Enhancement of Epithelial Barrier Function by Probiotics

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Abstract: Probiotics are microbial organisms that are administered in supplements or foods to enhance the well-being of the host. There exists substantial evidence that in a strain and dose-dependent manner, probiotics can modulate systemic and mucosal immune function, improve intestinal barrier function, alter gut microecology, and exert metabolic effects on the host. Several strains of Lactobacillus and Bifidobacterium are able to compete with pathogens for binding to intestinal epithelial cells, and can displace pathogens. Epithelial cell signalling pathways are stimulated by whole microbes, structural components, and microbial-produced metabolites. In particular, the NF-κB pathway is modulated by probiotics at many different levels with effects seen on IκB degradation and ubiquitination, proteasome function, and nuclear-cytoplasmic movement of RelA through a PPAR-gamma dependent pathway. In a strain and dose-dependent manner, probiotic strains have been shown to alter tight junction protein expression and/or localization in both in vivo and in vitro models. Probiotics can also enhance gut barrier function via increased production of cytoprotective molecules such as heat-shock proteins. In addition, probiotics are able to prevent cytokine- and oxidant-induced epithelial damage by promoting cell survival. Lactobacillus GG and soluble factors (p75 and p40) released from LGG prevented epithelial cell apoptosis through activating anti-apoptotic Akt in a phosphatidylinositol-3’-kinase (PI3K)-dependent manner and inhibiting pro-apoptotic p38/MAPK activation. It is clear that host-microbial interactions at the gut mucosal surface are critical for health and overall homeostasis and probiotics may possibly be harnessed to enhance barrier function in order to maintain health and protect against disease.

Keywords: Epithelium, Lactobacillus, Bifidobacterium, Tight junctions.

INTRODUCTION

Probiotics are microbial organisms that are administered in supplements or foods to enhance the well-being of the host [1]. A growing body of work now exists describing the role of various probiotic strains in ameliorating chronic intestinal inflammation, diarrhoea, constipation, irritable bowel syndrome, and liver disease [2]. There exists substantial evidence that in a strain and dose-dependent manner, probiotics can modulate systemic and mucosal immune function, improve intestinal barrier function, alter gut microecology, and exert metabolic effects on the host [1]. Probiotic strains have been shown to enhance intestinal barrier function by stimulating mucus and antimicrobial peptide production [3-7]; increasing mucosal IgA responses [8]; enhancing tight junction protein expression and/or localization [9-18], preventing epithelial cell apoptosis [19] and by the induction of cytoprotective molecules [20].

Intestinal epithelial cells have both a barrier and immunomodulatory role in the gut through their interactions with each other and influence over underlying immune cells. Epithelial cell signalling pathways are stimulated by whole microbes, microbial structural components, and microbial-produced metabolites. In particular, the NF-κB pathway is modulated by probiotics at many different levels with probiotic-induced effects seen on IκB degradation and ubiquitination [21-23], proteasome function [23, 24]; and nuclear-cytoplasmic movement of RelA through a PPAR-gamma dependent pathway [16, 25]. Effects of probiotics are not limited to live bacteria, but include modulation of cellular homeostasis by microbial DNA [23, 26, 27], soluble proteins released from live microbes [10, 20], cell wall structural components including polysaccharides [28, 29], and metabolites produced by probiotic fermentation of luminal nutrients [30].

Competitive Exclusion and Mucus Secretion

Several strains of Lactobacillus and Bifidobacterium are able to compete with pathogens for binding to intestinal epithelial cells, and are able to displace pathogens even if the pathogens have already attached [31-34]. Some strains of lactobacilli express human mucus-binding pili, which would enhance their ability for colonization [35]. Probiotic inhibition of pathogen adherence to epithelial cells is mediated partially by competition for lectin binding sites on glycoconjugate receptors on the brush border surface [36, 37]. Probiotics also express microorganism-associated molecular patterns (MAMPs) that bind to the same pattern recognition receptors as pathogens do, thus preventing access via competitive exclusion [35]. Probiotics also induce mucus secretion, which would aid in preventing pathogenic bacterial adhesion [4, 7, 38].

Effects on Epithelial Tight Junctions

In a strain and dose-dependent manner, numerous probiotic strains have been shown to directly alter tight junction

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protein expression and/or localization in both in vivo and in vitro models [9-18, 39]. Resta-Lenert and Barrett [11] showed that Streptococcus thermophilus and Lactobacillus acidophilus both increased epithelial resistance of HT-29 and Caco-2 cells. The effect of these strains on epithelial resistance was accompanied by either maintenance (actin, ZO-1) or increased (occludin, occludin) levels of tight junctional protein phosphorylation [11]. Treatment of HT-29 and Caco-2 cells with S. thermophilus and L. acidophilus also resulted in an activation of p38, ERK, phosphatidylinositol 3-kinase (PI3K), and JNK pathways [11, 40]. In these studies, cultured medium and heat-killed bacteria did not alter resistance, indicating that live S. thermophilus and L. acidophilus were required. In a similar fashion, Lactobacillus rhamnosus and Bifidobacterium lactis increased epithelial resistance in conjunction with increased phosphorylation of ZO-1 and occludin [15]. In contrast, both live organisms and conditioned media from bacteria strains found in the probiotic mixture, VSL#3, Bifidobacterium longum, Bifidobacterium infantis, Bifidobacterium breve, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus plantarum, Streptococcus salivarius subsp. Thermophilus) increased resistance of T84 cells, with Bifidobacterium infantis exerting the largest effect [41]. This increase in resistance was accompanied by decreased Claudin-2 protein expression and increased protein expression of ZO-1 and occludin [10]. B. infantis-conditioned medium increased levels of phospho-ERK1 and 2 and decreased phospho-p38, suggesting a role for the MAPK pathway [10]. B. infantis conditioned media was also effective in reducing intestinal permeability and improving disease in a mouse model of colitis [10].

Gene expression studies [12] have demonstrated that Lactobacillus plantarum MB452 altered expression levels of numerous tight junction-related genes, including those encoding occludin and cytoskeleton anchoring proteins. Tubulin and proteasome-associated genes were also altered by L. plantarum [12].

E. coli Nissle 1917 has also demonstrated direct effects on tight junctional proteins in epithelial cells. Zyrek et al., [13] showed that E. coli Nissle increased resistance of T84 cells and increased expression and tight junction localization of ZO-2. Germ-free mice colonized with E. coli Nissle had an upregulation of ZO-1 in intestinal epithelial cells at both the mRNA and protein levels [9]. Interestingly, a recent study showed both E.coli Nissle conditioned media and isolated LPS from E. coli Nissle to have similar effects on increasing epithelial resistance [28].

Various factors, including oxidative stress, pathogenic organisms, and pro-inflammatory cytokines, can reduce epithelial barrier function, [16, 18, 39, 42]. A common finding is that probiotics are able to protect tight junctions from disruptions induced by these factors. IFNγ and TNFα have been shown to alter barrier function in an apoptosis-independent manner by inducing an internalization of tight-junction transmembrane proteins, rearranging the internal actin cytoskeleton, causing a redistribution of JAM-1 from membrane raft-containing fractions, and inducing a redistribution of the adherent junction protein E-cadherin [43]. IFNγ and TNFα also reduce occludin and ZO-1 gene expression in HT-29/B6 cells, suggesting that cytokines can also modulate barrier function at gene level [44]. Pretreatment of T84 cells with B. infantis-conditioned medium prevented a decrease in resistance induced by TNF-α or IFN-γ treatment, and was blocked by ERK inhibition [10]. A similar finding was seen by Resta-Lenert, where pretreatment of T84 cells with S. thermophilus and L. acidophilus also prevented the deleterious effects of IFN-γ or TNF-α treatment [40]. This protection was associated with ERK, p38, and PI3K
activation by the probiotic [40]. Donato et al. [16] showed that Lactobacillus rhamnosus GG attenuated IFNγ and TNFα induced barrier disruption in Caco2 cells by reducing the nuclear translocation of p65. Lactobacillus GG and LGG-derived soluble proteins (p40 and p75) maintained epithelial barrier function in the presence of hydrogen peroxide-induced disruption by increasing membrane translocation of ZO-1, occludin, PKCβ1, and PKCε in an extracellular signal-related kinase (ERK1/2) and mitogen-activated protein kinase (MAPK)-dependent manner [45].

Similar results are seen in animal models. In two different animal models of colitis, (IL-10 deficient mice and mdm1 null mice), treatment with VSL#3 or S. thermophiles and L. acidophilus respectively resulted in a decrease in gut permeability and improvement of disease [41, 46]. In an animal model of acute pancreatitis, pre-treatment of rats with a multispecies probiotic mixture (Lactobacillus acidophilus (W70), Lactobacillus casei (W56), Lactobacillus salivarius (W24), Lactococcus lactis (W58), Bifidobacterium bifidum (W23), and Bifidobacterium lactis (W52) protected against an oxidative stress-induced increase in gut permeability, bacterial translocation, epithelial cell apoptosis, and tight junction protein disruption [39]. This was associated with a probiotic-induced increase in mucosal glutathione and glutamate-cysteine-ligase activity [39]. An interesting study by Pagnini et al., [22] demonstrated that treatment of SAMP1/YitFc (SAMP) mice with chronic gut inflammation with VSL#3 (Bifidobacterium longum, B. infantis, and B. breve, L. Acidophilus, L. casei, L. delbrueckii subsp. L. bulgaricus and L. planetarium, Streptococcus salivarius subsp. Thermophilus) resulted in a decrease in gut permeability in conjunction with a stimulation of innate immunity as evidenced by enhanced TNFα secretion.

Cytoprotective Molecules

Probiotics can also enhance epithelial gut barrier function via increased production of cytoprotective molecules such as heat-shock proteins. Heat-shock proteins are constitutively expressed in epithelial cells and are induced in cells by stress in order to help maintain homeostasis [24]. Soluble factors released from Lactobacillus GG induced cytoprotective heat shock protein synthesis in intestinal epithelial cells in a p38- and JNK/MAPK-dependent manner [20]. Quorum-sensing molecules secreted by Bacillus subtilis also induced epithelial expression of cytoprotective heat shock proteins [47].

Effects on Apoptosis

In addition to their effects on tight junction proteins, probiotics are also able to prevent cytokine- and oxidant-induced epithelial damage by promoting cell survival. Lactobacillus GG and soluble factors (p75 and p40) released from LGG prevented epithelial cell apoptosis through activating anti-apoptotic Akt in a phosphatidylinositol-3'-kinase (PI3K)-dependent manner and inhibiting pro-apoptotic p38/MAPK activation [48, 49]. This reduction in apoptosis would help to maintain epithelial barrier integrity and increase resistance to pathogens by reducing breaks in the mucosal barrier. In an in vivo study, Lactobacillus plantarum 299v increased expression of members of the inhibitor of apoptosis protein family (HIAP2/cIAP) [3]. In another study, administration of Bifidobacterium bifidum increased expression of TLR-2, COX2, and PGE2 and significantly reduced apoptosis in the intestinal epithelium in a necrotizing enterocolitis (NEC) model [19]. Further, inhibition of COX2 signalling blocked the protective effect of B. bifidum suggesting that a probiotic-induced increased expression of COX2 and elevated production of PGE2 was responsible for the protection. This elevation of PGE2 production subsequently protected against epithelial cell apoptosis associated with NEC thus preserving intestinal barrier function. [19]

Clinical Applications of Probiotics

The ability of the gut epithelium to act as a barrier between the external environment and the closely regulated internal milieu is absolutely essential for human health [50]. When the gut barrier is disrupted, not only does a greater amount of microbial antigenic material transverse the gut, but the altered route of passage can also significantly alter the immunogenicity of the microbial antigen. Increased gut permeability is associated with several different human diseases, including inflammatory bowel disease [51], graft vs host disease [52], type 1 diabetes [53], and celiac disease [54]. Increased gut permeability also has a role in the pathogenesis of sepsis and multiple organ failure in patients following surgery or trauma [55]. Using probiotics to prevent and/or treat pro-inflammatory and oxidant induced breakdown in gut barrier function is an attractive therapeutic option. However, in various clinical trials, probiotics have been shown to reduce gut permeability under some conditions but not others [56]. In addition, probiotic-induced alterations in gut permeability are not always associated with changes or improvement in clinical symptoms [56]. It is clear that at this time, more studies are required to determine the exact relationship which exists between probiotic use, alterations in gut permeability, and clinical symptoms.

CONCLUSION

A barrier between luminal microorganisms and the host immune system is maintained through intestinal epithelial cells, mucus and anti-microbial production, and IgA secretion. In a strain-dependent manner, probiotics have the capacity to enhance gut barrier function through numerous different mechanisms. It is clear that host-microbial interactions at the gut mucosal surface are critical for health and overall homeostasis and further, that probiotics may be harnessed to enhance barrier function in order to maintain health and protect against disease. In the future, larger clinical trials will need to be carried out to determine how best to use probiotics in the prevention or treatment of various diseases. The particular strains of probiotics best suited to the treatment of specific diseases will need to be determined, along with the amount of bacteria to use, the time of dosing, and whether combinations or single strains are preferable.

CONFLICT OF INTEREST

None declared.

ABBREVIATIONS

AJ = Adherens junction
ERK = Extracellular signal regulated kinase
IFN = Interferon


