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TOPIC HIGHLIGHT

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Modulation of immunity and inflammatory gene expression in the gut, in inflammatory diseases of the gut and in the liver by probiotics

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Abstract

The potential for the positive manipulation of the gut microbiome through the introduction of beneficial microbes, as also known as probiotics, is currently an active area of investigation. The FAO/WHO define probiotics as live microorganisms that confer a health benefit to the host when administered in adequate amounts. However, dead bacteria and bacterial molecular components may also exhibit probiotic properties. The results of clinical studies have demonstrated the clinical potential of probiotics in many pathologies, such as allergic diseases, diarrhea, inflammatory bowel disease and viral infection. Several mechanisms have been proposed to explain the beneficial effects of probiotics, most of which involve gene expression regulation in specific tissues, particularly the intestine and liver. Therefore, the modulation of gene expression mediated by probiotics is an important issue that warrants further investigation. In the present paper, we performed a systematic review of the probiotic-mediated modulation of gene expression that is associated with the immune system and inflammation. Between January 1990 to February 2014, PubMed was searched for articles that were published in English using the MeSH terms "probiotics" and "gene expression" combined with "intestines", "liver", "enterocytes", "antigen-presenting cells", "dendritic cells", "immune system", and "inflammation". Two hundred and five original articles matching these criteria were initially selected, although only those articles that included specific gene expression results (77) were later considered for this review and separated into three major topics: the regulation of immunity and inflammatory gene expression in the gut, in inflammatory diseases of the gut and in the liver. Particular strains of *Bifidobacteria*, *Lactobacilli*, *Escherichia coli*, *Propionibacterium*, *Bacillus* and *Saccharomyces* influence the gene expression of mucins, Toll-like receptors, caspases, nuclear factor-κB, and interleukins and lead mainly to an anti-inflammatory response in cultured enterocytes. In addition, the interaction of commensal bacteria and probiotics with the surface of antigen-presenting cells *in vitro* results in the downregulation of pro-inflammatory genes that are linked to inflammatory signaling pathways, whereas other anti-inflammatory genes are upregulated. The effects of probiotics have been extensively investigated in animal models ranging from fish to mice, rats and piglets. These bacteria induce a tolerogenic and hyporesponsive immune response in which many genes that are related to the immune system, in particular those genes expressing anti-inflammatory cytokines, are upregulated. By contrast, information related to gene expression in human intestinal cells mediated by the action of probiotics is scarce. There is a need for further clinical studies that evaluate the mechanism of action of probiotics both in healthy humans and in patients with chronic diseases. These types of clinical studies are necessary for addressing the influence of these microorganisms in gene expression for different pathways, particularly those

that are associated with the immune response, and to better understand the role that probiotics might have in the prevention and treatment of disease.

Key words: Gene expression; Immunity; Immunotolerance; Inflammation; Microbiota; Probiotics; Gut; Liver

Core tip: Probiotics, which include live microorganisms as well as dead bacteria and bacterial molecular components, confer a health benefit to the host when administered in adequate amounts. Most of the published research articles that are devoted to probiotics evaluate the effects of probiotics on the prevention and treatment of diseases. However, only a few of these articles address the mechanism of action of these microorganisms. This paper reviews the mechanisms of action that have been proposed to explain the beneficial effects of probiotics, most of which involve gene expression regulation in specific tissues, particularly the intestine and liver. Several strains of *Lactic acid bacteria*, *Escherichia coli*, *Propionibacterium*, *Bacillus* and *Saccharomyces* influence the gene expression in gut and liver cells, leading mainly to anti-inflammatory responses and to the enhancement of immunotolerance to foreign antigens.

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INTRODUCTION

The potential for the positive manipulation of the gut microbiome through the introduction of beneficial microbes, as also known as probiotics, is currently an active area of investigation[1,2]. Probiotics are generally recognized as live microorganisms that confer a health benefit to the host when administered in adequate amounts[3], although dead bacteria and bacterial molecular components may also exhibit probiotic properties[4]. In particular, strains belonging to *Bifidobacterium* and *Lactobacillus* are the most widely used probiotic bacteria[5] and exert health-promoting properties, including, for example, the maintenance of the gut barrier function and the local and systemic modulation of the host immune system[6,7].

Clinical studies have demonstrated the clinical potential of probiotics against many diseases[5], such as allergic pathologies (including atopic eczema[8] and rhinitis[9]), diarrhea[10], inflammatory bowel disease (IBD)[11] and viral infection[7]. However, generalizations concerning the potential health benefits of probiotics should not be made because probiotic effects tend to be strain-specific[12,13].

Several important mechanisms underlying the beneficial effects of probiotics include the modification of the gut microbiota, the competitive adherence to the mucosa and epithelium, the strengthening of the gut epithelial barrier and the regulation of the immune system and inflammation[5,13]. Most of these mechanisms involve gene expression regulation in specific tissues, particularly the intestine and liver.

In this sense, the probiotic-mediated modulation of gene expression is an important issue that needs to be addressed. The expression of mucin genes (*MUC*) can be affected by probiotics. Likewise, toll-like receptor (*TLR*) and nucleotide-binding oligomerization domain (*NOD*)-receptor genes as well as pro-inflammatory transcription factors, cytokines, and apoptosis- related enzyme genes can also be affected by commensal bacteria.

In the present paper, we performed a systematic review of the probiotic-mediated modulation of gene expression that is associated with the immune system and inflammation. Between 1990 to February 2014, PubMed was searched for articles that were published in English using the MeSH terms "probiotics" and "gene expression" combined with "intestines", "liver", "enterocytes", "antigen-presenting cells", "dendritic cells", "immune system", and "inflammation". Two hundred and five original articles matching these criteria were initially selected, although only those articles that included specific gene expression results (77) were later considered for the review and separated into three major topics: the regulation of immunity and inflammatory gene expression in the gut, in inflammatory diseases of the gut and in the liver.

REGULATION OF IMMUNITY AND INFLAMMATORY GENE EXPRESSION IN THE GUT BY PROBIOTICS

The intestinal epithelium is constantly exposed to high levels of food and bacterial antigens. Under normal physiological conditions, the intestinal epithelial monolayer facilitates a controlled and selective flux of components between the lumen and the underlining mucosa[14]. The intestine and the gut-associated lymphoid tissue (GALT) are essential components of the immune defense, protecting the body from foreign antigens and pathogens while tolerating commensal bacteria and dietary antigens. The balance between tolerance and immunity in the intestine is, in part, dictated by antigen-presenting cell (APCs) populations in the gut. The dysregulation of this balance can contribute to the pathogenesis of numerous inflammatory conditions[15]. The inflammatory response in the intestinal tract is abrogated or avoided by the complex and well-regulated tolerance-inducing mechanisms in the GALT.

Several cells that are capable of antigen presentation exist in the GALT, including enterocytes and other intestinal epithelial cells (IEC), such as M cells, dendritic cells

(DCs), macrophages, and T and B cells[16]. Microbes activate DCs directly *via* the DCs' pattern recognition receptors (PRR) or indirectly by capturing the apoptotic/necrotic products of other cells that are dying in response to microbial exposure[16]. PRRs are comprised of TLRs, NOD-like receptors (NLRs), adhesion molecules and lectins[13].

Commensal bacteria and probiotics can interact with these cells, thereby exerting immunomodulatory effects. Below we review the probiotic modulation of the genes that are involved in inflammation and immunity in intestinal cultured cells, as well as in animals and humans.

Intestinal cultured cells

Although most studies regarding probiotics have reported anti-inflammatory effects, certain probiotic strains have been shown to exert pro-inflammatory effects. The effects of selected probiotics on the gene expression in intestinal cells, namely HT-29, T84, Caco-2, APCs (*e.g.*, RAW264.7 macrophages) and DCs, are reviewed below.

Enterocytes

Otte and Podolsky[17] (2004) provided insight into the molecular mechanisms by which probiotic bacteria interact with the intestinal surface. The effects of *Escherichia coli* (*E. coli*) Nissle 1917 (EcN), the probiotic mixture VSL#3, bacterial cell lysates, and conditioned media on monolayer resistance, interleukin (IL)-8 secretion, mucin gene expression, and tight junction proteins were evaluated by these authors in T84 and HT-29 cells. The EcN as well as debris and cell extracts induced pro-inflammatory IL-8 secretion from the IEC, whereas no such effect was observed with VSL#3. A soluble factor that was released from VSL#3 increased monolayer resistance, prevented the pathogen-induced decrease in monolayer resistance, and stabilized tight junctions. VSL#3 induced the expression of mucins in intraepithelial cells, and these organisms as well as EcN diminished *Salmonella dublin*-induced cell death[17].

Similarly, Mack *et al*[18] (1999) showed that *Lactobacillus plantarum* (*L. plantarum*) 299v and *Lactobacillus rhamnosus* GG (LGG) quantitatively inhibited the adherence of an attaching and effacing pathogenic *E. coli* to HT-29 IEC but did not inhibit the adherence to non-intestinal HEp-2 cells. Media enriched with MUC2 and MUC3 mucins were added exogenously to binding assays and were shown to inhibit the enteropathogen adherence to HEp-2 cells. The incubation of *L. plantarum* 299v with HT-29 cells increased the *MUC2* and *MUC3* mRNA expression levels. These authors proposed that probiotic agents, which can bind to epithelial cells *in vitro* and colonize the intestinal tract *in vivo*, induce epithelial cells to secrete mucins that diminish enteric pathogens that are bound to mucosal epithelial cells.

In another study using trans-epithelial electrical resistance (TEER) across Caco-2 cell layers, Anderson *et al*[19] (2010) described the effect of *L. plantarum* MB452 on tight junction integrity. *L. plantarum* MB452 caused a dose-dependent TEER increase across Caco-2 cell monolayers compared to a control medium. Nineteen tight junction-related genes had altered expression levels in response to *L. plantarum* MB452. *L. plantarum* MB452 also caused changes in tubulin and proteasome gene expression that may be linked to the intestinal barrier function.

Audy *et al*[20] (2013) investigated the differential gene expression of potential probiotics, LPS, and enteropathogenic bacteria on human IEC using a custom-designed expression microarray evaluating 17 specific host-response pathways. The main outcome was the differential regulation of the central mitogen-activated protein kinases (MAPK) signaling pathway in response to these probiotics, validated later with quantitative real-time PCR (qPCR).

Different strains of bifidobacteria were tested for their effects on HT-29 in *in vitro* models of the non-inflamed and inflamed intestinal epithelium. None of the tested bifidobacteria induced the activation of nuclear factor-kappa beta (NF-κB), indicating that bifidobacteria themselves do not induce inflammatory events. However, six out of eight tested bifidobacteria inhibited the LPS-induced NF-κB activation in a dose- and strain-dependent manner. By contrast, the NF-κB activation in response to challenge with tumor necrosis factor-alpha (TNF-α) was not affected by any of the tested bifidobacteria, indicating that the inhibitory effect of bifidobacteria is specific for LPS-induced inflammation in IECs. As shown with two of the six inhibition-positive bifidobacteria, the LPS-induced inhibition of NF-κB activation was accompanied by a dose-dependent decrease in IL-8 secretion and by lower mRNA levels for IL-8, TNF-α, cyclooxygenase 2 (COX-2), and intercellular adhesion molecule 1 (ICAM-1)[21].

Ruiz *et al*[22] (2005) characterized the molecular mechanisms for the initial interaction of probiotic *Bifidobacterium lactis* (*B. lactis*) strain BB12 with native and IEC lines. *B. lactis*-monoassociated Fisher F344 rats transiently induced the phosphorylation/activation of the NF-κB transcriptionally active subunit RelA and the MAPK p38 in native IECs 5 d after the initial bacterial colonization. Additionally, *IL-6* gene expression significantly increased after 5 d. The adenoviral delivery of the mutant IKK-beta and the inhibition of the p38 MAPK pathway significantly blocked the *B. lactis*-induced *IL-6* gene expression in IECs, suggesting that *B. lactis* triggers NF-κB and MAPK signaling to induce gene expression in the intestinal epithelium[22]. Likewise, the inhibition of IL-8 secretion by intestinal epithelial INT-407 cells that were incubated with *B. lactis* HN019 has been reported by Liu *et al*[23] (2010).

Three species of *Bifidobacterium* and *Enterococcus faecalis* differentially modulate the *in vitro* production of cytokines from LPS-stimulated RAW264.7 macrophages. The three species of *Bifidobacterium* significantly inhibited the phosphorylation of IκB-alpha that had been previously induced by LPS and modulated the *IL12p40*, *IL-1β*, and *TNF-α* mRNA levels. The mRNA levels of suppressor

of cytokine signaling (*SOCS*)1 or *SOCS3* increased in response to exposure to *Bifidobacterium* species combined with LPS. Conversely, *E. faecalis* combined with LPS induced significantly lower levels of *SOCS* mRNA than those in those cells that were induced by *Bifidobacterium* species combined with LPS[24].

Imaoka *et al*[25] (2008) co-cultured peripheral blood mononuclear cells (PBMNC) that were isolated from ulcerative colitis (UC) patients or HT-29 cells with heat-killed probiotic bacteria or the culture supernatant of *Bifidobacterium breve* (*B. breve*) strain Yakult (BbrY) or *Bifidobacterium bifidum* strain Yakult (BbiY) to estimate the amount of secreted IL-10 or IL-8. Both strains of the probiotic bifidobacteria induced IL-10 production in the peripheral blood mononuclear cells (PBMNC), although BbrY was more effective than was BbiY. The inhibitory effect of the conditioned medium (CM) that was derived from BbiY was greater than that of the CM that was derived from BbrY. The DNAs of the two strains had a comparable inhibitory activity against the secretion of IL-8. The conditioned medium of BbiY induced a repression of the *IL-8* gene with a higher expression of *IκB-zeta* mRNA 4 h after the culture of HT-29 cells compared to that in the absence of CM.

Boesten *et al*[26] (2011) determined the genome-wide transcriptional response of HT-29 cells to TNF-α following exposure to *B. breve* strains M-16V, NR246 and UCC2003. Approximately 54% of the TNF-α induced genes were solely suppressed by the presence of *B. breve* M-16V. These genes included apoptosis-related cysteine protease caspase 7 (*CASP7*), interferon regulatory factor 3 (*IRF3*), amyloid beta (A4) precursor protein-binding family A member 1 (*APBA1*), NADPH oxidase (*NOX5*), and leukemia inhibitory factor receptor (*LIFR*). The extracellular IL-8 concentration did not change, indicating that *B. breve* M-16V only partially modulates the TNF-α pathway.

Anti-inflammatory effects by *Lactococcus lactis* subsp. *cremoris* FC have been shown by Nishitani *et al*[27] (2009) in both *in vivo* and *in vitro* experimental models. *L. lactis* subsp. *cremoris* FC showed preventive and therapeutic effects with the amelioration of colon length and histological score and an attenuation of pro-inflammatory cytokine mRNA expression in inflamed colon tissue. In an *in vitro* gut inflammation model consisting of a co-culture of IEC (Caco-2) and macrophages (RAW264.7), treatment with the probiotic downregulated the pro-inflammatory *IL-8* mRNA expression in Caco-2 cells and inhibited the nuclear translocation of NF-κB in RAW264.7 cells.

Likewise, O'Flaherty and Klaenhammer (2012) demonstrated that the exposure time to *L. acidophilus* impacted the immune-related gene expression profiles of IECs. In this study, a 1-h rather than a 4- or 8-h exposure time resulted in the maximal differential expression of immune-related genes and genes that are targeted by the NF-κB complex. After an initial exposure to *L. acidophilus*, the expression of the immune-related genes returned to baseline levels[28].

Oksaharju *et al*[29] (2011) examined the effects of LGG, *L. rhamnosus* Lc705, *Propionibacterium freudenreichii* ssp. *shermanii* JS, *Bifidobacterium animalis* (*B. animalis*) spp. *lactis* Bb12 and their combination on human mast cell gene expression. The LGG and *L. rhamnosus* Lc705 suppressed genes that encoded the allergy-related high-affinity IgE receptor subunits α and γ (*FCER1A* and *FCER1G*, respectively) and the histamine H4 receptor. The LGG, *L. rhamnosus* Lc705 and the combination of the four probiotics had the strongest effect on the expression of genes involved in mast cell immune system regulation and on several genes that encoded proteins with a pro-inflammatory impact, such as *IL-8* and *TNF-α*, whereas genes that encoded proteins with anti-inflammatory functions, such as *IL-10*, were upregulated.

Paszti-Gere *et al*[30] (2012) investigated the immunomodulatory effect of the culture supernatant of five bacterial strains in a non-transformed cell line that was derived from porcine jejunal epithelial IPEC-J2 cells that had been previously subjected to oxidative stress with hydrogen peroxide. *L. plantarum* 2142 had significantly decreased the *IL-8* and *TNF-α* mRNA levels with the concomitant upregulation of *HSP70* gene expression. However, *B. animalis* subsp. *lactis* BB-12 and *Bacillus amyloliquefaciens* CECT 5940 had the opposite effect, increasing the gene expression of either *IL-8*, *TNF-α* or both. No effects were observed with *Enterococcus faecium* CECT 4515 or *Lactobacillus casei* Shirota.

Zanello *et al*[31] (2011) reported that the yeast *Saccharomyces cerevisiae* (strain CNCM I-3856) modulates transcript and protein expression in the inflammation, recruitment and activation of immune cells in differentiated porcine intestinal epithelial (PIE) IPEC-1 cells and demonstrated that viable *S. cerevisiae* inhibits the enterotoxigenic *E. coli* (ETEC)-induced expression of pro-inflammatory transcripts (*IL-6*, *IL-8*, *CCL20*, *CXCL2*, and *CXCL10*) and proteins (IL-6, IL-8). This inhibition was associated with a decrease in ERK1/2 and p38 MAPK phosphorylation, an agglutination of ETEC by *S. cerevisiae* and an increase in the anti-inflammatory *PPAR-γ* nuclear receptor mRNA level.

Latvala *et al*[32] (2011) investigated which non-pathogenic bacteria could stimulate the expression of *SOCS3*, which controls the expression of pro-inflammatory cytokine genes in human primary macrophages. *Lactobacillus* and *Streptococcus* species induced *SOCS3* mRNA expression directly in the absence of protein synthesis and indirectly *via* bacteria-induced IL-10 production. The MAPK p38 signaling pathway played a key role in the bacteria-induced *SOCS3* gene expression.

The gene expression profiles of Caco-2 cells that were treated with EcN were analyzed *via* a microarray analysis by Ukena *et al*[33] (2005). The results revealed 126 genes that were specifically regulated after treatment with EcN. A second human intestinal cell line as well as pieces of small intestine from BALB/c mice were used to confirm the regulatory data of selected genes by qPCR. Among others, the expression of genes encoding the

pro-inflammatory molecules monocyte chemo-attractant protein-1 (*MCP-1*), macrophage inflammatory protein-2 alpha (*MIP-2α*) and macrophage inflammatory protein-2 beta (*MIP-2β*) increased up to 10-fold. Elevated levels of *MCP-1* and *MIP-2α* mRNA were confirmed using LoVo cells. *MCP-1* gene expression was also upregulated in mouse intestinal tissue.

Wang *et al*[34] (2013) analyzed the immunomodulatory effects of *Lactobacillus casei* Zhang (LcZ) in RAW264.7 macrophages. The immunostimulating effects of live LcZ were significantly attenuated in heat-killed LcZ. The live LcZ promoted *TLR2* mRNA transcription, whereas the heat-killed LcZ enhanced transcription of *TLR2*, *TLR3*, *TLR4*, and *TLR9*.

The effects of *L. plantarum* genomic DNA on the LPS-induced MAPK activation, NF-κB activation, and the expressions of TNF-α, IL-1 receptor-associated kinase M, and the pattern recognition receptor were studied in human monocyte-like cells. *L. plantarum* genomic DNA inhibited this signaling pathway and TNF-α production accompanied by the suppression of *TLR2*, *TLR4*, and *TLR9* and the induction of IL-1 receptor-associated kinase M, a negative regulator of TLR[35].

Cammarota *et al*[36] (2009) analyzed the probiotic potential of *L. plantarum* DSMZ 12028 *in vitro* using the pathogen *E. coli* K4 and a certified probiotic, *L. paracasei* F19, as controls in Caco-2 and HT-29 cells. Real-time PCR was used to monitor the expression of TLRs and cytokines in a monocytic cell line (THP-1) following bacterial exposure. *L. plantarum* downregulated *TLR* mRNA levels with the exception of *TLR2*, while *L. paracasei* F19 and *E. coli* K4 significantly upregulated *TLR2* and 4, respectively.

Ghadimi *et al*[14] (2010) tested the effects of DNA from LGG and *Bifidobacterium longum* on the TLR9 signaling cascade and the barrier integrity of polarized HT-29 and T84 cells that had been previously treated with TNF-α. The HT-29 and T84 cells enhanced expression of TLR9 in a specific manner, which was subsequently associated with the attenuation of TNF-α-induced NF-κB activation and NF-κB mediated *IL-8* expression. *TLR9* silencing abolished this inhibitory effect. Apically applied LGG DNA attenuated the TNF-α enhanced NF-κB activity by reducing the IκBα degradation and p38 phosphorylation. Likewise, LGG DNA diminished the TNF-α-induced membrane integrity reduction.

Eleven different probiotic strains with immunoregulatory capabilities used a common mechanism to induce tolerance in PIE cells. Immunoregulatory strains interacted with TLR2, upregulated the expression of *ubiquitin-editing enzyme A20* in PIE cells, and beneficially modulated the subsequent TLR4 activation by reducing the activation of MAPK and NF-κB pathways and the production of pro-inflammatory cytokines[37].

Gao *et al*[38] (2012) analyzed the myeloid differentiation primary response protein 88 (*MyD88*) expression using small interfering RNA in HT-29 cells. The knockdown of *MyD88* did not affect *Clostridium butyricum*-induced elevated levels of NF-κB, IL-8, IL-6, and TNF-α, suggesting a MyD88-independent route for TLR signal transduction in human epithelial cells. However, a significant reduction in the levels of NF-κB, *IL-8*, *IL-6*, and *TNF-α* was evident in the absence of *TLR2* expression, indicating the need for TLR2 in *C. butyricum* recognition.

In addition, the modulation of *TLR-4* gene expression by *Bacillus mesentericus* TO-A, *Clostridium butyricum* TO-A, and *Streptococcus faecalis* T-110 in human colonic epithelial cells HT-29 was investigated by Isono *et al*[39] (2007). Culture supernatants or heat-killed bacteria were added to HT-29 cells. Treatment with *C. butyricum* TO-A culture supernatant downregulated the *TLR4* mRNA and protein levels but only in the presence of butyrate. This effect seems to be mediated by the transcription factor PU.1.

Bacillus species, non-pathogen spore-forming microorganisms, are being used as probiotics owing to evidence indicating that these species are important for the development of a robust gut-associated lymphoid system[40]. Huang *et al*[40] (2008) tested the ability of six *Bacillus* strains (*B. subtillis* PY79, HU58 and HU68, *B. licheniformis* HU14 and HU53, and *B. flexus* HU37), from the human gut to induce the pro-inflammatory cytokines TNF-α, IL-6 and IL-1α in cultured RAW264.7 macrophages. The highest levels of induction were with TNF-α followed by IL-6, for which all of the strains induced expression. IL-1α was only induced by *B. subtillis* PY79 and HU68 and *B. licheniformis* HU14. The authors also determined the expression of *TLR-2* and *TLR-4* in RAW264.7 macrophages that were co-incubated with either spores or vegetative cells of *B. subtillis* PY79, demonstrating a clear temporal increase in the expression of both *TLR-2* and *TLR-4* over time. A closer inspection of TLR-2 and TLR-2 induction indicated that for vegetative cells, there was a progressive increase in the expression during the 6-h period of evaluation. However, for induction by spores, there was a significant increase in the expression only between h 4 and 6.

In summary, specific strains of bifidobacteria, lactobacilli, EcN, *Propionibacterium*, *Bacillus* and *Saccharomyces* influence the gene expression of *mucins*, *TLRs*, *caspases*, *NF-κB*, and *ILs*, leading mainly to an anti-inflammatory response. Notwithstanding, specific responses are dependent on particular strains and intestinal cell types.

Dendritic cells

Dendritic cells (DCs) are potent APCs that induce a primary immune response against microbial infection and other stimuli[41]. Upon activation, DCs upregulate co-stimulatory molecules and migrate to secondary lymphoid organs where they activate antigen-specific T cells. The types of cytokines and other factors that are secreted by DCs and other innate immune cells program the differentiation of naïve Th0 into Th1, Th2 or Th17 effector cells or Treg cells[16]. Understanding the direct interaction between commensal bacteria and DCs is particularly important in determining how the immune system of the

gut is able to distinguish these bacteria from pathogens and elicit a tolerogenic response[42].

In this sense, the mixture probiotic VSL#3 induces the release of significant levels of IL-10 in DC culture supernatants if added over a period of 3 d. IL-10 is a critical Th2 cytokine that suppresses IL-12 production and therefore other cytokines, such as interferon gamma (IFN-γ) and TNF-α[43]. Drakes *et al*[41] (2004), using DCs that were generated from mice bone marrow showed that VSL#3 induces the release of higher amounts of IL-10 and more modest levels of IL-12. This study highlighted the fact that the presence of probiotic bacteria during the development of DCs influences the outcome of the immune response[41]. Changes in the DC cell surface phenotypes may result in altered DC function or cytokine production[44]. To determine the action of probiotic VSL#3 on the DC surface phenotypes, Drakes *et al*[41] (2004) added the probiotic mixture during the DC generation phase, observing that the addition of 10^3 and 10^5 organisms/mL did not alter the immature phenotype of DC; however, higher concentrations (10^7 organisms/mL) upregulated the DC co-stimulatory molecule expression of CD80, CD86, CD40, and major histocompatibility complex class (MHC) class Ⅱ I-A^d.

In agreement with the aforementioned study, Mastrangeli *et al*[45] (2009) demonstrated that the effects of VSL#3 on BALB/c mice bone marrow (BM) DC maturation were time- and dose-dependent and peaked after 18 h of co-culture at the 10^7-organisms/mL dose. Live and sonicated VSL#3 induced a significant upregulation of CD83, CD86 and MHC class Ⅱ. Moreover, both of the VSL#3 preparations were as effective as the LPS control in inducing DC maturation. In addition, live VSL#3 at the same dose induced a significant production by BMDCs of high levels of IL-12p70 and IL-10, significantly higher than those obtained after LPS stimulation. However, sonicated VSL#3 induced lower levels of IL-12 and IL-10, which were in any case significantly higher than those that were induced by LPS and higher than the control (medium only) levels.

In addition, D'Arienzo *et al*[46] (2009), using BMDCs that were generated from a DQ8 tg mouse, a well characterized model of food antigen sensitivity, demonstrated that incubation with 10^8 colony-forming units (CFU)/mL of *L. paracasei* IMPC2.1, *L. plantarum* ITM21B, *L. fermentum* DRL38 and *B. lactis* NCCC2818 two days before cell harvesting in the presence/absence of LPS stimulated CD86 expression, with *L. plantarum* and *L. paracasei* inducing higher expression levels. However, no strains modulated the expression of CD11c or CD80 or further enhanced the LPS-induced CD86 expression. These data clearly indicate that probiotic exposure alters the immature phenotype of DCs. Regarding the cytokine analysis, immature BMDCs revealed no significant differences for IL-12, whereas in mature BMDCs (LPS-treated), *L. plantarum* and *L. fermentum* enhanced IL-12 production. In addition, the IL-10 levels were low in both un-stimulated and LPS-induced DCs. Moreover, the *L. fermentum, L. plantarum* and *B. lactis* strains induced a significant increase in TNF-α in immature BMDCs, while *L. fermentum* and *B. lactis* induced a significant increase in mature BMDCs[46].

Furthermore, the incubation of murine BMDCs with heat-killed *L. lactis* subsp. *cremoris* FC and *L. lactis* subsp. *cremoris* ATCC 19257 (as a control strain) enhanced the production of IL-10, IL-12, IL-6 and TNF-α in a dose-dependent manner[47]. Compared to *L. lactis* subsp. *cremoris* ATCC 19257, *L. lactis* subsp. *cremoris*, FC induced higher levels of IL-12 production, whereas treatment with *L. lactis* subsp. *cremoris* ATCC 19257 induced higher levels of IL-6 production. Moreover, the production of IL-10, IL-12, IL-6 and TNF-α that was induced by *L. lactis* subsp. *cremoris* FC was almost completely depleted in the culture supernatants of BMDCs that were derived from $MyD88^{-/-}$ mice. This result suggests that *L. lactis* subsp. *cremoris* FC activates DCs and induces cytokine production through a MyD88-dependent pathway[47].

Certain *Lactobacillus* spp. posses strong IL-12- inducing properties whose production depends on the up-regulation of type I IFNs that are mainly involved in the immune response against viral infection[48]. In this sense, Weiss *et al*[48] (2010) investigated whether *L. acidophilus* NCFM could induce anti-viral defense gene expression in immature murine DCs. A genome-wide microarray analysis revealed that the induction of virus-related genes was most prominent for the *RSAD2* gene (radical S-adenosyl methionine domain-containing 2). The genes encoding IFN-induced T cell-specific GTPase (*TGTP2*), IFN-stimulated gene 15 (*ISG15*), IFN-regulatory factor (*IRF-7*) and *TLR-3*, all of which are involved in the viral immune defense that is induced by IFN-β, were similar among the highest significantly upregulated genes. The upregulation of viral response genes seems to be caused by a rapid, strong and transient upregulation of *IFN-β*, which in turn stimulates the transcription of a number of other genes that are involved in viral defense. Moreover, the upregulation of *IFN-β* in DCs was much stronger upon stimulation with *L. acidophilus* NCFM compared with that of cells that were stimulated with Poly I:C, EcN and *B. bifidum* Z9. In addition, *IFN-β* expression was markedly reduced in $TLR\text{-}2^{-/-}$ DCs, dependent on endocytosis, the major cause of the induction of *IL-12* and *TLR-3* in DCs that were stimulated with *L. acidophilus* NCFM. These results reveal that certain lactobacilli trigger the expression of viral defense genes in DCs in a TLR-2-dependent manner.

The stimulation of chicken bone marrow dendritic cells (chi-BMDCs) with LPS, *Saccharomyces boulardii* (Sb), *Bacillus subtillis* B10 (Bs), and a co-culture of Sb+Bs and phosphate-buffered saline (PBS) as a control group revealed that the treatment groups modulate the phenotype and biological functions of chi-BMDCs. The gene expression levels of *MHC-II*, *CD40*, *CD80* and *CD86* were upregulated in the stimulated groups. Furthermore, the cell surface receptors *TLR*-1, 2, 4 and 15 showed significant upregulation at the mRNA levels. In addition, the levels of the associated factors *MyD88*, *TRAF6*, *TAB1*

and *NF-κB* mRNA increased in all of the treatment groups compared to those of the control group. However, the NF-κB response was significantly higher in the LPS treatment. Regarding the cytokine production levels, the probiotics improved the production of IL-1β, IL-17, IL-4, transforming growth factor beta (TGF-β) and IL-10, whereas IL-8 and IFN-γ were downregulated[49].

In an interesting study, Latvala *et al*[50] (2008) stimulated human monocyte-derived DCs (moDCs) with nine probiotic bacteria (two well-characterized probiotics, *L. rhamnosus* GG and *B. animals* Bb12, and seven potentially probiotic bacteria). These authors showed that *S. thermophilus* THS efficiently induced TNF-α, IL-6 and IL-12. *B. animalis* Bb12 and *B. breve* Bb99 were potent inducers of *TNF-α, IL-1β, IL-6, IL-10, IL-12*, and *IFN-γ*. However, *B. longum* strain 1/10 was not as efficient as *B. animalis* Bb12 and *B. breve* Bb99 in inducing cytokine production. *L lactis* subsp. *cremoris* ARH74 and *L. helveticus* 1129 were as efficient as bifidobacteria. By contrast, LGG and LC705 as well as *L. mesenteroides* subsp. *cremoris* PIA2 were poor inducers of cytokine production in moDCs. The cytokine responses were directly associated with the bacterial dose; a 40:1 bacteria:host cell ratio showed the highest cytokine production levels. In addition, all of the studied bacteria induced CCL20 production in a dose-dependent manner, whereas none of the aforementioned bacteria were able to induce CCL19 production[50]. According to the gene expression levels, *L. mesenteroides* subsp. *cremoris* PIA2, LGG and LC705 were weak inducers of moDC cytokine responses. By contrast, bifidobacteria, *S. thermophilus* THS, *L. lactis* ARH74 and *L. helveticus* 1129 induced the production of the pro-inflammatory cytokines and chemokines TNF-α, IL-6, IL-12, CCL20 and CXCL10. In addition, *S. thermophilus* THS and *B. breve* Bb99 stimulated the highest upregulation of human leukocyte antigen (HLA) class Ⅱ (ratio 10:1) in the moDCs, whereas a ratio of 40:1 for *L. lactis* subsp. *cremoris* ARH74 was required to maximize HLA class Ⅱ induction. MoDCs that were stimulated with probiotic bacteria matured equally well as cells that were stimulated with pathogenic *S. pyogenes,* a known inducer of moDC maturation[50].

Furthermore, human myeloid DCs that were isolated from PBMCs and treated with a ratio of 10:1 for *B. infantis* or *L. salivarius* for 48 h stimulated a significant increase in IL-10 and TNF-α secretion compared to that of the untreated DCs[51].

In an elegant study, Evrard *et al*[52] (2011) investigated the effects of *Lactobacillus rhamnosus* 35 (Lcr35) on human PBMNC, using a multiplicity of infection (MOI) ranging from 100 to 0.01. These authors' flow cytometry data indicated the Lcr35-induced semi-maturation of DCs with the upregulation of the expression of HLA-DR, CD86 and CD83 as well as the upregulation of CCR7. The Lcr35-induced phenotype was intermediate between that of immature DCs and that of fully mature LPS-induced DCs, a so-called semi-mature DCs phenotype. In addition, a gene array analysis showed great dose-dependent variations. At a MOI of 10823 genes were overexpressed (with a 3-fold change threshold), and 859 were downregulated. Most of these genes were involved in four main biological processes: immune and inflammatory responses, antigen processing and presentation *via* MHC, intracellular signaling and signal transduction. At an MOI of 0.01, the expression of 58 genes was upregulated, while that of 138 genes was downregulated. In addition, at an MOI of 10, these authors observed that the expression of genes directing a Th1 (*IL12A, IL12B* and *TNF-α*) or a Th17 (*IL1B, IL6, IL23A, Il12B* and *TGFB1)* profile was strongly upregulated. A comparison of these results with the Torri's model[53] of the molecular signature of inflammation indicated that at an MOI of 10, Lcr35 exhibited a pro-inflammatory DC phenotype in response to 76% of the genes, neither a pro- nor an anti-inflammatory DC phenotype in response to 11% and an anti-inflammatory DC phenotype in response to 13%. A quantitative reverse transcriptase PCR analysis revealed that at an MOI of 10, the transcription of the *CCL20*, *IL1B*, *IL12B* and *TNF-α* genes increased by approximately 100-, 300-, 400- and 200-fold, respectively. In addition, the expression of *IL-23* and *PYSG2* increased, although less strikingly, and the *CCR7*, *FCAR*, and *IL-8* genes were upregulated[52]. Regarding cytokine production, a strong dose-dependent increase of IL-12p40, TNF-α and, to a lesser extent, IL-10 was induced by Lcr35 compared to the untreated immature DCs[52].

Most of the studies on probiotic activity have been performed in human moDCs or murine DCs, which are different from human gut DCs[54]. Recently, our research group[55] co-incubated intestinal-like human DCs from cord blood CD34+ progenitor cells[56] with *B. breve* CNCM I-4035 or its cell-free supernatant (CFS), *S. typhi* or a combination of these treatments for 4 h. These treatments upregulated *TLR-9* gene transcription. In addition, CFS was a more powerful inducer of *TLR-9* expression than were the probiotic bacteria in the presence of *S. typhi.* In addition, both of the treatments induced Toll-interacting protein (*TOLLIP*) gene expression. Furthermore, CFS decreased the pro-inflammatory cytokines and chemokines in DCs that were challenged with *S. typhi.* By contrast, *B. breve* CNCM I-4035 was a potent inducer of the pro-inflammatory cytokines TNF-α, IL-8 and RANTES (regulated upon activation normal T cell expressed, and presumably secreted) as well as of anti-inflammatory cytokines, including IL-10. CFS restored the TGF-β levels in the presence of *S. typhi.* These results indicate that *B. breve* CNCM I-4035 affects the intestinal immune response, whereas its supernatant exerts anti-inflammatory effects that are mediated by DCs[55]. Likewise, *Lactobacillus paracasei* and its CFS also decreased the pro-inflammatory cytokines and chemokines in human intestinal DCs that were challenged with *Salmonella.* CFS was as effective as the bacteria in reducing pro-inflammatory cytokine expression. These treatments strongly induced the transcription of the *TLR-9* gene. In addition, an upregulation of the *CASP8* and *TOLLIP* genes was

also observed. *L. paracasei* CNCM I-4034 was a potent inducer of TGF-β2 secretion, whereas the supernatant enhanced the innate immunity through the activation of TLR signaling[7]. Giahi *et al*[4] (2012) investigated the effect of heat-inactivated LGG and *Lactobacillus delbrueckii* subsp. *bulgaricus* on the expression of *TLR4* and signaling factors, such as *p38 MAPK* and *IκB*, at the transcription level in human monocyte-derived DCs. LGG significantly downregulated the expression of *p38*, while the *IκB* expression was significantly reduced in the *Lactobacillus delbrueckii* subsp. *bulgaricus*-treated DCs.

In summary, the interactions of commensal bacteria and probiotics with the surface of APCs, mainly through TLR, in most studies result in the downregulation of pro-inflammatory genes that are linked to inflammatory signaling pathways, whereas other anti-inflammatory genes are upregulated. The probiotic-mediated increase in TGF-β and IL-10 expression can help to explain the immunotolerance process that is mediated by these microorganisms.

Animal studies

Matsumoto *et al*[57] (2011) supplemented the diet of 10-mo-old Crj:CD-1 female mice with *B. animalis* subsp. *lactis* LKM512 for 11 mo. The colonic mucosal function was better in LKM512 mice, with increased mucus secretion and better maintenance of tight junctions. *B. animalis* subsp. *lactis* LKM512 also downregulated the expression of aging-associated and inflammation-associated genes. The gene expression levels in 21-mo-old *B. animalis* subsp. *lactis* LKM512-treated mice resembled those in 10-mo-old untreated (younger) mice.

Ohtsuka *et al*[58] (2012) examined the immunomodulatory effects of *B. breve* M-16V during early infancy in rat pups during the newborn or weaning period. The numbers of upregulated and downregulated genes were greater during the weaning period than during the newborn period, and these were greatest in the colon, with fewer genes altered in the small intestine and the fewest in the spleen. The expression of inflammation-related genes, including lipoprotein lipase (*LPL*), glutathione peroxidase 2 (*GPX2*), and lipopolysaccharide-binding protein (*LBP*), was significantly reduced in the colon during the newborn period. In weaning rat pups, the expression of CD3d, a cell surface receptor-linked signaling molecule, was significantly enhanced in the colon; however, the expression of co-stimulatory molecules was not enhanced.

Trevisi *et al*[59] (2008) investigated the potential synergic action of one prebiotic with increasing dietary doses of a probiotic strain of *B. animalis* on the translocation of bifidobacteria and on TLR gene expression in different organs of weaned piglets. The linear effect of the dose of *B. animalis* on the expression of the TLR2-encoding gene in the lymph nodes was observed when fructo-oligosaccharides were added to the diet. TNF-α-encoding gene expression was positively correlated with the *TLR4*- and *TLR2*-encoding genes.

The effect of live *L. plantarum* 299v (Lp299v), *Lactobacillus rhamnosus* R0011 (LrR0011), and *Bifidobacterium bifidum* R0071 (BbR0071) were analyzed in rats. After killing the rats *via* CO_2 suffocation, the *MUC2*, *MUC3*, neuronal apoptosis inhibitor protein (*NAIP*), human inhibitor of apoptosis protein 1/cellular inhibitor of apoptosis 2 (*HIAP1/cIAP2*), and human inhibitor of apoptosis protein 2/cellular inhibitor of apoptosis 1 (*HIAP2/cIAP1*) mRNA and protein levels were analyzed *via* qPCR and immunohistochemistry. Live Lp299v, BbR0071, and LrR0011 increased MUC3 protein and mRNA expression in the jejunum and ileum. A heat-killed non-adherent derivative of Lp299v failed to induce *MUC3* expression. Lp299v did induce the expression of *HIAP2/cIAP1* and *NAIP* expression. *MUC3* mucin expression was elevated for 5 d after the oral administration of Lp299v; however, this effect was not sustained despite ongoing daily ingestion of a probiotic[60].

Three groups of rats orally received LGG, *B. animalis* MB5, or PBS for 28 d. Each group was divided into two subgroups of tolerized or immunized rats receiving ovalbumin (OVA; 7 mg) or PBS on days 7, 9, and 11. All of the rats were immunized with OVA (300 mg) on days 14 and 21. In the tolerized rats, the OVA-induced proliferative response of mesenteric lymph nodes (MLN) and spleen cells did not differ from those of the control, indicating that the two probiotics maintained the tolerance. *Lactobacillus rhamnosus* GG and *B. animalis* MB5 in the immunized rats reduced the OVA-induced proliferative response in the MLN but not in the spleen, whereas the proliferative response to anti-CD3 and concanavalin A of the MLN and spleen cells as well as the delayed-type hypersensitivity reaction were not affected by probiotic treatment, indicating that the OVA-specific hyporesponsiveness is restricted to intestinal immunity. This hyporesponsiveness was associated with CD4+CD25+Foxp3+T cell expansion, increased IL-10 and TGF-β after LGG, and increased apoptosis after *B. animalis* MB5 in MLN[61].

The effects of *L. acidophilus*, inulin, or both (synbiotic) on pathogen-induced inflammatory responses, NF-κB, and Smad 7 signaling were evaluated in a murine model to parallel infantile enteric disease. Newborn mice were inoculated bi-weekly for 4 wk with *L. acidophilus*, inulin, or synbiotic and challenged with *Citrobacter rodentium* (Cr) at 5 wk. The results showed that the host defense against Cr infection correlated with enhanced colonic *IL-10* and *TGF-β* expression and the inhibition of NF-κB in synbiotic-treated mice, whereas mice that were pretreated with synbiotic, *L. acidophilus*, or inulin had an attenuation of Cr-induced Smad 7 expression[62].

Deng *et al*[63] (2013) evaluated the ability of the co-administration of *Bacillus subtillis* RJGP16 and *Lactobacillus salivarius* B1 to stimulate local immune responses. Thirty two newborn piglets were divided into four groups and were orally administered with different combinations of probiotics (none; RJGP16; B1; RJGP16 and B1) at the ages of 0, 7 and 11 d. These authors analyzed the parameters of the mucosal immunity of piglets one week after weaning. The results showed that the expressions of *IL-6*

in the duodenum and ileum and of porcine beta-defensins (*pBD*)-*2* in the duodenum significantly increased with the co-administration of RJGP16 and B1. Additionally, the expression and release of TLR-2 and the number of IgA-producing cells increased.

In addition, the cytokine gene expression in the spleen and in Peyer's patches of mice that received dahi supplemented with *L. casei* was analyzed. The mRNA levels of *IFN-γ* in both the spleen and in the Peyer's patches were significantly increased in the probiotic dahi group after 14 and 28 d compared with those of the control and dahi groups. The abundance of *IL-2* mRNA also significantly increased in the Peyer's patches of probiotic-fed animals[64].

The effects of lactic acid bacteria on the control of lactococcosis and the impact of probiotics on the expression of immune-related genes were investigated in the head kidney and intestine of rainbow trout. *L. plantarum*, *Lactococcus lactis* and *Leuconostoc mesenteroides* were administered orally for 36 d. Twenty-one days after the start of the feeding period, the fish were challenged with *Lactococcus garvieae*. Only the fish that were fed the diet containing *L. plantarum* showed significantly improved protection against *L. garvieae* compared to that of the control. Subsequently, qPCR was used to measure the mRNA levels of *IL-1β*, *IL-8*, *IL-10* and *TNF-α* in the head kidney and of *IL-8*, *TLR5* and *IgT* in the intestine of the control and *L. plantarum* groups. The expression of *IL-1β, IL-10* and *TNF-α* was significantly upregulated by *L. plantarum*. Moreover, the mRNA levels of *IL-10*, *IL-8* and *IgT* were significantly higher in the *L. plantarum* group after *L. garvieae* infection, suggesting that *L. plantarum* can stimulate the immune response of rainbow trout. These findings demonstrate that direct probiotic-host interactions with the intestine are not always necessary to induce host stimulatory responses that ultimately enhance disease resistance[65].

Pirarat *et al*[66] (2011) investigated the modulation of immunity in Nile tilapia by LGG and found higher levels of *TNF-α* and *IL-1* gene expression. As described before for intestinal cultured cells, probiotic bacteria influence the immune response and inflammation by controlling TLR, NF-κB and cytokine gene expression in animal models.

In summary, the effects of probiotics have been extensively investigated in animal models ranging from fish to mice, rats and piglets. These bacteria induce a tolerogenic and hyporesponsiveness immune response in which many genes that are related to the immune system, in particular those expressing anti-inflammatory cytokines, are upregulated.

Human studies

Compared to intestinal cultured cells and animal models, there are only a few studies in humans evaluating the effects of probiotic bacteria on the expression of genes that are involved in immunity and inflammation.

Van Baarlen *et al*[67] (2011) obtained transcriptomes in an intervention study after a double-blind placebo-controlled cross-over study to investigate the *in vivo* mucosal responses of healthy adults to probiotics. In the mucosa of the proximal small intestine of healthy volunteers, probiotic strains from the species *Lactobacillus acidophilus*, *L. casei*, and *L. rhamnosus* each induced differential gene-regulatory networks and pathways in the human mucosa. Comprehensive analyses revealed that these transcriptional networks regulate major basal mucosal processes and uncovered remarkable similarity to the profiles that were obtained in response to specific bioactive molecules and drugs.

Lammers *et al*[68] (2005) analyzed the expression of *IL-1β*, *IL-6*, *IFN-γ*, *TNF-α*, *IL-12*, *IL-10*, *TGF-β* and *IL-8* in endoscopic samples. The data showed that patients who were treated with probiotics had significantly lower mucosal mRNA expression levels of *IL-1β*, *IL-8*, and *IFN-γ* compared with those of the placebo-treated patients.

Di Caro *et al*[69] (2005) evaluated the gene expression pattern that was induced by *Bacillus clausii* in the intestinal mucosa of healthy individuals. Six male patients who were affected by mild esophagitis were treated for one month with esomeprazole and were randomly selected to receive or not *B. clausii* (groups Ⅰ and Ⅱ, respectively). Duodenal biopsies were taken pre- and post-treatment to identify the modification of gene expression. After *B. clausii* administration, a total of 158 and 265 genes were upregulated and downregulated, respectively. *Bacillus clausii* mainly affected the expression of genes that are involved in the immune response and inflammation, apoptosis and cell growth, cell differentiation, cell-cell signaling, cell adhesion, signal transcription and transduction.

Information regarding gene expression in human intestinal cells that are mediated by the action of probiotics is very scarce (Table 1 summarizes the principal results). Hence, new studies should consider this aspect to ascertain the mechanism of action of specific strains in the modulation of the immune response and inflammation, mainly in chronic disorders of the gut.

REGULATION OF GENE EXPRESSION BY PROBIOTICS IN INFLAMMATORY DISEASES OF THE GUT

The intestinal microbiota plays essential roles in nutrient absorption and metabolism, immune stimulation, satiety and pain. An altered composition of intestinal microbiota has been reported in IBD patients[70]. IBD is linked to post-inflammatory and stress-correlated factors that cause changes in the perception of visceral events.

Probiotic bacteria may be effective in treating IBD symptoms[71]. The effects of *B. breve* (DSMZ 20213) and LGG on the expression of IL-17 and IL-23, which play an important role in IBD, and on the epigenetic machinery were evaluated in a 3D co-culture model that was composed of human intestinal HT-29/B6 or T84 cells and PBMCs. The cells were treated with LPS in the pres-

Table 1 Regulation of immunity and inflammatory gene expression in the gut by probiotics

Study	Probiotic strain	Genes involved
Intestinal cultured cells		
Enterocytes		
Ghadimi *et al*[14]	DNA from *L. rhamnosus* GG and B. longum	*TLR-9* and *IL-8*
Otte *et al*[17]	*E. coli* Nissle 1917 and VSL#3	Mucins genes
Mack *et al*[18]	*L. plantarum* 299v and *L. rhamnosus* GG	*MUC2* and *MUC3*
Anderson *et al*[19]	*L. plantarum* MB452	Tight junction-related genes
Audy *et al*[20]	Lactobacilli and bifidobacteria strains	MAPK signaling pathway
Riedel *et al*[21]	Bifidobacteria strains	NF-κB activation, *IL-8*, *TNF-α*, *COX-2*, and *ICAM-1*
Ruiz *et al*[22]	*B. lactis* BB12	NF-κB, MAPK signaling, and *IL-6*
Liu *et al*[23]	*B. lactis* HN019	*IL-8*
Okada *et al*[24]	Bifidobacteria	*IL12p40*, *IL-1β*, *TNF-α*, and *SOCS1*
Imaoka *et al*[25]	*B. breve* strain Yakult and *B. bifidum* strain Yakult	*IL-8* and *IκB-zeta*
Boesten *et al*[26]	*B. breve* strains M-16V, NR246 and UCC2003	*CASP7*, *IRF3*, *A4*, *APBA1*, *NOX5*, and *LIFR*
Nishitani *et al*[27]	*L. lactis* subsp. *cremoris* FC	*IL-8*
O'Flaherty *et al*[28]	*L. acidophilus*	NF-κB signaling
Oksaharju *et al*[29]	Bifidobacteria, lactobacilli, and *P. freudenreichii*	*FCER1A*, *FCER1G*, *IL-8*, *TNF-α*, and *IL-10*
Paszti-Gere *et al*[30]	*L. plantarum* 2142 and bifidobacteria	*IL-8* and *TNF-α*
Zanello *et al*[31]	*Saccharomyces cerevisiae* CNCM I-3856	*PPAR-γ*
Latvala *et al*[32]	*Lactobacillus* and *Streptococcus* species	*SOCS3*
Ukena *et al*[33]	*E. coli* Nissle 1917	*MCP-1*, *MIP-2alpha* and *MIP-2beta*
Wang *et al*[34]	*L. casei* Zhang	*TLR2*, *TLR3*, *TLR4*, and *TLR9*
Kim *et al*[35]	*L. plantarum* genomic DNA	*TLR2*, *TLR4*, and *TLR9*
Cammarota *et al*[36]	*L. plantarum* DSMZ 12028	*TLR2* and *TLR4*
Tomosada *et al*[37]	Eleven different probiotic strains	MAPK and NF-κB pathways
Gao *et al*[38]	*C. butyricum*	*IL-8*, *IL-6*, and *TNF-α*
Isono *et al*[39]	*C. butyricum* TO-A	*TLR-4*
Huang *et al*[40]	*Bacillus* species	*TLR-2* and *TLR-4*
Dendritic cells		
Bermudez-Brito *et al*[7]	*L. paracasei* CNCM I-4034	*TLR9*, *CASP8*, and *TOLLIP*
Weiss *et al*[48]	*L. acidophilus*	Genes encoding *IFN*, *TLR-3*, and *IL-12*
Rajput *et al*[49]	*S. boulardii* and *B. subtillis* B10	*MyD88*, *NF-κB*, *TLR-1*, *2*, *4*, and *15*
Latvala *et al*[50]	Bifidobacteria, lactobacilli, and *S. thermophilus* THS	*TNF-α*, *IL-1β*, *IL-6*, *IL-10*, *IL-12*, and IFN-γ
Evrard *et al*[52]	*L. rhamnosus* 35	*IL12*, *TNF-α*, *IL1B*, *IL6*, *TGFB1*, *IL-23*, and *IL-8*
Bermudez-Brito *et al*[55]	*B. breve* CNCM I-4035	*TLR-9* and *TOLLIP*
Ayehunie *et al*[56]	*L. delbrueckii* subsp. *bulgaricus*	*TLR-4*, *p38*, and *IκB*
Animal studies		
Matsumoto *et al*[57]	*B. animalis* subsp. *lactis* LKM512	Aging-associated and inflammation-associated genes
Ohtsuka *et al*[58]	*B. breve* M-16V	*LBP*
Trevisi *et al*[59]	*B. animalis*	*TLR-2* and *TLR-4*
Dykstra *et al*[60]	Bifidobacteria and lactobacilli	MUC2, MUC3, NAIP, HIAP1/cIAP2, and HIAP2/cIAP1
Foye *et al*[62]	*L. acidophilus*	*IL-10* and *TGF-β*
Deng *et al*[63]	*B. subtillis* RJGP16 and *L. salivarius* B1	*IL-6* and *pBD-2*
Jain *et al*[64]	*L. casei*	*IFN-γ* and *IL-2*
Pérez-Sánchez *et al*[65]	*L. plantarum*, *L. lactis*, and *L. mesenteroides*	*IL-1β*, *IL-8*, *IL-10*, *TNF-α*, *IL-8*, *TLR5*, and *IgT*
Pirarat *et al*[66]	*L. rhamnosus* GG	*TNF-α* and *IL-1*
Human studies		
van Baarlen *et al*[67]	*L. acidophilus*, *L. casei*, and *L. rhamnosus*	Gene-regulatory networks and pathways in human mucosa
Lammers *et al*[68]	VSL#3	*IL-1β*, *IL-6*, *IFN-γ*, *TNF-α*, *IL-12*, *IL-10*, *TGF-β*, and *IL-8*
Di Caro *et al*[69]	*B. clausii*	Genes involved in the immune response and inflammation

A4: Amyloid beta; APBA1: Precursor protein-binding family A member 1; CASP7: Cysteine protease caspase 7; COX2: Cyclooxygenase 2; FCER1A: Allergy-related high-affinity IgE receptor subunits α; FCER1G: Allergy-related high-affinity IgE receptor subunits γ; HIAP1/cIAP2: Human inhibitor of apoptosis protein 1/cellular inhibitor of apoptosis 2; HIAP2/cIAP1: Human inhibitor of apoptosis protein 2/cellular inhibitor of apoptosis 1; ICAM-1: Intercellular adhesion molecule 1; IFN-γ: Interferon gamma; IL: Interleukin; IRF3: Interferon regulatory factor 3; LBP: Lipopolysaccharide-binding protein; LIFR: Leukemia inhibitory factor receptor; MAPK: Mitogen-activated protein kinases; MCP-1: Monocyte chemo-attractant protein-1; MIP-2α: Macrophage inflammatory protein-2 alpha; MIP-2β: Macrophage inflammatory protein-2 beta; MUC: Mucins; MyD88: Myeloid differentiation primary response protein 88; NAIP: Neuronal apoptosis inhibitor protein; NF-κB: Nuclear factor-kappa beta; NOX5: NADPH oxidase; PPAR-γ: Peroxisome proliferator-activated receptor gamma; SOCS: Suppressor of cytokine signaling; p-BD2: Porcine beta-defensins 2; TGF-β: Transforming growth factor beta; TLR: Toll-like receptor; TNF- α: Tumor necrosis factor-alpha; TOLLIP: Toll-interacting protein; VSL#3: mixture of *Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium breve*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus plantarum*, and *Streptococcus salivarius* subsp, *thermophilus*.

ence or absence of bacteria for 48 h, and the expression of *IL-17*, *IL-23*, and CD40 at the mRNA and protein levels was assessed using qPCR. The NF-κB activity was assessed by NF-κB-dependent luciferase reporter gene assays. *B. breve* and LGG diminished the LPS-induced expression of *IL-17*, *IL-23*, and CD40 as well as histone acetylation and slightly enhanced DNA methylation. These effects were paralleled by a decrease in the nuclear

translocation of NF-κB, as demonstrated by a decrease in the expression of MyD88, IRAK-1, IκBα, the nuclear NF-κB p50/p65 subunits, p-p38 MAPK and p-MEK1 and the NF-κB-dependent luciferase reporter gene activity in LPS-stimulated cells[72].

To mimic the IBD response to Gram-negative bacteria, Grimoud *et al*[73] (2010) used HT-29 cells that were sensitized to the inflammatory response to LPS by IFN-γ, which increased the expression of *TLR4*, the LPS biosensor, and were then treated by probiotics, prebiotics and synbiotics. Only three probiotic strains induced a proliferation decrease but with a lack of reproducibility. Binary or ternary probiotic associations, complemented or not by prebiotics, significantly decreased proliferation, especially with a synbiotic association of *B. breve*, *Lactococcus lactis* and *L. oligoalternan*.

Angiogenesis is an integral process of inflammatory responses in IBD and is required for mucosal remodeling during restitution. Chen *et al*[74] (2013) indicated that *Saccharomyces boulardii* modulates angiogenesis to limit intestinal inflammation and promote mucosal tissue repair by regulating vascular endothelial growth factor (VEGF) receptor signaling using an adenovirus expressing VEGF-A(164) in the ears of adult nude mice.

IBD increases the risk of colorectal cancer. Bassaganya-Riera *et al*[75] (2012) studied the cellular and molecular mechanisms underlying the efficacy of probiotic bacteria in mouse models of inflammation-driven colorectal cancer. Immune cell subsets in the MLN, spleen and colonic lamina propria lymphocytes (LPL) were phenotypically and functionally characterized. The mice were treated with conjugated linoleic acid (CLA) or VSL#3 and recovered faster from the acute inflammatory phase of disease and had lower disease severity in the chronic, tumor-bearing phase of disease. VSL#3 increased the mRNA expression of *TNF-α*, angiostatin and *PPAR-γ*, whereas CLA decreased *COX-2* levels. Moreover, the VSL#3-treated mice had increased IL-17 expression in the MLN CD4+ T cells and an accumulation of Treg LPL and memory CD4+ T cells. Finally, IBD in a rat model with male neonatal maternal separation (NMS) was reported and treated orally with placebo or VSL#3 from days 3 to 60, while normal, not-separated rats were used as controls. A microarray analysis demonstrated that NMS induced a robust change in the expression of subsets of genes (*CCL2, NOS3, THP1, NTRK1, CCR2, BDRKRB1, IL-10, TNFRSF1B, TRPV4, CNR1* and *OPRL1*) that are involved in pain transmission and inflammation. TPH1, tryptophan hydroxylase 1, a validated target gene in IBD treatment, was markedly upregulated by NMS; this effect was reversed by VSL#3 intervention[71].

Ulcerative colitis

Garrido-Mesa *et al*[76] (2011) tested the association of minocycline and EcN in a mouse model of reactivated colitis. The mice were assigned to different groups: non-colitic and dextran sodium sulfate (DSS) control groups (without treatment), and minocycline, EcN, and minocycline plus EcN treated groups. Colitis was induced by adding DSS to the drinking water (3%) for 5 d; 2 wk later, the colitis was reactivated by subsequent exposure to DSS. The inflammatory status was evaluated daily by a disease activity index (DAI), and the colonic damage was assessed histologically and biochemically by the mRNA relative expression of different mediators. Minocycline and EcN exerted an intestinal anti-inflammatory effect and attenuated the reactivation of the colitis, as shown by the reduced DAI values; these effects were greater when both of the treatments were combined. These effects were evidenced histologically and biochemically by the reduced expression of *TNFα*, *IL-1β*, *IL-2*, *MIP-2*, *MCP-1*, *ICAM-1*, *iNOS* and *MMP-9* together with an increased *MUC-3* and *ZO-1* expression. In the same model, Claes *et al*[77] (2010) utilized a *dltD* mutant of the model probiotic LGG in its lipoteichoic acid molecules. The mice received either PBS, LGG wild-type or the *dltD* mutant *via* drinking water. The macroscopic parameters, histological abnormalities, and cytokine and TLR expression levels were analyzed to assess the disease activity. The mice that were treated with the *dltD* mutant showed an improvement of some of the colitic parameters compared to the LGG wild-type-treated mice in both experimental models. In addition, treatment with the *dltD* mutant correlated with a significant downregulation of *TRL-2* expression and of downstream pro-inflammatory cytokine expression in the colitic mice.

Lactobacillus rhamnosus OLL2838 was employed in the DSS model. The barrier function was restored by the administration of live and heat-killed OLL2838 to the DSS-treated animals, and an increased expression of *ZO-1* (4.8-fold) and myosin light-chain kinase (3.1-fold) was found in IECs that were isolated from mice of the heat-killed OLL2838 group[78].

The efficacy of probiotics in the recurrent trinitrobenzenesulfonic (TNBS)-induced colitis model in BALB/c mice has been tested. A microarray analysis revealed differences in expression of genes that are related to inflammation and immune processes between untreated mice and those that were treated with the probiotics *L. plantarum* NCIMB8826 or VSL#3. The effects of probiotics on colonic gene expression were most profound during active inflammation, in particular on gene clusters that are related to mast cells and antimicrobial peptides[79].

Amit-Romach *et al*[80] (2010) evaluated and compared the effects of two probiotic regimens, LGG and a mixture of *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium lactis* in both normal and TNBS acid colitis-induced rats. Colonic tissues were used for mRNA analysis *via* qPCR. The administration of both of the probiotic regimens reduced the expression of the pro-inflammatory cytokines *TNF-α* and *IL-6* and increased the expression of *MUC2* compared with the that of the colitis group[80]. Using the same model, Duary *et al*[81] (2012) examined the effects of *L. plantarum* Lp91 on the gene expression of cytokines and other molecules. *L. plantarum* Lp91 downregulated *TNF-α* and *COX-2* in mice with

colitis. IL-10 was significantly upregulated in colitis and non-colitis mice that were treated with *L. plantarum* Lp91, while other anti-inflammatory markers, *i.e.*, *COX-1, IL-4* and *IL-6,* were significantly upregulated in the colitis mice that were treated with *L. plantarum* Lp91. The MUC2 gene was also significantly up regulated in the non-colitis group.

The antioxidant potential of *Lactobacillus rhamnosus* CNCM I-3690 using the nematode *Caenorhabditis elegans* as host was investigated. The transcriptomic analysis of *C. elegans* that were fed this strain showed that an increased lifespan is correlated with the differential expression of the DAF-16/insulin-like pathway, which is highly conserved in humans. In addition, this *Lactobacillus* strain reduced inflammation in a murine model of colitis[82].

Finally, the synbiotic (*Bifidobacterium longum* and inulin-oligofructose) was tested in UC patients. The treatment was administered for a period of one month in a double blind, randomized, controlled trial using 18 patients with active UC. The sigmoidoscopy scores were reduced in the test group (start 4.5, end 3.1) compared with those of the placebo group (start 2.6, end 3.2). The mRNA levels for *human beta defensins 2*, *3*, and *4*, which are strongly upregulated in active UC, were significantly reduced in the test group after treatment. *TNF-α* and *IL-1α*, which are inflammatory cytokines that drive inflammation and induce defensin expression, were also significantly reduced after treatment[83].

Necrotizing enterocolitis

Necrotizing enterocolitis (NEC) afflicts extremely low-birth-weight neonates, and probiotics reduce its incidence and severity. Nitric oxide (NO) is involved in the pathogenesis of NEC, and caveolin-1 regulates NO signaling. D'Souza *et al*[84] (2010) evaluated the importance of NO in formula-fed neonatal rats that were supplemented with "Florastar Kids" and/or galacto-oligosaccharides and fructo-oligosaccharides. Samples from the terminal ileum were analyzed for total NO metabolites, growth factors, and gene expression of *caveolin-1*, *NOS isoforms*, and antioxidants. The data showed that formula feeding with and without supplementation resulted in significant growth restriction. *Caveolin-1, endothelial NOS*, and *neuronal NOS* were simultaneously downregulated with formula feeding, while the inducible NOS was upregulated. Superoxide dismutase and glutathione peroxidase were upregulated with supplementation. Moreover, Lin *et al*[85] (2008) evaluated probiotics in the incidence of NEC. *Lactobacillus rhamnosus* GG reduced the chemically induced intestinal epithelial apoptosis, demonstrating that LGG upregulates a battery of genes with known and likely cytoprotective effects.

Other inflammatory disorders

There are many inflammation-based intestinal diseases. However, probiotics have been tested in only a few of these diseases.

The gastroprotective potential of *Bifidobacterium bifidum* BF-1 in a rat model of acid-ethanol-induced acute gastric injury was investigated to elucidate its potential compared with *Streptococcus thermophilus* YIT 2021. Living *B. bifidum* BF-1 and *S. thermophilus* YIT 2021 or vehicle was orally administered to rats, and acid-ethanol gastric injury was induced 2 h later. Mucin 5ac (muc5ac) gene expression in gastric corpus samples and gastric mucin production in stomach samples from the *B. bifidum* BF-1 group, but not the *S. thermophilus* YIT 2021 group, were significantly higher than those in the respective samples from the vehicle group[86].

Mirpuri *et al*[87] (2012) evaluated the enteral administration of LGG in mice with intestinal injury due to the administration of platelet-activating factor (PAF) and LPS. The probiotic strain downregulated the expression of *TNF-α* and *MIP-2* but failed to alter *IL-10* mRNA and protein expression. LGG did however induce the mRNA expression of the *IL-10R2* subunit of the IL-10 receptor. IL-10 receptor activation has been associated with the signal transducer and activator of transcription (STAT) 3-dependent induction of members of the SOCS family. In 2-wk-old mice, LGG also induced STAT3 phosphorylation, increased the colonic expression of SOCS-3, and attenuated the colonic production of MIP-2 and TNF-α. These LGG-dependent changes in phospho-STAT3, SOCS3, MIP-2 and TNF-α were inhibited by the antibody-mediated blockade of the IL-10 receptor. Thus LGG decreased the baseline pro-inflammatory cytokine expression in the developing colon *via* the upregulation of IL-10 receptor-mediated signaling, most likely due to the combined induction of phospho-STAT3 and SOCS3. The principal findings concerning gene expression in inflammatory diseases of the gut mediated by probiotics appear in Table 2.

Although studies evaluating the mechanism of action of probiotics in IBD are heterogeneous because of the different methodological approaches, basically probiotics lead to the downregulation of a number of pro-inflammatory genes and the upregulation of others, *e.g.*, mucin genes, which can help explain the beneficial effects of probiotics in decreasing the activity of these gut diseases.

REGULATION OF IMMUNITY AND INFLAMMATION GENE EXPRESSION IN THE LIVER BY PROBIOTICS

A large body of evidence has highlighted the concept that putative intestinal bacteria-derived compounds may affect liver metabolism and, therefore, cause systemic diseases[88,89]. Serum LPS levels have been proposed to increase upon obesity and steatosis, leading to a metabolic endotoxemia that can modulate pro-inflammatory cytokines as well as glucose and lipid metabolism in the liver or adipose tissue[90-93]. Endotoxemia is considered a major risk for inducing liver inflammation in nonalcoholic steatohepatitis (NASH) and nonalcoholic fatty liver disease (NAFLD) in humans[94-97]. NASH and NAFLD are as-

Table 2 Regulation of gene expression by probiotics in inflammatory diseases of the gut

Study	Probiotic strain	Genes involved
Inflammatory bowel disease		
Disfrutti *et al*[71]	VSL#3	*IL-10, TNFRSF1B*
Ghadimi *et al*[72]	*B. breve* (DSMZ 20213) and *L. rhamnosus* GG	*IL-17* and *IL-23*
Grimoud *et al*[73]	*B. breve* and *L. lactis*	*TLR-4*
Chen *et al*[74]	*Saccharomyces boulardii*	*VEGF*
Bassaganya-Riera *et al*[75]	VSL#3	*TNF-α*, *COX-2*, and *PPAR-γ*
Ulcerative colitis		
Garrido-Mesa *et al*[76]	*E. coli* Nissle 1917	*TNF-α, IL-1β, IL2, MIP-2, MCP-1, ICAM-1, MUC3*, and *ZO-1*
Claes *et al*[77]	*L. rhamnosus* GG wild type and mutant	*TLR-2*
Miyauchi *et al*[78]	*L. rhamnosus* OLL2838	*ZO-1*
Mariman *et al*[79]	*L. plantarum* NCIMB8826 and VSL#3	Inflammation and immune genes
Amit-Romach *et al*[80]	*L. rhamnosus* GG and a mixture of probiotics	*MUC2, IL-6*, and *TNF-α*
Duary *et al*[81]	*L. plantarum* Lp91	*IL-4, IL-6,COX-1, COX-2*, and *TNF-α*
Grompone *et al*[82]	*L. rhamnosus* CNCM I-3690	DAF-16/insulin-like pathway
Furrie *et al*[83]	*B. longum*	*Human beta defensins* 2, 3, and 4, *TNF-α*, and *IL-1α*
Necrotizing enterocolitis		
D'Souza *et al*[84]	*S. boulardii*	*Caveolin-1* and *NOS-isoforms*
Lin *et al*[85]	*L. rhamnosus* GG	Genes with cytoprotective effects
Other inflammatory disorders		
Gomi *et al*[86]	*B. bifidum* BF-1	*MUC5*
Mirpuri *et al*[87]	*L. rhamnosus* GG	*IL-10, MIP-2*, and *TNF-α*

COX1: Cyclooxygenase 1; COX2: Cyclooxygenase 2; ICAM-1: Intercellular adhesion molecule 1; IL: Interleukin; MCP-1: Monocyte chemo-attractant protein-1; MIP-2: Macrophage inflammatory protein-2 alpha; MUC: Mucins; NOS isoforms: Constitutional neuronal isoform (nNOS), the inducible isoform (iNOS), and the endothelial isoform (eNOS); PPAR-γ: Peroxisome proliferator-activated receptor gamma; TLR: Toll-like receptor; TNF-α: Tumor necrosis factor-alpha; TNFRSF1B: Tumor necrosis factor receptor superfamily, member 1b; TOLLIP: Toll-interacting protein; VEGF: Vascular endothelial growth factor; VSL#3: Mixture of *Bifidobacterium longum, Bifidobacterium infantis, Bifidobacterium breve, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus plantarum,* and *Streptococcus salivarius* subsp, *thermophilus*; ZO: Zona occludens.

sociated with increased gut permeability in humans[98,99]. Cani *et al*[100] (2009) demonstrated the alteration of gut-barrier function in genetic models of obesity. Overall, these studies strongly suggest a direct link between the gut microbiota, the gut barrier, and hepatic changes.

Few papers have been published regarding the probiotic-mediated modulation of genes that are involved in immunity and inflammation in the liver. These few papers are reviewed below and are organized by pathology.

Sepsis

Bu *et al*[101] (2006) described a bacteria-free, lysozyme-modified probiotic product that was obtained by treating the probiotic bacteria, *Lactobacillus* spp., with lysozyme (LzMPC), which might be beneficial for the treatment of sepsis owing to the potent immunomodulatory effects of lysozyme on macrophages. The oral administration of LzMPC effectively protected rats against lethality from polymicrobial sepsis that was induced by cecal ligation and puncture. LzMPC was engulfed by macrophages in the liver after crossing the intestinal barrier. The LzMPC-induced protection was associated with an increase in the bacterial clearance in the liver. Surgical stress or cecal ligation and puncture caused a decrease in the cathelicidin-related peptide (*CRAMP*) expression in the liver, whereas the enteral administration of LzMPC restored *CRAMP* gene expression in these animals. In addition, macrophages from LzMPC-treated rats had an enhanced capacity of cytokine production in response to LPS or LzMPC stimulation.

Inflammation

Mair *et al*[102] (2010) evaluated the 4-wk administration of a probiotic mixture (*Enterococcus faecium*, *Lactobacillus salivarius*, *L. reuteri* and *Bifidobacterium thermophilum*) on cell turnover, growth and inflammatory marker gene expression [*caspase-3*; cyclin-dependent kinase-4, *CDK-4*; insulin-like growth factor I , (*IGF-I*); *NF-κB; TNF-α*; and *TGF-β*] in piglets' intestines and liver. The gene expression of *CDK-4* and *TGF-β* was upregulated in the jejunum and the mesenteric lymph nodes, respectively, in the probiotic group. In addition, the probiotic group exhibited an upregulation in cell turnover marker genes in the colon and blood. No significant differences were observed in gene expression in the liver tissue.

The administration of *L. ingluviei* to mice promotes alterations in the intestinal microbiota, weight gain increase, and accelerated metabolism as well as liver enlargement and inflammation. Angelakis *et al*[103] (2012) studied the mRNA expression of genes that are involved in lipogenesis and inflammation in the liver of BALB/c mice gavaged for different periods of time with this probiotic strain. The mRNA expression of fatty acyl synthase (*FAS*), sterol regulatory element binding protein 1 (*SREBP-1*), cytochrome P450 2E1, 3-phosphoinositide-dependent protein kinase-1 (*PDPK1*), acyl-Coenzyme A dehydrogenase-11 (*Acad11*), ATP-binding cassette sub family member G (*ABCG2*), and DEAD box polypeptide 25 (*DDX25*) was significantly higher in the probiotic-fed mice compared with that of the control mice. This result was accompanied by a low-grade in-

Table 3 Regulation of immunity and inflammation gene expression in the liver by probiotics

Study	Probiotic strain	Genes involved
Sepsis		
Bu *et al*[101]	*Lactobacillus* spp.	*CRAMP*
Inflammation		
Mair *et al*[102]	*E. faecium, L. salivarius, L. reuteri* and *B. thermophilum*	*CDK-4* and *TGF-β*
Angelakis *et al*[103]	*L. ingluviei*	*TNF-α*
Experimental liver disease		
D'Argenio *et al*[104]	*L. paracasei* B21060	*TNF-α, TGF-β, IL-10, TLR4, TLR2, iNOS, eNOS*, and *αSMA*
Zuo *et al*[105]	*L. casei, B. subtillis*, and *Pichia anomala*	Genes involved in immunity

CDK-4: Cyclin-dependent kinase-4; CRAMP: Cathelicidin-related peptide; IL: Interleukin; TGF-β: Transforming growth factor beta; iNOS: Inducible isoform; eNOS: Endothelial isoform; TLR: Toll-like receptor; TNF-α: Tumor necrosis factor-alpha.

flammatory state in the liver, suggested by a significantly increased mRNA expression of liver *TNF-α* in the mice that received probiotics.

Experimental liver disease

D'Argenio *et al*[104] (2013) specifically examined the effects of a synbiotic formulation on an experimental model of CCl4-induced liver fibrosis in rats. The synbiotic product was a mixture of a probiotic strain (*L. paracasei* B21060) with L-glutamine, arabinogalactan and xylo-oligosaccharides as prebiotics. The serum ALT and AST activities as well as liver histology and collagen deposition improved in fibrotic mice with the synbiotic mixture compared with those of the placebo group. The serum levels of the pro-inflammatory cytokine TNF-α were significantly increased in rats with liver fibrosis compared with those of normal rats, whereas the synbiotic treatment normalized the plasma levels of TNF-α and significantly enhanced the anti-inflammatory cytokine IL-10. In the liver, *TNF-α*, *TGF-β*, *TLR4*, *TLR2*, *iNOS* and *αSMA* mRNA levels were upregulated in rats with CCl4-induced liver fibrosis and downregulated by the synbiotic treatment. Moreover, the *IL-10* and *eNOS* mRNA levels increased in the fibrotic rats that received synbiotics.

Aflatoxins are naturally occurring toxins that are produced by *Aspergillus flavus* and *Aspergillus parasiticus*, which are species of fungi. Aflatoxin exposure produces an acute hepatic necrosis that results in cirrhosis or hepatocarcinoma. Because aflatoxin-producing members of *Aspergillus* are common and widespread in nature, these compounds pose serious hazards to human and animal health, and chemoprevention strategies aimed at reducing their toxicity in animal diets are needed. Zuo *et al*[105] (2013) investigated one such strategy that was based on the administration of a mixture of three aflatoxin-degrading probiotic strains (*Lactobacillus casei*, *Bacillus subtillis*, and *Pichia anomala*) along with the aflatoxin-degrading enzyme from *Aspergillus oryzae* to Arbor Acres broilers that were fed an aflatoxin-supplemented diet. The administration of this mixture to chickens that were fed the aflatoxin-supplemented diet resulted in the restorations of (1) the antioxidant enzymatic defense in the serum and liver; and (2) the hepatic expression of an array of genes that are involved in apoptosis, cell growth, immunity and metabolism. Table 3 summarizes the reported investigation related to gene expression in the liver by probiotics.

CONCLUSION

Probiotics exert their actions through interaction with intestinal cells, which in turn modify the expression of many genes that are mainly related to the gut-associated immune system. Although the specific actions are dependent on the particular bacteria and strains, probiotics mainly induce a tolerogenic response to external antigens by interacting with TLR and down-regulating the expression of NF-κB and pro-inflammatory cytokines.

There is a need for further clinical studies that evaluate the mechanism of action of probiotics both in healthy humans and patients with chronic diseases. These types of clinical studies are necessary for addressing the influence of these microorganisms in gene expression for different pathways, particularly those that are associated with the immune response, and to better understand the role that probiotics might have in the prevention and treatment of disease.

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