	0.90	Regulation of transcription
NM_012074	1.03	Regulation of transcription
NM_016310	0.64	Transcription from RNA polymerase III promotor
AF112345	0.95	Cell–matrix adhesion, integrin-mediated signalling pathway
NM_033100	0.60	Homophilic cell adhesion
BC002828	0.73	Cell differentiation, angiogenesis
NM_002039	−0.76	Cell proliferation
AF411609	0.90	Cytokinesis
NM_002241	0.72	Potassium ion transport
NM_013356	*0.83*	Lactate transport
BC013419	0.69	Intracellular protein transport
NM_001439	1.25	*N*-acetylglucosamine metabolism
NM_003356	−0.92	Transport
NM_005038	−0.61	Protein folding
AK027403	0.54	Induction of apoptosis
BC011614	1.02	Amino acid metabolism
AF249277	0.96	Formate metabolism
AF297710	1.30	Thiamin metabolism

Given are the GeneBank accessions, *M*-value as a measure of the change in expression and the biological process the gene is involved.

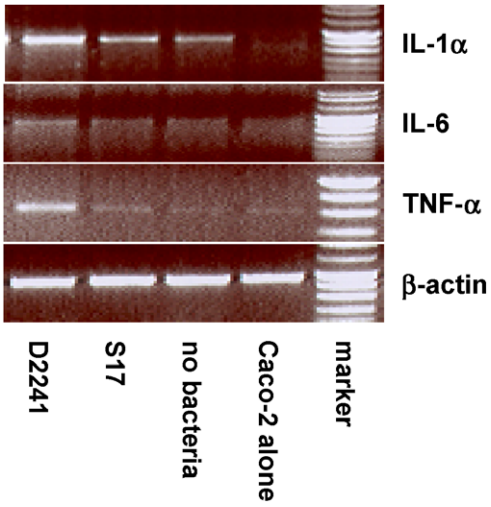

Fig. 2. Semi-quantitative RT-PCR on different cytokine RNAs isolated from co-culture IECs stimulated with different bacterial strains. Caco-2 cells were stimulated with bacteria in the apical compartment of the transwell cultures in the presence of PBMCs (basolateral). Relative levels of cytokine mRNA were measured by RT-PCR and β-actin served as a control for total RNA quantity.

3.3. Cytokine induction by *B. bifidum* S17 and *E. coli* D2241

Finally, Caco-2/PBMC co-cultures were used to investigate mRNA responses of a series of immune markers to a 16-h challenge with highly adherent *B. bifidum* S17 and *E. coli* D2241 using RT-PCR. The results for expression of the genes for TNF-α, IL-6, and IL-1α are shown in Fig. 2. *E. coli* D2241 induced high amounts of these transcripts while no expression above baseline was seen after challenge with *B. bifidum* S17. A similar differential expression was observed for other pro-inflammatory cytokine mRNAs like IL-8 and MCP-1 (data not shown). These results further support the data obtained by microarray experiments in that challenge with *E. coli* D2241 resulted in an activation of the epithelial cell whereas no response could be observed after challenge with *B. bifidum* S17. Moreover, the changes in mRNA expression after 16 h of challenge with *E. coli* D2241 clearly indicate a pro-inflammatory response of IECs towards this strain. This is in line with previous publications showing a pro-inflammatory response of PBMC-sensitised Caco-2 cells after challenge with non-pathogenic *E. coli* (Haller et al., 2000) and an active involvement of IEC in immune responses (Philpott et al., 2001).

4. Conclusions

Our results provide further evidence that adhesion of bifidobacteria to human IECs is strain dependent. Adhesion is influenced by the pH in a strain-dependent manner suggesting that different mechanisms of adhesion exist among bifidobacteria. Challenge with non-pathogenic *E. coli* induced a marked response on mRNA level in PBMC-sensitised Caco-2 cells. At an early time point this response involves genes implicated in gene transcription, signal transduction and immune and cellular

defence as shown by microarray experiments. At a later time point the response of Caco-2 cells to *E. coli* D2241 is clearly pro-inflammatory as indicated by induction of mRNA for IL-6, IL-1α, and TNF-α. By contrast, any of four tested strains of bifidobacteria, showing different levels of adhesion, failed to induce a measurable response in these cells. This indicates that under normal conditions, intestinal epithelial cells are specifically unresponsive to bifidobacteria, thus suggesting that, in contrast to the inflamed epithelium, in homeostasis IECs are not affected by probiotic bifidobacteria.

Acknowledgements

The authors thank D. Matteuzzi for strain MB226, Christine Staudt for kindly providing and typing the intestinal isolates and Dominik Grathwohl for statistical analysis of adhesion data. C.U.R. was supported by a grant from the Nestlé Research Center PhD Training Fellowship Program.

References

Adlerberth, I., Ahrne, S., Johansson, M.L., Molin, G., Hanson, L.A., Wold, A.E., 1996. A mannose-specific adherence mechanism in *Lactobacillus plantarum* conferring binding to the human colonic cell line HT-29. Appl. Environ. Microbiol. 62, 2244–2251.

Asahara, T., Shimizu, K., Nomoto, K., Hamabata, T., Ozawa, A., Takeda, Y., 2004. Probiotic bifidobacteria protect mice from lethal infection with Shiga toxin-producing *Escherichia coli* O157:H7. Infect. Immun. 72, 2240–2247.

Bernet, M.F., Brassart, D., Neeser, J.R., Servin, A.L., 1993. Adhesion of human bifidobacterial strains to cultured human intestinal epithelial cells and inhibition of enteropathogen–cell interactions. Appl. Environ. Microbiol. 59, 4121–4128.

Coconnier, M.H., Klaenhammer, T.R., Kerneis, S., Bernet, M.F., Servin, A.L., 1992. Protein-mediated adhesion of *Lactobacillus acidophilus* BG2FO4 on human enterocyte and mucus-secreting cell lines in culture. Appl. Environ. Microbiol. 58, 2034–2039.

Crociani, J., Grill, J.P., Huppert, M., Ballongue, J., 1995. Adhesion of different bifidobacteria strains to human enterocyte-like Caco-2 cells and comparison with in vivo study. Lett. Appl. Microbiol. 21, 146–148.

Del Re, B., Sgorbati, B., Miglioli, M., Palenzona, D., 2000. Adhesion, autoaggregation and hydrophobicity of 13 strains of *Bifidobacterium longum*. Lett. Appl. Microbiol. 31, 438–442.

Furrie, E., Macfarlane, S., Kennedy, A., Cummings, J.H., Walsh, S.V., O'neil, D.A., Macfarlane, G.T., 2005. Synbiotic therapy (*Bifidobacterium*longum/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. Gut 54, 242–249.

Gionchetti, P., Rizzello, F., Venturi, A., Brigidi, P., Matteuzzi, D., Bazzocchi, G., Poggioli, G., Miglioli, M., Campieri, M., 2000. Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. Gastroenterology 119, 305–309.

Gionchetti, P., Rizzello, F., Helwig, U., Venturi, A., Lammers, K.M., Brigidi, P., Vitali, B., Poggioli, G., Miglioli, M., Campieri, M., 2003. Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. Gastroenterology 124, 1202–1209.

Granato, D., Perotti, F., Masserey, I., Rouvet, M., Golliard, M., Servin, A., Brassart, D., 1999. Cell surface-associated lipoteichoic acid acts as an adhesion factor for attachment of *Lactobacillus johnsonii* La1 to human enterocyte-like Caco-2 cells. Appl. Environ. Microbiol. 65, 1071–1077.

Granato, D., Bergonzelli, G.E., Pridmore, R.D., Marvin, L., Rouvet, M., Corthesy-Theulaz, I.E., 2004. Cell surface-associated elongation factor Tu mediates the attachment of *Lactobacillus johnsonii* NCC533 (La1) to human intestinal cells and mucins. Infect. Immun. 72, 2160–2169.

Greene, J.D., Klaenhammer, T.R., 1994. Factors involved in adherence of lactobacilli to human Caco-2 cells. Appl. Environ. Microbiol. 60, 4487–4494.

Guarner, F., Malagelada, J.R., 8-2-2003. Gut flora in health and disease. Lancet 361, 512–519.

Haller, D., Bode, C., Hammes, W.P., Pfeifer, A.M., Schiffrin, E.J., Blum, S., 2000. Non-pathogenic bacteria elicit a differential cytokine response by intestinal epithelial cell/leucocyte co-cultures. Gut 47, 79–87.

Harmsen, H.J., Wildeboer-Veloo, A.C., Raangs, G.C., Wagendorp, A.A., Klijn, N., Bindels, J.G., Welling, G.W., 2000. Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. J. Pediatr. Gastroenterol. Nutr. 30, 61–67.

Harmsen, H.J., Raangs, G.C., He, T., Degener, J.E., Welling, G.W., 2002. Extensive set of 16S rRNA-based probes for detection of bacteria in human feces. Appl. Environ. Microbiol. 68, 2982–2990.

Hooper, L.V., Wong, M.H., Thelin, A., Hansson, L., Falk, P.G., Gordon, J.I., 2-2-2001. Molecular analysis of commensal host-microbial relationships in the intestine. Science 291, 881–884.

Jacobsen, C.N., Rosenfeldt Nielsen, V., Hayford, A.E., Moller, P.L., Michaelsen, K. F., Paerregaard, A., Sandstrom, B., Tvede, M., Jakobsen, M., 1999. Screening of probiotic activities of forty-seven strains of *Lactobacillus* spp. by in vitro techniques and evaluation of the colonization ability of five selected strains in humans. Appl. Environ. Microbiol. 65, 4949–4956.

Lievin, V., Peiffer, I., Hudault, S., Rochat, F., Brassart, D., Neeser, J.R., Servin, A.L., 2000. *Bifidobacterium* strains from resident infant human gastrointestinal microflora exert antimicrobial activity. Gut 47, 646–652.

Lonnstedt, I., Speed, T., 2002. Replicated microarray data. Stat. Sin. 12, 31–46.

Madsen, K., Cornish, A., Soper, P., McKaigney, C., Jijon, H., Yachimec, C., Doyle, J., Jewell, L., De Simone, C., 2001. Probiotic bacteria enhance murine and human intestinal epithelial barrier function. Gastroenterology 121, 580–591.

Man, A.L., Prieto-Garcia, M.E., Nicoletti, C., 2004. Improving M cell mediated transport across mucosal barriers: do certain bacteria hold the keys? Immunology 113, 15–22.

McCarthy, J., O'Mahony, L., O'Callaghan, L., Sheil, B., Vaughan, E.E., Fitzsimons, N., Fitzgibbon, J., O'Sullivan, G.C., Kiely, B., Collins, J.K., Shanahan, F., 2003. Double blind, placebo controlled trial of two probiotic strains in interleukin 10 knockout mice and mechanistic link with cytokine balance. Gut 52, 975–980.

O'Mahony, L., McCarthy, J., Kelly, P., Hurley, G., Luo, F., Chen, K., O'Sullivan, G.C., Kiely, B., Collins, J.K., Shanahan, F., Quigley, E.M., 2005. *Lactobacillus* and *Bifidobacterium* in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. Gastroenterology 128, 541–551.

Philpott, D.J., Girardin, S.E., Sansonetti, P.J., 2001. Innate immune responses of epithelial cells following infection with bacterial pathogens. Curr. Opin. Immunol. 13, 410–416.

Pinto, M., Robineleon, S., Appay, M.D., Kedinger, M., Triadou, N., Dussaulx, E., Lacroix, B., Simonassmann, P., Haffen, K., Fogh, J., Zweibaum, A., 1983. Enterocyte-like differentiation and polarization of the human-colon carcinoma cell-line Caco-2 in culture. Biol. Cell 47, 323–330.

Reddy, B.S., 1999. Possible mechanisms by which pro- and prebiotics influence colon carcinogenesis and tumor growth. J. Nutr. 129, 1478S–1482S.

Rescigno, M., Urbano, M., Valzasina, B., Francolini, M., Rotta, G., Bonasio, R., Granucci, F., Kraehenbuhl, J.P., Ricciardi-Castagnoli, P., 2001. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nat. Immunol. 2, 361–367.

Resta-Lenert, S., Barrett, K.E., 2003. Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive *Escherichia coli* (EIEC). Gut 52, 988–997.

Saavedra, J.M., Bauman, N.A., Oung, I., Perman, J.A., Yolken, R.H., 15-10-1994. Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. Lancet 344, 1046–1049.

Simmering, R., Blaut, M., 2001. Pro- and prebiotics—the tasty guardian angels? Appl. Microbiol. Biotechnol. 55, 19–28.

Smyth, G.K., 2004. Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. Stat. Appl. Genet. Mol. Biol. 3 (1) Article 3.

Tuomola, E.M., Salminen, S.J., 5-5-1998. Adhesion of some probiotic and dairy *Lactobacillus* strains to Caco-2 cell cultures. Int. J. Food Microbiol. 41, 45–51.

Tuomola, E., Crittenden, R., Playne, M., Isolauri, E., Salminen, S., 2001. Quality assurance criteria for probiotic bacteria. Am. J. Clin. Nutr. 73, 393S–398S.

Yang, Y.H., Dudoit, S., Luu, P., Lin, D.M., Peng, V., Ngai, J., Speed, T.P., 15-2-2002. Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. Nucleic Acids Res. 30, e15.