Anti-inflammatory effects of bifidobacteria by inhibition of LPS-induced NF-κB activation

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INTRODUCTION

Inflammatory bowel diseases such as Crohn’s disease (CD) and ulcerative colitis (UC) are multifactorial disorders characterized by chronic inflammation of the intestinal epithelium. In addition to the genetic predisposition[1-4], the intestinal microflora seems to be involved in triggering inflammation, as observed in several animal models, where colitis was not induced in germ-free animals[2-4]. It has been suggested, that breakdown of tolerance towards the intestinal bacterial flora and dysfunction of regulatory T-cells are implicated in the development of chronic intestinal inflammation.[5,6].

One of the central transcription factors mediating inflammatory responses is nuclear factor KB. Upon activation, through a wide range of stimuli such as TNF-α, IL-1β, or LPS, NF-κB translocates to the nucleus[9,10], where it regulates the transcription of a series of genes involved in acute responses to injury and in chronic intestinal inflammation including the genes for IL-1, IL-6, IL-8, IL-12, Cox-2, ICAM-1, vascular endothelial growth factor-1, T-cell receptor-α, and major histocompatibility complex class II molecules[11,12]. LPS activates NF-κB through toll-like receptor 4 (TLR4)[13,14]. The events preceding the actual binding of LPS to TLR4 involve LPS-binding protein (LBP) and the intermediate receptor CD14[14,15]. LBP conveys LPS to CD14, which then promotes binding of LPS to the LPS receptor complex composed of TLR4 and MD-2, a co-receptor essential for LPS signalling via TLR4[16]. On monocytes and macrophages CD14 was shown to be membrane-bound by a glycosylphophoinositol anchor[17]. Stimulation of endothelial and epithelial cells with LPS requires the second form of CD14, i.e. soluble CD14 (sCD14)[18].

There is increasing evidence that in chronic intestinal inflammation expression of TLR4 and CD14 on IECs is abnormal. Under normal conditions, TLR4 and CD14
are expressed in IECs at very low levels in vivo\textsuperscript{[19,20]} and are confined to undifferentiated cells of the crypts, which are not exposed to the overwhelming quantity of luminal antigens\textsuperscript{[21]}. By contrast, expression of TLR4 and CD14 is significantly increased in IECs of both, animal models of colitis\textsuperscript{[22,23]} and IBD patients\textsuperscript{[19]}. A possible explanation for this increased expression could come from genetic studies. Higher frequencies of the Asp299Gly allele in the extracellular domain of TLR4 and the T allele and TT genotype at position -159 in the gene for CD14 have been positively associated with CD and UC\textsuperscript{[24–27]}. Abnormal LPS signalling through increased levels of TLR4 and CD14 in IECs could contribute to the sustained mucosal inflammation in IBD.

Bifidobacteria are Gram-positive, anaerobic microorganisms that inhabit mainly the colon of healthy infants and adults. In breast-fed infants, soon after birth, up to 90% of all bacteria in faecal samples detected by fluorescence in situ hybridisation are bifidobacteria\textsuperscript{[28]} and they still make up 3%-5% of the adult microbiota\textsuperscript{[29]}. Thus, bifidobacteria undoubtedly constitute one of the predominant species of the human colonic microflora\textsuperscript{[30]}. The analysis and annotation of the genome sequence of \textit{B. longum} NCC2705 illustrated the close relationship of this bacterium with its human host\textsuperscript{[31]}. Several beneficial health effects have been claimed to be related to the presence of bifidobacteria in the colon\textsuperscript{[12–21]}. Based on these properties, bifidobacteria have become increasingly interesting for probiotic use, both in pharmaceutical application and dairy products\textsuperscript{[32–34]}. One of these applications is their use in probiotic intervention in chronic intestinal inflammation. Probiotics containing bifidobacteria have been shown to be effective in reducing the severity of inflammation in several rodent models and patients with IBD\textsuperscript{[18,40–47]}. In this study, the effects of a selection of bifidobacteria on IECs were investigated using an \textit{in vitro} approach mimicking the situation of increased responsiveness of IECs to LPS. In this model, the antagonistic potential of several bifidobacteria on LPS-induced inflammatory responses in IECs was shown to be mediated by blocking NF-κB activation. This suggests that several \textit{Bifidobacterium} strains, especially of \textit{B. bifidum}, might be good candidates for probiotic intervention in IBD.

### MATERIALS AND METHODS

#### Bacterial strains, cell lines and culture conditions

All bacterial strains used are shown in Table 1. For experiments, bacteria were cultured for 16 h at 37°C (bifidobacteria: \textit{de Man-Rogosa Sharpe} medium with 0.5 g/L cysteine, anaerobic; \textit{E. coli}: brain heart infusion medium, aerobic; all media BD Difco, Basel, Switzerland). HT-29 cells were maintained at 50% CO\textsubscript{2} in DMEM (4.5 g/L glucose; Amimed, Basel, Switzerland) and cultured in the respective cell culture medium until they reached 90%-100% confluence. At this stage, approximately 1 × 10\textsuperscript{5} cells/well were counted.

#### LPS and TNF-α challenge of IEC lines

HT-29 or HT-29 clone 34 cells were grown as described above. At 90%-100% confluence, cells were washed and the medium was changed to 0.9 mL of DMEM with 100 mL/L FCS, 10 mL/L NEAA, 50 mmol/L Hepes (Invitrogen), and penicillin G (100 kU/L; Fluka). Bacteria were grown as described above and added at the indicated multiplicity of infection (m.o.i) in 0.1 mL of cell culture medium. In some experiments, cells were pre-incubated for 1 h with bacteria and subsequently stimulated with TNF-α (10 μg/L) or LPS (10 μg/L, serotype B55:O5; Sigma). Where indicated, a challenge was performed in the presence of 50 mL/L of human milk (HM). Milk was

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### Table 1 Bacterial strains used in this study

| Strain or oligonucleotides | Relevant characteristics | Source or PCR product |
|---------------------------|--------------------------|-----------------------|
| E. coli                   |                          |                       |
| TG1                       | Cloning host             | DSMZ\textsuperscript{1} |
| D2241                     | Non-pathogenic intestinal isolate, control | NCC\textsuperscript{2} |
| \textit{Bifidobacterium}  |                          |                       |
| NCC362                    | B. lactis, type strain    | NCC\textsuperscript{3} |
| NCC2705                   | B. longum, type strain    | NCC\textsuperscript{3} |
| NCC2251                   | B. adolescentis, type strain | NCC\textsuperscript{3} |
| NCC189                    | B. bifidum               | NCC\textsuperscript{3} |
| S16                       | B. bifidum, intestinal isolate from a breast-fed infant | [57] |
| S17                       | B. bifidum, intestinal isolate from a breast-fed infant | [57] |
| E18                       | B. infantis/longum, intestinal isolate from an adult | [57] |
| MB226                     | B. breve, type strain     | D. Matteuzzi          |

\textsuperscript{1} DSMZ: Deutsche Sammlung von Mikroorganismen und Zellkulturen; \textsuperscript{2} NCC: Nestlé Culture Collection.

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sampled from different mothers (d 20 of lactation) and pooled. The pool of HM used for this study contained 26 mg/L of sCD14 (determined by ELISA; P. Serrat, personal communication). After 16 h of incubation, supernatants were collected by centrifugation and used for quantification of SEAP or IL-8. Viability of the eukaryotic cells was continuously checked by microscopic examination of trypan blue (Sigma) exclusion and was > 95% in all experiments.

Detection of SEAP and IL-8 protein
Phosphatase activity of SEAP was quantified by bioluminescence using the PhosphaLight™ kit (Tropix, MA, USA). Bioluminescence was measured as relative light units (RLU) in a TECAN SPECTRAFluor Plus spectrometer with an integration time of 100 milliseconds and gain set to 100. Data were analysed using the XFLOUR software version 4.40. RLU varied between experiments due to the nature of the assay. Where more than one experiment was used to present figures and for statistical analysis, correction for these day to day variations was performed by normalizing data to set 100 RLU units for the positive control (LPS + HM). In one experiment, levels of NF-κB activation were similar for 10 μg/L of TNF-α and 10 μg/L of LPS in combination with HM. Secreted IL-8 protein was quantified by ELISA using the IL-8 Eli-pair kit (Diaclone, CT, USA). Absorbance was measured in a DyneX MRX microplate reader (DyneX Technologies, Worthing, England) at 450 nm.

Real-time PCR
For real-time PCR, RNA of HT-29 cells was isolated after bacterial pre-treatment and 4 h of LPS challenge in the presence of 5% (v/v) HM using the NucleoSpin® RNAII kit (Macherey-Nagel, Düren, Germany). RNA was quantified using Ribogreen® RNA quantification kit (Molecular Probes, Basel, Switzerland). Quality of RNA was verified using Agilent RNA 6000 Nano Assays and the 2100 Bioanalyzer (Agilent Technologies, CA, USA) with corresponding software (version A.01.16). 1 μg of total RNA was transcribed to cDNA using TaqMan® Reverse Transcription Reagents. Real time reactions were set up using 5 μL TaqMan® 2 x PCR Master Mix, 0.5 μL of TaqMan® assays-on-demand primer/probe mixture (glyceraldehyde-3 phosphate dehydrogenase (GAPDH): Hs99999905_mL; IL-8: Hs00174103_mL; TNF-α: Hs00174128_mL; COX-2: Hs00153133_mL; ICAM-1: Hs00164932_mL; all reagents were purchased from Applied Biosystems) and 4.5 μL H2O containing cDNA corresponding to 10-20 ng RNA, depending on the target gene. Real-time PCR was performed in an ABI Prism 7900HT Sequence Detection System (Applied Biosystems) and analysed with the SDS software package version 2.1. Results of the different RNA samples were normalised for RNA quantity using the gene for GAPDH.

RESULTS

Bifidobacteria do not induce NF-κB-dependent reporter gene activity in HT-29 clone 34 cells
To study the effect of bifidobacteria on inflammatory events in IECs, i.e. on NF-κB activation, a reporter gene assay in HT-29 clone 34 cells was used. Cells were incubated with bacteria (moi = 100) for 16 h. Subsequently, activity of the NF-κB-driven reporter protein SEAP was measured in culture supernatants (Figure 1). While non-pathogenic E. coli D2241 induced significant reporter gene activity, all bifidobacteria tested failed to induce any activation of NF-κB above background levels. The same cell-based assay was used to investigate the effect of bifidobacteria on LPS-induced inflammation in IECs. HT-29 clone 34 cells were challenged with LPS for 16 h and subsequently SEAP activity was measured in the culture supernatants (Table 2). Only a 2.5-fold induction of NF-κB activation by LPS alone compared to unstimulated cells was observed. To mimic the situation in the intestinal epithelium of IBD patients where expression of CD14 is increased, 50 mL/L of HM, containing sCD14, were added to the assay. Addition of HM led to a 50-fold increase in LPS-induced NF-κB activation, whereas HM alone did not result in activation of NF-κB. Also, in the presence of HM none of the tested bifidobacteria induced NF-κB-dependent reporter gene activity above background levels while stimulation with E. coli D2241 was enhanced about 200-fold (data not shown), further supporting the results indicating that bifidobacteria do not induce inflammatory events in IECs.
Strain- and dose-dependent inhibition of LPS-induced NF-κB activation by bifidobacteria

Several reports suggest anti-inflammatory effects of probiotics containing bifidobacteria. Therefore, all bifidobacteria used in this study were assessed for their potential inhibitory effect on LPS-induced NF-κB activation at different bacterial doses. At a moi of 1, none of the bifidobacteria tested had an effect on NFκB-dependent reporter gene activity (data not shown). As shown in Figure 2, at a moi = 10, however, pre-treatment with B. lactis NCC362 and B. bifidum NCC189, S16, and S17 significantly decreased NF-κB-dependent reporter gene activity to 47%-71% of the positive control. When the bacterial dose for pre-treatment was further increased to 100 bacteria per cell (moi = 100), the inhibitory effect of B. bifidum NCC189 and that of B. bifidum S17 was almost complete (5% of positive control), while that of B. lactis NCC362, B. longum NCC2705, B. adolescentis NCC251 and B. bifidum S16 was intermediate (20%-52% of positive control). In contrast, B. longum/infantis E18 and B. breve MB226 had no or only very slight inhibitory effect on SEAP activation at either moi. To assess whether physical presence of bifidobacteria was required for their inhibitory effect, further experiments were carried out using cell culture medium pre-treated for 16 h with bifidobacteria followed by removal of bifidobacterial cells using filter sterilization. Use of this conditioned medium did not result in any inhibition of LPS-induced NF-κB activation in HT-29 clone 34 cells (data not shown). These results show that six out of eight bifidobacteria tested significantly inhibited LPS-induced NF-κB activation in IECs and that this inhibitory capacity of bifidobacteria is strain- and dose-dependent. Furthermore, presence of bifidobacterial cells during LPS challenge is required for their anti-inflammatory effects indicating that this inhibition is not due to secreted compounds. To prove that decreased activity of the reporter protein was not a consequence of apoptosis due to high bacterial doses in combination with LPS, viability of the cells was confirmed by trypan blue exclusion. Microscopic examination of cells pre-treated with two representative strains (B. longum NCC2705 and B. bifidum S17), challenged with LPS + HM showed that viability was >95% in all experiments demonstrating that inhibition of LPS-induced reporter gene activity by bifidobacteria is not due to cell death (Figure 3).

Inhibition of LPS-induced inflammatory events by B. bifidum S17 and B. longum NCC2705

To further investigate the effect of bifidobacteria on inflammatory events following NF-κB activation, IL-8 secretion and mRNA level of the genes encoding IL-8, TNF-α, COX-2, and ICAM-1 were monitored in normal HT-29 cells after bacterial pre-treatment with B. bifidum S17 and B. longum NCC2705 and a subsequent LPS challenge. Pre-incubation with B. bifidum S17 reduced IL-8 secretion of the IECs to about 20% of the positive control already at a moi = 10 and was able to completely inhibit IL-8 secretion at 100 bacteria per cell (Table 3). Effective inhibition with B. longum NCC2705 was only observed at a moi = 100. Thus, the dose-response of the IECs for IL-8 secretion was similar to that observed for NF-κB-driven SEAP reporter gene activity.

The effect of pre-treatment of HT-29 cells with bifidobacteria on mRNA levels for genes known to be regulated by NF-κB was investigated by real-time PCR. Pre-treatment with B. longum NCC2705 and B. bifidum S17 at a moi = 100 severely inhibited transcriptional activation of the genes for IL-8, TNF-α, COX-2, and ICAM-1 in

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**Table 3** IL-8 protein in the supernatants of HT-29 cells (mean ± SD, n = 3)

| Treatment                  | IL-8 [ng/L] |
|----------------------------|-------------|
| None (untreated control)   | 2 ± 1       |
| LPS+HM                     | 36 ± 3      |
| NCC2705/LPS+HM 1           | 30 ± 2      |
| S17/LPS+HM 1               | 7 ± 2       |

1 Pre-incubation with B. longum NCC2705 or B. bifidum S17 at moi = 10 or 100 was followed by stimulation with LPS + HM for 16 h.

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**Figure 2** Dose- and strain-dependent inhibition of LPS-induced NF-κB activation by different bifidobacteria. SEAP activity was quantified in the supernatants of HT-29 clone 34 cells pre-incubated with different bifidobacteria at moi = 10 (dark grey bars) or moi = 100 (light grey bars) and subsequently stimulated with LPS + HM for 16 h. Cells treated with medium only served as negative control (neg; white bar) and positive controls (pos; black bar) were cells challenged with LPS + HM without bacterial pre-treatment. Results are means ± standard error of the mean (SEM) of three independent experiments performed in triplicate. RLU of individual experiments were normalised to give 100 RLU for the positive control.

**Figure 3** Microscopic examination of cellular viability. Trypan blue exclusion was monitored in HT-29 clone 34 cells challenged with LPS + HM for 16 h after pre-incubation with bifidobacteria (B. longum NCC2705 or B. bifidum S17; moi = 100). Positive control (pos) was cells incubated with LPS + HM without bacterial pre-treatment. As a control for trypan blue staining cells were incubated for 16 h in cell culture medium at pH 4 (dead).
HT-29 cells challenged with LPS in the presence of HM (Table 4). Again, B. bifidum S17 was more effective than B. longum NCC2705. Taken together, these results indicate that selected strains of bifidobacteria are able to inhibit LPS-induced inflammatory events in IECs.

**TNF-α-induced NF-κB activation is not inhibited by bifidobacteria**

NF-κB is activated through a wide range of stimuli other than LPS (see introduction). Therefore, it was interesting to test whether a similar inhibition of bifidobacteria could be observed for a pro-inflammatory stimulus other than LPS, such as TNF-α. At moi = 100, none of the tested bifidobacteria had any inhibitory effect on NF-κB-dependent reporter gene activity when 10 μg/L TNF-α was used to challenge HT-29 clone 34 cells (Figure 4). Further experiments were performed using a lower dose of TNF-α (1 μg/L) as stimulus and different doses of bacteria (moi = 10 and 100). In none of the tested conditions, an inhibitory effect of bifidobacteria on TNF-α induced NF-κB activation was observed (data not shown). These results suggest that the inhibitory capacity of bifidobacteria is specific for LPS-induced NF-κB activation in IECs.

**DISCUSSION**

Previous studies have shown that the intestinal microflora is implicated in the pathogenesis of IBD[5-4]. Increased expression of CD14 and TLR4 in IECs[5] might contribute to the sustained inflammation in the epithelium of patients of IBD. On the other hand, for several probiotic formulations, effects on inflammation in cultured IEC lines and in mouse models of chronic intestinal inflammation have been reported, yet there is a significant lack in our understanding of the molecular mechanisms by which probiotics downmodulate intestinal inflammation. Most studies on anti-inflammatory effects of probiotics were performed using mixtures of different probiotic strains, mainly lactobacilli and bifidobacteria[9]. For HT-29 and T84 cells, it was shown that incubation of these cells with VSL#3, a formulation containing eight different strains including 3 strains of bifidobacteria, resulted in decreased secretion of IL-8 in response to challenge with non-pathogenic E. coli or pathogenic Salmonella dublin[31]. In the IL-10 knock-out model of colitis, introduction of VSL#3 in the diet had an inhibitory effect on basal and LPS-stimulated TNF-α secretion in biopsies obtained from the colonic mucosa[44]. In the same mouse model, feeding a diet containing B. infantis 35624 was superior to L. salivarius UCC118 in effectively reducing the total gastrointestinal inflammatory score[38]. Finally, VSL#3 has been successfully used in the treatment of some IBDs[43,44].

Here, the effects of bifidobacteria on IECs were investigated with particular emphasis on LPS-induced inflammation. To our knowledge this represents the first attempt to characterize anti-inflammatory effects of whole cells of a single bifidobacterial strain. None of the tested bifidobacteria induced activation of NF-κB in HT-29 clone 34 cells, whereas a challenge with E. coli D2241 resulted in significant induction of NF-κB driven reporter gene activity in this cell line. Challenge of HT-29 clone 34 cells with LPS alone resulted in only a 2.5-fold induction of NF-κB-dependent reporter gene activity above background levels. This is consistent with results from other groups, which showed that colonic epithelial cells are poorly responsive to LPS[28]. However, using HM as a source of sCD14[32], the NF-κB response of HT-29 IECs to LPS was dramatically increased. This result supports previous studies showing increased responsiveness of HT-29 cells to LPS in the presence of HM[32,54]. This increased responsiveness of HT-29 cells to LPS in the presence of milk could be blocked by an anti-CD14 antibody[52].

NF-κB-dependent reporter gene activation by LPS in the presence of milk could be inhibited by pre-incubation with bifidobacteria in a strain- and dose-dependent manner, with the three B. bifidum strains (NCC189, S16, and S17) being most effective. To achieve this inhibitory effect, the presence of bifidobacterial cells was required, as filter-sterilized cell culture medium pre-treated with bifidobacteria did not show any inhibition of LPS-induced NF-κB activation. This indicates that the anti-inflammation caused by bifidobacteria might be species-specific as we only observed significant inhibitory effect in the presence of B. bifidum strains.
inflammatory compound is not actively secreted by bifidobacteria or released from lysed cells. Furthermore, incubation of HT-29 cells with *B. longum* NCC2705 and *B. bifidum* S17 prior to LPS challenge reduced IL-8 secretion and had a significant inhibitory effect on mRNA levels of the TNF-α, IL-8, ICAM-1, and COX-2 genes, all of which have been shown to be regulated by NF-κB [57]. In a similar study, VSL#3 inhibited NF-κB reporter gene activity and MCP-1 secretion after a challenge with TNF-α in conditionally immortalised mouse colon cells [49]. In the presented experiments, none of the tested strains of bifidobacteria inhibited NF-κB activation in response to TNF-α. These results suggest that bifidobacteria interfere with pro-inflammatory signals upstream of the pathway of NF-κB activation common for LPS and TNF-α.

Lipoteichoic acids (LTA) of two lactobacilli strains were able to inhibit IL-8 secretion in response to LPS in HT-29 cells [38]. In a recent study, it was shown that the composition of LTA have an impact on the immunogenicity and anti-inflammatory capacity of *L. plantarum* [43]. Of note, a mutant lacking D-alanine substitutions to the back bone of LTA showed dramatically improved anti-inflammatory properties compared to the wild type. The mechanism by which bifidobacteria exert their inhibitory effect remains to be elucidated. One study investigating DNA from the VSL#3 mixture showed that the bifidobacterial strains’ DNA was effective in limiting epithelial pro-inflammatory responses in IL-10-deficient mice and in HT-29 cells challenged with TNF-α. However, the pro-inflammatory response to LPS in HT-29 cells was not inhibited by the probiotic DNA [10]. This rules out, to a certain degree, that the effects observed in the presented experiments stem from DNA leaking from lysed bifidobacteria. Furthermore, it suggests that the mechanisms by which whole cells of bifidobacteria inhibit inflammatory responses are distinct from those described for their DNA.

Although anti-inflammatory activity of probiotics has been reported previously, to our knowledge, this is the first report on a specific inhibitory effect of bifidobacteria on LPS-induced inflammatory events in IECs, suggesting a role for bifidobacteria in down-modulation of pro-inflammatory cytokines [52]. Blocking of LPS-induced NF-κB activation by bifidobacteria could, at least in part, explain their positive effects on chronic intestinal inflammation in vivo through prevention of further amplification of the pro-inflammatory signal after exposure to LPS. Our results suggest that the capacity to inhibit LPS-induced NF-κB activation is strain-dependent. Especially, the strains of *B. bifidum* are promising candidates for probiotic intervention in inflammatory disorders of the gastrointestinal tract.

**ACKNOWLEDGMENTS**

C.U.R. was funded by a Nestlé PhD fellowship. *B. breve* MB226 was generously provided by D. Matteuzzi. P. Serrant kindly provided the pool human milk.

**REFERENCES**

1. Bouma G, Strober W. The immunological and genetic basis of inflammatory bowel disease. Nat Rev Immunol 2003; 3: 521-533
2. Dianda L, Hanby AM, Wright NA, Sebesteny A, Hayday AC, Owen MJ. T cell receptor-alpha beta-deficient mice fail to develop colitis in the absence of a microbial environment. Am J Pathol 1997; 150: 91-97
3. Contractor NV, Bassiri H, Reyta T, Park AY, Baumgart DC, Wasik MA, Emerson SG, Carding SR. Lymphoid hyperplasia, autoimmunity, and compromised intestinal intraepithelial lymphocyte development in colitis-free gnotobiotic IL-2-deficient mice. J Immunol 1998; 160: 385-394
4. Sellon RK, Tonkonogy S, Schultz M, Dieleman LA, Granther W, Balish E, Rennick DM, Sartor RB. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infect Immun 1998; 66: 5224-5231
5. Kühn R, Löbler J, Rennick D, Rajewsky K, Müller W. Interleukin-10-deficient mice develop chronic enterocolitis. Cell 1993; 75: 263-274
6. Duchmann R, Kaiser I, Herrmann E, Mayet W, Ewe K, Meyer zum Büschenfelde KH. Tolerance exists towards resident intestinal flora but is broken in active inflammatory bowel disease (IBD). Clin Exp Immunol 1995; 102: 448-455
7. Duchmann R, Schmitt E, Knolle P, Meyer zum Büschenfelde KH, Neurath M. Tolerance towards resident intestinal flora in mice is abrogated in experimental colitis and restored by treatment with interleukin-10 or antibodies to interleukin-12. Eur J Immunol 1996; 26: 934-938
8. Neurath MF, Fuss I, Kehsall BL, Presky DH, Waegell W, Strober W. Experimental granulomatous colitis in mice is abrogated by induction of TGF-beta-mediated oral tolerance. J Exp Med 1996; 183: 2605-2616
9. Li Q, Verma IM. NF-kappaB regulation in the immune system. Nat Rev Immunol 2002; 2: 725-734
10. Hayden MS, Ghosh S. Signaling to NF-kappaB. Genes Dev 2004; 18: 2195-2224
11. Barnes PJ, Karin M. Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. N Engl J Med 1997; 336: 1066-1071
12. Neurath MF, Becker C, Barbulescu K. Role of NF-kappaB in immune and inflammatory responses in the gut. Gut 1998; 43: 856-860
13. Akira S, Takeda K. Toll-like receptor signalling. Nat Rev Immunol 2004; 4: 499-511
14. Wright SD, Tobias PS, Ulevitch RJ, Ramos RA. Lipopolysaccharide (LPS) binding protein opsonizes LPS-bearing particles for recognition by a novel receptor on macrophages. J Exp Med 1989; 170: 1231-1241
15. Schumann RR, Leong SR, Flags GW, Gray PW, Wright SD, Mathison JC, Tobias PS, Ulevitch RJ. Structure and function of lipopolysaccharide binding protein. Science 1990; 249: 1429-1431
16. Shimazu R, Akashi S, Ogata H, Nagai Y, Fukudome K, Miyake K, Kimoto M. MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. J Exp Med 1999; 189: 1777-1782
17. Haziot A, Chen S, Ferrero E, Low MG, Silber R, Goyert SM. The monocyte differentiation antigen, CD14, is anchored to the cell membrane by a phosphatidylinositol linkage. J Immunol 1998; 141: 547-552
18. Pugin J, Schürer-Maly CC, Leturcq D, Mioriarty A, Ulevitch RJ, Tobias PS. Lipopolysaccharide activation of human endothelial and epithelial cells is mediated by lipopolysaccharide-binding protein and soluble CD14. Proc Natl Acad Sci U S A 1993; 90: 2744-2748
19. Cario E, Podolsky DK. Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. Infect Immun 2000; 68: 7010-7017
20. Abreu MT, Fukata M, Arditi M. TLR signaling in the gut in health and disease. J Immunol 2005; 174: 1435-1443
21. Furrer E, Macfarlane S, Thomson G, Macfarlane GT. Toll-like receptors-2, -3 and -4 expression patterns on human colon and their regulation by mucosal-associated bacteria. Immunology

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Purification, amino acid sequence, and mode of action of bifidocins B produced by Bifidobacterium bifidum NCIB 1454. J Appl Microbiol 1999, 86: 45-54

Brady LJ, Gallaher DD, Basta FF. The role of probiotic cultures in the prevention of colon cancer. J Nutr 2000: 130: 4105-4108

Asahara T, Nomoto K, Shimizu K, Watanuki M, Tanaka R. Increased resistance of mice to Salmonella enterica serovar Typhimurium infection by syndiotactic administration of Bifidobacteria and transglutaminylated oligosaccharides. J Appl Microbiol 2001, 91: 985-996

McCarthy J, O'Mahony L, O'Callaghan L, Sheil B, Vaughan L, Fitzsimons N, Fitzgibbon J, O'Sullivan GC, Kiely B, Collins JK, Shanahan F. Double blind, placebo controlled trial of two probiotic strains in the prevention of colon cancer. Lancet 1999; 354: 1539-1546

Yildirim Z, Winters DK, Johnson MG. Purification, amino acid sequence and mode of action of bifidocin B produced by Bifidobacterium bifidum NCIB 1454. J Appl Microbiol 1999, 86: 45-54

Brady LJ, Gallaher DD, Basta FF. The role of probiotic cultures in the prevention of colon cancer. J Nutr 2000: 130: 4105-4108

Asahara T, Nomoto K, Shimizu K, Watanuki M, Tanaka R. Increased resistance of mice to Salmonella enterica serovar Typhimurium infection by syndiotactic administration of Bifidobacteria and transglutaminylated oligosaccharides. J Appl Microbiol 2001, 91: 985-996

McCarthy J, O'Mahony L, O'Callaghan L, Sheil B, Vaughan EE, Fitzsimons N, Fitzgibbon J, O'Sullivan GC, Kiely B, Collins JK, Shanahan F. Double blind, placebo controlled trial of two probiotic strains in the prevention of colon cancer. J Nutr 2000: 130: 4105-4108

Resta-Lenert S, Barrett KE. Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive Escherichia coli (EIEC). Gut 2003; 52: 975-980

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

O'Mahony L, McCarthy J, O'Callaghan L, Kelly P, Hurley G, Luo F, Chen K, O'Sullivan GC, Kiely B, Collins JK, Shanahan F, Quigley EM. Lactobacillus and bifidobacteria in Irritable Bowel Syndrome: symptom responses and relationship to cytokine profiles. Gastroenterology 2005; 128: 541-551

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

Gionchetti P, Rizzello F,VENTURI A, Lammers H, Wildeboer-Veloo AC, Raangs GC, Wagendorp J, Berendt A, Vermeire S, El Housni H, Pierik M, Van Steen K, O'Mahony L, O'Callaghan L, Sheil B, Vaughan L. Probiotics enhance murine and human intestinal epithelial barrier function. Gastroenterology 2001; 121: 580-591

Gionchetti P, Rizzello F, Helwig U, Venturi A, Lammers KM, Brigi Di P, Vitali B, Poggioli G, Miglioli M, Campieri M. Oral bacterotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. Gastroenterology 2000; 119: 305-309

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

Gionchetti P, Rizzello F, Helwig U, Venturi A, Lammers KM, Brigi Di P, Vitali B, Poggioli G, Miglioli M, Campieri M. Oral bacterotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. Gastroenterology 2000; 119: 305-309

Bibiloni R, Fedorak RN, Tanock GW, Madsen KL, Gionchetti P, Campieri M, De Simone C, Sartor RB. VSL#3 probiotic mixture induces remission in patients with active ulcerative colitis. Am J Gastroenterol 2005; 100: 1539-1546

Furrie E, Macfarlane S, Kennedy A, Cummings JH, Walsh SV, O’Neill DA, Macfarlane GT. Symbiotic therapy (Bifidobacterium longum/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. Gut 2005; 54: 1242-1250

Moon KY, Hahn BS, Lee J, Kim YS. A cell-based assay system for monitoring NF-kappaB activity in human Hacat transfectant cells. Anal Biochem 2001; 292: 17-21

Petros EF, Kojima K, Roepelski MJ, Musch MW, Tao Y, De Simone C, Chang EB. Probiotics inhibit nuclear factor-kappaB and induce heat shock proteins in colonic epithelial cells through proteasome inhibition. Gastroenterology 2004; 127: 1474-1487

Picard C, Fioramonti J, Francois A, Robinson T, Neant F, Machuchsny C. Review article: bifidobacteria as probiotic agents -- physiological effects and clinical benefits. Aliment Pharmacol Ther 2005; 22: 495-512

Otte JM, Podosky DK. Functional modulation of enterocytes by gram-positive and gram-negative microorganisms. Am J Physiol Gastrointest Liver Physiol 2005; 288: G613-G626

Labota MO, Vidal K, Nores JE, Arias M, Vita N, Morgan BP, Guillen JT, Loyaux D, Ferraera P, Schmid D, Affolter M, Borysiewicz LK, Donnet-Hughes A, Schirrfin EJ. Innate recognition of bacteria in human milk is mediated by a milk-derived highly expressed pattern recognition receptor, soluble CD14. J Exp Med 2000; 191: 1807-1812

Vidal K, Labota MO, Schirrfin EJ, Donnet-Hughes A. Soluble CD14 in human breast milk and its role in innate immune responses. Acta Odontol Scand 2001; 59: 330-334

Vidal K, Donnet-Hughes A, Granato D. Lipoteichoic acids from Lactobacillus johnsonii strain La1 and Lactobacillus acidophilus strain La10 antagonize the responsiveness of human intestinal epithelial HT29 cells to lipopolysaccharide and gram-negative bacteria. Infect Immun 2002; 70: 2057-2064

Grangerette C, Nutten S, Palombo E, Morath S, Hermann C, Dufwulf J, Pot B, Hartung T, Hols P, Mercenier A. Enhanced antiinflammatory capacity of a Lactobacillus plantarum mutant synthesizing modified teichoic acids. Proc Natl Acad Sci U S A 2005; 102: 10321-10326

Jijon H, Backer J, Díaz H, Yeung H, Thiel D, McKaigney C, De Simone C, Macfarlane GT. Lactobacillus acidophilus strain La10 from mice and rat cecal bacteria modulate murine and human epithelial and immune function. Gastroenterology 2004; 126: 1358-1373

Staudt C. Wechselwirkungen von bifidobakterien mit darmepitelzellen und mit extrazellulären matrix- und plasmamolekülten(Thesis). German: University of Ulm, 2002