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Fighting Intestinal Infections with Immunobiotic Lactic Acid Bacteria

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ABSTRACT

Intestinal infections are the most common diseases in humans. These infections account for high morbidity and mortality and are considered to be the fifth leading cause of death at all ages worldwide. Thus, significant efforts have been directed toward the detection, control and prevention of intestinal diseases. In this regard, the field of application for probiotics has increased significantly in the past decades. The mechanisms of action of probiotics are better understood now, thanks to detailed cellular and molecular *in vitro* and *in vivo* studies. It has been clearly demonstrated that probiotics can act directly against pathogenic bacteria by producing antimicrobial agents, or by competing for nutrients. They can also be effective against intestinal pathogens by interacting with the host, either by reinforcing the function of the epithelium barrier or by modifying the immune system response. The purpose of this work is to review the current knowledge on the effects of probiotics on intestinal infections and to provide insights on the possible cellular and molecular mechanisms of probiotics' action, especially those affecting the intestinal immune system.

Keywords: Intestinal infections; Probiotic; Lactic acid bacteria; Intestinal immune system; Immunobiotics

ABBREVIATIONS

LAB-Lactic Acid Bacteria; MALT- Mucosal-Associated Lymphoid Tissue; GALT- Gut Associated Lymphoid Tissue; BALT-Bronchus-Associated Lymphoid Tissue; NALT-Nasopharinx-Associated Lymphoid Tissue; Ig-Immunoglobulin; DCs-Dendritic Cells; PPs-Peyer's Patches; SED-Subepithelial Dome; TLRs-Toll-Like Receptor; MHC-Major Histocompatibility Complex; TGF-Transforming Growth Factor; IL-Interleukin; PAMPs-Pathogen Associated Molecular Patterns; PRRs-Pattern Recognition Receptors; TNF-Tumor Necrosis Factor; IFN-Interferon; IECs-Intestinal Epithelial Cells; LPS-Lipopolysaccharide; NK-Natural Killer; ETEC-Enterotoxigenic *Escherichia coli;* PIE-Porcine Intestinal Epitheliocyte; MCP-Monocyte Chemotactic Protein; APC-Antigen-Presenting Cell; EPEC-Enteropathogenic *Escherichia coli*; EHEC-Enterohaemorrhagic *E. coli*; EPS-Exopolysaccharide

INTRODUCTION

Intestinal infections account for high morbidity and mortality and are considered to be the fifth leading cause of death at all ages worldwide. Thus, significant efforts have been directed toward the detection, control and prevention of intestinal diseases. Many antimicrobials including antibiotics have been used for their control and prevention. However, probiotics offer a potential alternative intervention strategy owing to their general health beneficial properties and inhibitory effects against pathogens. For some decades now, bacteria known as probiotics have been added to various foods because of their beneficial effects for human health. In recent years, enormous efforts have been made to unravel the mechanisms of probiotic actions, and various

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experimental approaches have been used to characterize the molecular basis of probiotic effects, especially those associated to the improvement against intestinal pathogens. Probiotic lactic acid bacteria (LAB) represent a promising resource for the development of prevention strategies against gastrointestinal infections that could be effective tools for medical application. This review summarizes the interplay existing between the host immune system and probiotic bacteria, and revises the impact of those interactions in the resistance against intestinalpathogens.

MUCOSAL IMMUNE SYSTEM

Mucosal surfaces provide chemical and mechanical mechanisms to remove foreign particles and invading microorganisms. In addition, the transport of molecules and antigens through the epithelial barrier is controlled by epithelial cells, antigen presenting cells and lymphoid cells [1] which constitute the mucosal immune system [2].

The mucosal immune system may be morphologically and functionally divided into:

-Inductor sites: responsible for the induction phase of the immune response. They are composed of lymphoid tissue associated with mucosa (MALT, mucosal-associated lymphoid tissue). According to their location MALT is denominated: GALT, gut associated lymphoid tissue, BALT, bronchus-associated lymphoid tissue, or NALT, nasopharinx-associated lymphoid tissue. Lymphoid tissue of the mammary and salivary glands and genitourinary organs are also part from MALT [3]. MALT consists of diffuse lymphoid cell clusters embedded in mucosal tissue parenchyma. These sites are populated by lymphocytes (T and B) and antigen presenting cells that have contact with environmental antigens [2].

-Effector sites: that is a diffuse lymphoid tissue associated with mucosa, comprised by leukocytes widely dispersed throughout the epithelium and lamina propria [4,5].

In the intestinal epithelium, whose intercellular spaces are sealed by tight junctions, antigens are mainly raised through specialized areas present in the GALT [6].

M cells of the intestinal epithelium capture antigens. In addition, dendritic cells (DCs) capture antigens from lumen through extensions emitted through the epithelium [7]. M cells have microvilli and small glycocalyx to transport certain types of antigens from the lumen towards organized lymphoid tissues such as Peyer's patches (PPs) in the gut. The basolateral surface of the M cell is invaginated forming a "pocket" in which the particles and macromolecules (intact antigens) are transported to the antigen presenting cells. These cells phagocyte and process antigens for their presentation to T lymphocytes, either the epithelium or subepithelial dome (SED) area located beneath the epithelium, rich in B and T lymphocytes, and plasma cells [6,8]. Then the immune response is initiated. The response elicited depends on the nature of antigen, the type of DCs, the local microenvironment involved, antigen dose, frequency of administration and the host genotype [9]. The resulting response can be induction of tolerance (against innocuous antigens and self-antigens) or stimulation of the specific immune response (against pathogens). In the case

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of pathogens, antigens are recognized by toll-like receptors (TLRs) expressed by macrophages, epithelial and mesenchymal cells, which synthesize cytokines and chemokines capable of inducing and mediating inflammatory response [10]. In this situation, DCs are mobilized into the germinal centers of the PPs where they present processed antigen to Th lymphocytes in the context of major histocompatibility complex (MHC, major histocompatibility complex) by (MHC) class II [5].

The activation of Th2 cells by DCs induces the expression of cytokine receptors and facilitate proliferation of IgM+ B cells and their differentiation into IgA+ B cells [11]. Subsequently, IgA+ B cells migrate to the mesenteric lymph node, then to the bloodstream via the thoracic duct and ultimately reach the gut lamina propria. The tissue specificity of the IgA+ B cells is the result of complex interactions between receptors on lymphocytes and their ligands expressed in the vascular wall endothelium of the lamina propria [6,12]. CD4+ T lymphocytes can also migrate from the inductor sites to the effector sites. In the lamina propria CD4+ T cells secrete cytokines such as transforming growth factor (TGF)-β, interleukin (IL)-4 and IL-5 that are essential for the differentiation of IgA+ B cells to IgA-producing plasma cells [11].

PROBIOTICS

The concept of probiotics was first established in 1907 by Metchnikoff, who suggested that the ingestion of fermented dairy products exerted beneficial effects on health [13,14]. In 1989, Fuller defined probiotics as "a live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance" [15]. Later in 2002, the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) defined probiotics as "live microorganisms which if administered in adequate amounts confer a health benefit on the host" [16]. Certain probiotic LAB strains can exert their beneficial effect on the host through their immunomodulatory activity. These strains, termed immunobiotics [17,18], have been used for the development of functional foods with the ability to stimulate mucosal immunity. Moreover, studies have demonstrated that some immunobiotic LAB can stimulate the common mucosal immune system to provide protection in other mucosal sites distant from the gut [19].

In order to be considered probiotics, microorganisms must meet a number of criteria related to security, functional effects and technological properties [20].

Probiotic microorganisms should be non-pathogenic, not be related to diarrhoea-causing bacteria, maintain their genetic stability and not transfer antibiotic resistance genes. Most probiotics fall into the group known as LAB and are normally consumed in the form of yogurt, fermented milk, or other fermented foods.

The physiological effects associated with probiotic LAB include: a) resistance to pH, acids and bile salts, b) stability against digestive enzymes, c) biosecurity, d) resistance to food processing and storage, e) ability to colonize the gastrointestinal tract and/or adhesion to the intestinal

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epithelium, and f) scientifically proved beneficial effect at reasonable doses. Not all probiotics fulfill the following requirements: a) human origin, b) active colonization of the gastrointestinal tract, or c) production of antimicrobial components and/or antagonism against pathogens.

A large variety of potentially beneficial effects have been reported for probiotics (Figure 1), including stabilization of the mucosal barrier and of the intestinal microbiota. It has been suggested that probiotics modulate and stabilize the gut microbiota through competitive exclusion, production of antagonistic substances (bacteriocins, hydrogen peroxide, organic acids, diacetyl, acetaldehyde, lactoperoxidase, lactones and other no identified substances) [21], regulation of intestinal motility, and enhance colonization resistance against enteric pathogens [22,23].

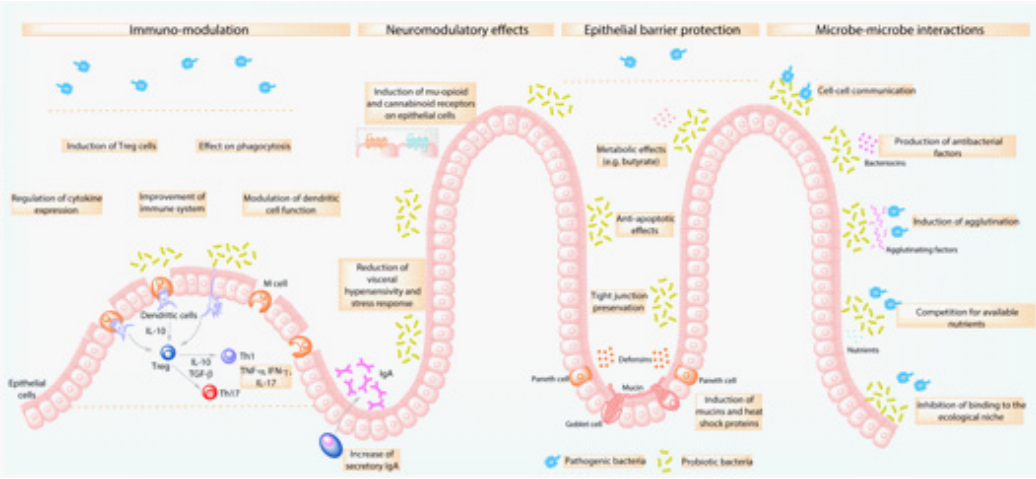

Figure 1: Proposed mechanisms for the anti-infectious activities of probiotics.

On the other hand, there are several studies of the ability of probiotics to limit cancer development in animal models of carcinogenesis due to lower production of pro-carcinogenic enzymes, anti-inflammatory and anti-mutagenic activities, and immune system stimulation [24,25].

Moreover, live probiotic strains can reduce lung [26,27], skin [28] or intestinal [29] allergic inflammation when orally administered. The anti-allergic effects can be due to immune system stimulation and prevention of food antigenic translocation at intestinal level [30,31].

Certain probiotic LAB strains can exert their beneficial effects on the host through their immunomodulatory activities with regulation of innate and adaptive immune responses [14,19,32-37]. These abilities of some probiotic strains have been used to improve resistance against intestinal pathogens. In this regard, the field of application for probiotics has increased significantly in the past decades. The mechanisms of action of probiotics are better understood now, thanks to detailed cellular and molecular *in vitro* and *in vivo* studies. It has been clearly demonstrated that probiotics can act directly against pathogenic bacteria by producing

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antimicrobial agents, or by competing for nutrients. They can also be effective against intestinal pathogens by interacting with the host, either by reinforcing the function of the epithelium barrier or by modifying the immune system response.

PROBIOTICS AND *SALMONELLA* INFECTION

Salmonella are a common source of food- or water-borne infection and cause a wide range of clinical disease in human and animal hosts. The molecular tools available to study *Salmonella* as well as suitable animal models for salmonellosis, have provided optimal conditions to drive scientists to generate a large expansion of our knowledge about the pathogenesis of *Salmonella*-induced enterocolitis as well as the immune response [38]. The key virulence traits that enable *Salmonella* to elicit inflammation are its ability to penetrate the intestinal epithelium and to survive within macrophages [39]. These interactions between bacteria and host cells result in the production of pro-inflammatory cytokines that have key roles in host defense. During invasive *Salmonella* infection, pathogen associated molecular patterns (PAMPs) are detected by several pattern recognition receptors (PRRs) that initiate the innate immune response leading to activation and recruitment of neutrophils and macrophages and the production of pro-inflammatory cytokines, most notably IL-6, IL-1β, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ. These cytokines plays a central role in the control of persistent infection by affecting the extent of neutrophils and macrophages activation [40].

Probiotics have been extensively used experimentally or therapeutically for treating *Salmonella*-induced diseases with predominantly positive outcomes. Several works provide experimental evidences indicating that probiotics may have a protective effect in mice experimentally challenged with *Salmonella* (Table 1).

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Table 1: Probiotics and *Salmonella* infection.

Strain	Viability	Mice	Route	Challenge	Protective effect	Immunoregulatory effect	Ref.
***Bifidobacterium animalis* subsp. lactis INL1**	Viable	Adult mice	Oral	*Salmonella* Typhimurium	-Reduction of pathogen translocation to liver and spleen. -Reduction the incidence and the severity of infection.	-Enhancement secretory IgA and IL-10 production in the small and large intestine, respectively.	[51]
***Saccharomyces boulardii* (Floratil®)**	Viable	Infant mice	Oral	*Salmonella* Typhimurium	-Increase of survival rate. -Reduction of pathogen translocation -Protection of liver damage.	-Decrease of inflammatory cytokines levels and activation of mitogen-activated protein kinases (p38, JNK and ERK1/2), phospho-IkB, p65-RelA, phospho-jun and c-fos in the colon, signal pathways involved in the activation of inflammation.	[95]
***Lactobacillus casei* CRL431**	Viable	Adult mice	Oral	*Salmonella* Typhimurium	-Improvement of animal survival. -Diminution of pathogen spreading outside the intestine.	-Attenuation of intestinal inflammation. -Modulation of cytokine profile. -Increase in expression and secretion of IgA in the gut. -Increase of peritoneal, Peyer'spatches and spleen macrophages' phagocytic activity. -Increase of MCP-1 production by intestinal epithelial cells *in vitro*.	[42]
***Bifidobacterium longum* subspecies infantis 35624**	Viable	Adult mice	Oral	*Salmonella* Typhimurium UK1	-Prevention of weight loss. -Protection of brush border enzyme activity. -Reduction of small intestinal damage.	-Regulation of IL-10 and IL-8 expressions.	[52]
***Lactobacillus rhamnosus* CRL1505**	Viable	Adult mice	Oral	*Salmonella typhimurium*	-Acceleration the recovery of the clinical nutritional parameters altered by malnutrition as body and thymus weights and serum proteins. -Increase resistance against intestinal infection in immunocompromised hosts.	-Improvement the hematological parameters. -Early normalization of leukocytes, neutrophils and lymphocytes in blood for the recovery of immunity against infections.	[96]
Multispecies probiotics (12 strains selected)	Viable	Adult mice	Oral	*Salmonella typhimurium*	-Significantly decrease of viable pathogen counts in the spleen and liver of the mice.	-Increase in the phagocytotic activity of macrophage cells.	[97]
***Lactobacillus fermentum* ME-3 plus ofloxacin treatment**	Viable	Adult mice	Oral	*Salmonella* Typhimurium	-Eradication of pathogen from tested sites, reduction of typhoid nodules in the liver, and decreased the values of LPO.	-Reduction of pro-inflammatory cytokines IFN-γ and TNF-α. -Increase of anti-inflammatory cytokine IL-10 in the liver.	[50]

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***Lactobacillus casei* DN-114-001**	Viable	Adult mice	Oral	*Salmonella* Typhimurium	-Improvement the intestinal microbiota in immunocompromised hosts. -Decrease the spread of pathogenic bacteria to liver and spleen.	-Increase the number of IgA+ cells, macrophages and dendritic cells. -Increase the production of different cytokine (IFN-γ, TNF-α, IL-12) and the phagocytic activity in cells of peritoneum and spleen.	[98]
***Lactobacillus casei* CRL 431**	Viable	Adult mice	Oral	*Salmonella* Typhimurium	-Decrease the severity of infection. -Improvement of animal survival. -Diminution of pathogen spreading to liver, spleen and large intestine.	-Modulation the inflammatory response (decreased TNF-α and increased IFN-γ, IL-6 and IL-10 production in the lamina propria of the small intestine).	[99]
***Lactobacillus rhamnosus* CRL1505 and *Lactobacillus rhamnosus* CRL1506**	Viable	Adult mice	Oral	*Salmonella typhimurium*	-Both strains improved resistance to infection.	-Both strains increased peroxidase activity of the phagocytic cells in the blood. -*L. rhamnosus* CRL1506 showed higher levels of cytokines (TNF-α, IFN-γ and IL-10) at intestine, while in serum higher cytokines levels were found in the *L. rhamnosus* CRL1505 mice. -*L. rhamnosus* CRL1505 increased blood leukocytes. -*L. rhamnosus* CRL1505 increased IgA at intestine and serum IgG.	[43]
***Lactobacillus casei* CRL431**	Viable	Adult mice	Oral	*Salmonella* Typhimurium	-Diminution of counts of the pathogen in the intestine and its spread outside this organ. -Decrease the severity of the infection.	-Decrease in the neutrophil infiltration with diminution of intestinal inflammation. -Activation the macrophage phagocytic activity in Peyer'spatches, spleen and peritoneum. -Increase in the number of IgA+cells in the lamina propria of the small intestine. -Increased release of s-IgA specific against the pathogen in the intestinal fluids.	[41]
***Lactobacillus casei* DN-114 001**	Viable	Adult and newborn mice	Oral	*Salmonella* Typhimurium	-Decrease of the severity of the infection. -Maintenance the intestinal barrier and the immune surveillance in optimal conditions.	-Regulation of immune maturity.	[100]
Saccharomyces boulardii	Viable	Infant mice	Oral	*Salmonella* Typhimurium	-Prevention of bacterial translocation to the liver. -Improvement of animal survival. -Abolition of pathogen invasion in T84 human colorectal cancer cells.	-Decrease of activation of Rac1. -Preservation of T84 cells barrier function. -Decrease of IL-8 synthesis and inhibitory effect on activation of the MAPKs (ERK1/2, p38 and JNK) and NF-kB.	[101]

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Studies in Mice

Several *in vivo* studies in mouse models of *Salmonella*-infection clearly demonstrated that beneficial effects of probiotics. The preventive administration of the probiotic strain *L. casei* CRL431 to adult immunocompetent mice diminished *Salmonella* Typhimurium counts in the intestine as well as its spread to blood, liver and spleen [41,42]. The probiotic administration decreased neutrophils infiltration with the consequent diminution of intestinal inflammation, activated macrophages' phagocytic activity, and increased the number of IgA^+ cells in the lamina propria of the small intestine which was correlated with increased release of intestinal anti-*Salmonella* IgA antibodies. Similarly, the preventive administration of the probiotics strains *L. rhamnosus* CRL1505 and *L. rhamnosus* CRL1506 enhanced the resistance against *Salmonella* infection through the improvement of macrophages' phagocytic activity, and intestinal anti-*Salmonella* IgA antibodies [43]. In addition, we observed a significant increase in IFN-γ in serum and intestine of mice fed with both strains of *L. rhamnosus*. IFN-γ produced by activated T cells and natural killer (NK) cells, has been shown to play an important role in host defence against intracellular pathogens such as *Salmonella* Typhimurium. *In vitro* studies have shown that, epithelial cells and fibroblasts are resistant to *Salmonella* Typhimurium invasion in the presence of IFN-γ and that this cytokine activates mouse peritoneal macrophages, resulting in enhanced *Salmonella* Typhimurium killing. We also demonstrated that the administration of both lactobacilli strains significantly augmented the expression of IFN-γ in PPs cells compared with control mice [44]. Moreover, *L. rhamnosus* CRL1505 was more efficient than *L. rhamnosus* CRL1506 for increasing the levels of IFN-γ, and IL-6 in the intestine. It is well established that a high IL-12 production of DCs by microbial stimuli gives rise to Th1 polarization and thus a strong stimulation of the adaptive immune defense. In fact, oral administration of LAB to mice has been reported to augment IL-12 and IFN-γ mRNA expressions and $CD4^+$ T cell-DCs interaction in PPs [45]. Studies showed that probiotics are captured by $CD11c^+$ DCs in PPs and increase IL-12 production by these antigen-presenting cells. Subsequently, T cells receive the information from DCs, resulting in the immune activation of $CD4^+$ T and increased production of IL-6 and IFN-γ [46]. Therefore, *L. rhamnosus* CRL1505 would be able to improve intestinal Th1 immune response through this mechanism and it would be more efficient than *L. rhamnosus* CRL1505. Recently, we studied how these two probiotic *L. rhamnosus* strains functionally modulated porcine PPs-derived adherent immune cells ($CD172a^+CD11R1^-$, $CD172a^-CD11R1^{low}$ and $CD172a^+CD11R1^{high}$ cells) [47]. The main effect of incubating *L. rhamnosus* with the single population of immune adherent cells resulted in differential mRNA expression of the key polarizing cytokines IL-6 and IFN-γ, confirming our previous results in mouse models. *L. rhamnosus* CRL1505 was the strain with the highest capacity to functionally modulate porcine antigen-presenting cells. On the other hand, *L. rhamnosus* CRL1505 also improved IL-10 in the gut of mice [43,48] and induced IL-10 mRNA and protein expression porcine PPs-derived adherent immune cells [47], which is an immunoregulatory cytokine that avoids inflammatory-tissue injury during infections. Then, the improved production of IL-10 induced by *L. rhamnosus* CRL1505 in

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antigen-presenting cells could have an important protective effect during intestinal infections. In our experiments, we also observed a clear involvement of TLR2 signalling pathway in the up-modulation of IL-6, IL-10 and IFN-γ in antigen-presenting cells exerted by both *L. rhamnosus* strains. In addition, the lactobacilli reported by Plantinga *et al*. [49] induced cytokines in DCs in a TLR9-dependent manner, contrasting our results which show no relationship between TLR9 and the immunoregulatory effect of *L. rhamnosus* CRL1505 or *L. rhamnosus* CRL1506 [47].

Our studies demonstrated that in addition to the improvement of Th1 response, immunoregulatory mechanisms would be necessary to fully protect against *Salmonella* infection. In line with this statement, some recent studies showed that probiotics with the capacity to improve immunoregulatory mechanisms are able to protect against this intestinal pathogen. Truusalu *et al*. [50] showed that the addition of *L. fermentum* ME-3 to ofloxacin treatment significantly increased the eradication of *Salmonella* Typhimurium in adult challenged mice. This effect was associated to the reduction of pro-inflammatory cytokines (TNF-α) and the increase in anti-inflammatory cytokine IL-10 in the liver of mice. Oral administration of *B. animalis* subsp. lactis INL1 to mice before the challenge with *Salmonella* Typhimurium, reduced the number of infected animals and the levels of translocation to liver and spleen [51]. The protective effect of the INL1 strain was attributed to enhanced secretory IgA levels and IL-10 production in the intestine. Interestingly, Symonds *et al.*[52] showed that *Salmonella* Typhimurium reduces the small intestinal brush border enzyme activity in mice in a dose- and time-dependent manner, being the level of reduction associated to weight loss of adult mice. Moreover, the study showed that *B. longum* subsp. infantis 35624 administration prevented weight loss, protected brush border enzyme activity, and reduced the small intestinal damage. Those effects were related to the capacity of the 35624 strain to modulate IL-10/IL-8 expression after *Salmonella* challenge.

In Vitro Studies

In vitro studies evaluating the activity of LAB strains in intestinal epithelial cells (IECs) demonstrated that probiotic bacteria are able to functionally modulate these cells and improve resistance against *Salmonella*. It was reported that *L. rhamnosus* GG was able to attenuate the barrier disruption of Caco-2 IECs caused by *Salmonella* lipopolysaccharide (LPS) administration [53]. LPS was specifically able to disrupt epithelial barrier and change the location of ZO-1 while *Lactobacillus* treatment was associated with the maintenance of the tight junction integrity and appearance of Caco-2 IECs. Vizoso Pinto *et al*. [54] showed that incubation of HT29 IECs with *L. plantarum* BFE 1685 or *L. rhamnosus* GG significantly increased IL-8 production in response to *Salmonella* Typhimurium. This effect was associated to the capacity of both strains to improve the expression levels of TLR2 and TLR5 in HT29 IECs. On the contrary, Malago *et al.* [55,56] reported a reduction of IL-8 production in response to *Salmonella enteritidis* in Caco-2 cells after the stimulation with *L. casei* Shirota, *L. plantarum* 299v, *B. infantis* W52, *L. casei* W56, or *L. lactis* W58. Authors attributed a beneficial effect to these lactobacilli considering that the decrease in IL-8

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levels could be associated to the control of intestinal inflammation. Moreover, authors concluded that suppression of *Salmonella*-induced IL-8 synthesis by Caco-2 cells exhibited by probiotics was related to the induction of Hsp70 expression. In support to the protective anti-inflammatory effects of probiotic LAB during *Salmonella* infection, some studies reported beneficial effects of probiotics through their interactions with DCs. Bermudez-Brito *et al*. [57] showed that *L. paracasei* CNCM I-4034, and its cell-free culture supernatant decreased pro-inflammatory cytokines and chemokines in DCs generated from umbilical cord blood $CD34^+$ progenitors or human intestinal DCs challenged with *Salmonella*. The supernatant was as effective as the bacterium in reducing pro-inflammatory cytokine expression. Interestingly, the bacterium was a potent inducer of TGF-β2 secretion, whereas the supernatant increased the secretion of TGF-β1 in response to *Salmonella*. The work also showed that both the bacterium and its supernatant strongly induced the transcription of the TLR9, CASP8 and TOLLIP genes. Similarly, it was demonstrated that supernatant of *B. breve* CNCM I-4035 decreased pro-inflammatory cytokines and chemokines in human intestinal DCs challenged with *Salmonella typhi*, and upregulated TLR9, CASP8, IRAK4 and TOLLIP genes expression in the presence of *Salmonella typhi* [58]. In contrast, the *B. breve* CNCM I-4035 strain was a potent inducer of the pro-inflammatory cytokines and chemokines tested (TNF-α, IL-8 and RANTES), as well as anti-inflammatory cytokines including IL-10. In addition, *B. breve* CNCM I-4035 upregulated TLR9, and TOLLIP gene expression. The work speculated that *B. breve* CNCM I-4035 may protect intestinal mucosa from highly infectious agents such as *Salmonella typhi* and modulate the immune system through the down-regulation of pro-inflammatory pathways at the same time.

PROBIOTICS AND *ESCHERICHIA COLI* INFECTION

Escherichia coli is one of the most important bacterial species in the human alimentary tract. In healthy humans, these bacteria are harmless commensals and live in a symbiotic relationship contributing to the welfare of the host. Some strains can also be hostile. Enterotoxigenic *Escherichia coli* (ETEC) are the most common bacterial pathogens causing diarrhea in developing countries where they lead to hundreds of thousands of deaths, mostly in children. These organisms are also a leading cause of diarrheal illness in travelers to endemic countries. Moreover, diarrhea due to ETEC is an important problem in neonatal and just weaned piglets and hence for the pig farming industry.

Studies in Animal Models

Several studies in mice models have demonstrated the capacity of probiotics to improve resistance against pathogenic *E. coli* (Table 2). Administration of probiotic strains such as *B. lactis* HN019, *B. thermacidophilum* RBL 71, *L. rhamnosus* HN001 or *L. casei* CRL431 are able to reduce bacterial translocation and the severity of infection and improve survival of mice infected with enteroinvasive or enterohemorrhagic *E. coli* [59-62]. Those effects were related to the enhancement of leucocyte phagocytic activity and improvement of secretory anti-*E. coli* IgA in

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intestinal fluid. Moreover, probiotics have been associated to improved levels of IFN-γ in the gut and blood, which correlated with increased protection against pathogenic *E. coli* [63,64]. In this regard, it was showed that *Lactobacillus casei* I-5 activated NF-kB pathway in macrophages and enhanced the production of IFN-γ, IL-12 and TNF-α in response to LPS challenge or pathogenic *E. coli* Juhl infection [64]. *L. rhamnosus* was able to increase of IFN-γ and decreased IL-4 production in splenocytes, beneficially modulating the immunosenescence-associated Th1/Th2 imbalance, which in turn enhanced the resistance of old mice to the challenge with pathogenic *E. coli* [63]. The improvement of the Th1 response, macrophages phagocytic activity and modulation of pro-inflammatory cytokines during pathogenic *E. coli* infection has been also described for infant mice [65]. Authors demonstrated that administration of *L. gasseri* TMC0356 to infant mice significantly reduced the symptoms of infection: piloerection, soft stool, diarrhea, and anal hyperemia; and decreased the mortality of infected mice.

Table 2: Probiotics and *Escherichia coli* infection.

Strain	Viability	Mice	Route	Challenge	Protective effect	Immunoregulatory effect	Ref.
***Lactobacillus gasseri* TMC0356 (TMC0356)**	Viable	Infant mice and rats	Oral	Enteropathogenic *Escherichia coli*	-Reduction of general symptoms (piloerection, soft stool, diarrhea, and anal hyperemia). -Reduction of mortality of infected mice in the early phase.	-Increased phagocytic activity of peritoneal macrophages -Significant increase of IL-6 and slightly increased of TNF-α, IL-1β, IL-10, and IL-12.	[65]
Lactobacillus rhamnosus	Viable	Old mice	Oral	Enteropathogenic *Escherichia coli* (ATCC 14948)	-Increase of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) in liver and red blood cells. -Reduction of pathogen translocation to organs (intestine, liver, spleen, peritoneal fluid).	-Increase of IFN-γ and decreased IL-4 and IL-10 production in splenocytes. -Increase of neutrophil respiratory burst enzymes and phagocytosis. -Enhancement of E. coli-specific antibodies (IgA and IgG1) and inflammatory proteins. -Alleviation of immunosenescence-associated Th1/Th2 imbalance.	[63]
***Lactobacillus paracasei* subsp. paracasei NTU 101**	Viable	Adult mice	Oral	Enterohemorrhagic *Escherichia coli* O157:H7	-Increased weight gain. -Promotion of survival.	-Up regulation of dendritic cells, helper T cell activation, and antibody production. -Down regulation of the expression of TLRs on macrophages and pro-inflammatory cytokines, and chemokines.	[78]

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***Lactobacillus casei* I-5**	Viable	Adult mice and rats	Oral	Pathogenic *Escherichia coli* Juhl	-Promotion of survival.	-Slightly increase of IFN-γ and decrease of IL-6 in plasma. -Increased phagocytic activity of peritoneal macrophages. -Activation of NF-kappaB, IL-12 and TNF-α in macrophages stimulated by LPS.	[64]
***Bifidobacterium thermacidophilum* RBL 71**	Viable	Adult mice	Oral	Enterohemorrhagic *Escherichia coli* O157:H7	-Reduction the severity of infection. -Decrease in the fecal pathogen counts. -Increased weight gain. -Attenuation of intestinal injuries.	-Increase of specific IgA in feces and IgG+IgM in serum. -Improvement of the lymphoid component in the mucosa of the ileum.	[62]
Lactobacillus casei, lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus	Viable	Adult mice	Oral	Enteroinvasive *Escherichia coli*	-Decrease of *E. coli* colonization of liver than control mice	-Enhancement the unspecific immune response. -Increase in the percentage of phagocytosis and number of IgA+ cells in the small intestine. -Increased secretory anti-E. coli IgA in intestinal fluids.	[59]
***Lactobacillus rhamnosus* HN001**	Viable	Adult mice	Oral	Enterohemorrhagic *Escherichia coli* O157:H7	-Promotion of survival. -Lower bacterial translocation rates.	-Significantly higher intestinal anti-E. coli IgA responses. -Increase of blood leucocyte phagocytic activity.	[60]
***Bifidobacterium lact*is HN0**19	Viable	Adult mice	Oral	Enterohemorrhagic *Escherichia coli* O157:H7	-Promotion of survival. -Reduction of the severity of infection. -Lower bacterial translocation.	-Increase of blood and peritoneum leucocyte phagocytic activity. -Increase of intestinal IgA anti-E. coli antibody responses.	[61]

PRRs signaling in the intestinal epithelium can trigger pro-inflammatory responses by underlying lamina propria immune cells [66,67]. This PRRs signaling is a crucial aspect of innate defense [68,69], but if uncontrolled at mucosal surfaces, it becomes pathological. Thus, robust mechanisms must be in place to avoid chronic stimulation of inflammatory signaling by the resident microbiota while maintaining responsiveness to pathogens. Several studies reported that in addition to their anti-pathogenic abilities, probiotics are capable of beneficially modulate the inflammatory response during pathogenic *E. coli* infections, avoiding tissue damage induced by the host response.

On the other hand, some studies have specifically evaluated the capacity of probiotics to improve the resistance of piglets against ETEC. Qiao *et al.* [70] conducted experiments to evaluate the effects of a complex Lactobacilli preparation on performance, resistance to *E. coli* infection and gut microbial flora of weaning pigs. The mix of four lactobacilli (*L. gasseri, L. reuteri, L. acidophilus and L. fermentum*) isolated from weaning pigs was able to reduce *E. coli* and anaerobe counts in the gut, and decrease diarrhea. Additionally, lactobacilli treatment significantly improved

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average daily feed intake of pigs compared to controls during the first two weeks after weaning and the average daily gain [70]. Herfel *et al*. [71] examined the impact of a novel probiotic strain of *Bifidobacterium longum* AH1206 on the health, growth and development of neonatal pigs. Authors found that ileal IL-10 expression increased progressively with AH1206 supplementation, which indicated the potential for modulation of the inflammatory tone of the intestinal mucosa of suckling piglets. However, no differences were found between AH1206-treated and control piglets when comparing body weight gain, feed efficiency (gain: intake). Another recent study evaluated the effect of the co-administration of *Bacillus subtilis* RJGP16 and *Lactobacillus salivarius* B1 on intestinal immunity in piglets [72]. Authors demonstrated that probiotic administration increased the expression of IL-6, porcine β-defensins and IgA producing cells in the intestine, clearly showing that co-administration of RJGP16 and B1 strains strongly enhances the intestinal mucosal immunity of piglets. It was shown that the probiotic strain *L. plantarum* CJLP243 may serve as a potential alternative to antibiotic supplementation to improve the growth and health performance of weaning pigs because of its capacity to reduce the severity of ETEC-induced diarrhea [73]. Li *et al*. [74] showed that pretreatment of piglets with *L. rhamnosus* ATCC7469 ameliorates $F4^+$ETEC-induced diarrhoea. In piglets exposed to $F4^+$ETEC, jejunal TLR4 and IL-8 expression were increased; however, these increases were attenuated by administration of *L. rhamnosus*. Notably, expression of jejunal TLR2, ileal TLR9, NOD1 and TNF-α was upregulated in the ATCC7469-treated piglets after $F4^+$ETEC challenge [74]. These results indicate that probiotic treatments would be able to beneficially modulate the overwhelming inflammatory response in infected piglets and improve resistance against ETEC.

In Vitro Studies

A study in Caco-2 cells demonstrated that *L. rhamnosus* GG counteracts the enterotoxigenic *Escherichia coli* (ETEC)-induced up-regulation of IL-1β and TNF-α and the down-regulation of TGF-β1 expression, consequently blocking cytokine deregulation [75]. In addition, comparative studies between *L. rhamnosus* GG and *Bifidobacterium longum* MB5 demonstrated that individual strains of probiotics have a different impact on the inflammatory response triggered in IECs [75]. Others studies evaluating the effect of immunobiotic yeasts have shown that *Saccharomyces cerevisiae* CNCM I-3856 decreases the expression of the pro-inflammatory mediators IL-6, IL-8, CCL20, CXCL2, and CXCL10 in porcine intestinal epithelial IPI-2I cells cultured with $F4^+$ ETEC [76]. Moreover, the CNCM I-3856 strain inhibits ETEC-induced expression of pro-inflammatory cytokines and chemokine transcripts and proteins, and this inhibition is associated with a decrease in ERK1/2 and p38 MAPK phosphorylation and an increase in the mRNA level of anti-inflammatory PPARγ [77]. These findings indicate that some immunobiotic strains could be beneficial for preventing inflammation-mediated damage in IECs. Moreover, studies of Tsai *et al*. [78] showed that *L. paracasei* subsp. *paracasei* NTU 101, administered to adult mice infected with enterohemorrhagic *E. coli* O157:H7, increased survival by upregulating DCs and Th1 cells activities, and downregulating the expression of TLRs on macrophages and pro-inflammatory cytokines, and chemokines (Table 2).

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To study the mechanisms by which IECs induce an immune response to pathogens and the potential immunoregulatory effect of immunobiotics, we previously established a clonal porcine intestinal epitheliocyte cell line (PIE cells) [79]. We observed that stimulation of PIE cells with porcine-specific ETEC significantly increases the mRNA levels of IL-1α, IL-6, IL-8, and monocyte chemotactic protein (MCP)-1 12 hours after the challenge and that the damage to PIE cells correlates with the mRNA levels of pro-inflammatory cytokines produced after stimulation with ETEC and LPS [80]. We selected lactobacilli and bifidobacteria strains able to regulate the inflammatory response induced by ETEC in PIE cells by evaluating the levels of IL-1α, IL-6, IL-8, and MCP-1. Interestingly, *L. jensenii* TL2937, a strain with a high capacity to activate TLR2, was the strain with the highest capacity to down-regulate IL-6 and IL-8 production by PIE cells in response to ETEC. For this reason, we became interested in *L. jensenii* TL2937 and examined the mechanisms behind the anti-inflammatory effect mediated by this strain, and demonstrated that *L. jensenii* TL2937 inhibits NF-κB and MAPK signaling pathways in ETEC-challenged PIE cells [81]. To dissect the mechanism(s) involved in the anti- inflammatory effect of *L. jensenii* TL2937, the effect of this strain on the expression of the negative TLR regulators in PIE cells was evaluated. The expression of SIGIRR, TOLLIP, A20, Bcl-3, MKP-1, and IRAK-M was studied, and it was found that MKP-1, A20, and Bcl-3 mRNA expression was upregulated in PIE cells stimulated with *L. jensenii* TL2937 [81]. Recently, we also demonstrated that *B. longum* BB536 and *B. breve* M-16V significantly downregulated levels of IL-8, MCP-1, and IL-6 in PIE cells challenged with ETEC by modulating the NF-κB and MAPK pathways [82]. Moreover, both bifidobacteria upregulated A20 in PIE cells in a TLR2-dependent manner. Then, the most effective anti-inflammatory strains evaluated in our laboratory, *L. jensenii* TL2937 and bifidobacteria strains BB536 and M-16V, strongly upregulated the ubiquitin-editing enzyme A20. This finding is of interest because it not only shows a common mechanism for the anti-inflammatory activity of immunobiotics but also provides a potential biomarker for the screening and selection of new immunoregulatory strains.

Two recent studies support our investigations by demonstrating the capability of probiotics to beneficially modulate ETEC infection/inflammation in porcine IECs. Zhou *et al. and* [83] showed that *L. reuteri* CL9 is capable of reducing the expression of enterotoxin genes (*estA*,*estB* and *elt*) in ETEC at the early stage of its infection to IPEC-J2 cells. Cell death of IPEC-J2 induced by STa and STb heat-stable enterotoxins was all remarkably reduced by CL9. The host responses of IPEC-J2 to ETEC infection in the absence and presence of *L. reuteri* CL9 was also investigated by measuring the level of IL-8 and IL-10 produced by IPEC-J2 cells. Authors found that CL9 was able to suppress the increase in IL-8 production induced by ETEC and substantially enhanced the production of IL-10. Finamore A *et al.* [84] demonstrated in intestinal explants isolated from 5 week-old piglets, that *L. amylovorus* suppress the activation of the different steps of TLR4 signaling, by inhibiting the ETEC induced increase in the level of TLR4 and MyD88, the phosphorylation of the IKKα, IKKβ, IκBα and NF-κB subunit p65, as well as the over-production of inflammatory cytokines IL-8 and IL-1β. Similarly to our previous results, authors showed that the anti-inflammatory effects of

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L. amylovorus were achieved through modulation of the negative regulators TOLLIP and IRAK-M in a TLR2-dependent manner.

Considering the anti-inflammatory effects of the TL2937 strain in IECs and the critical importance of antigen-presenting cell (APC) polarization in immunoregulation, it was also examined the effect of *L. jensenii* TL2937 on activation patterns of APCs from porcine PPs. *Ex vivo* experiments using porcine PPs APCs showed that the treatment with *L. jensenii* TL2937 increases the expression of IL-10 and TGF-β in $CD172a^{+}CD11R1^{high}$ and $CD172a^{+}CD11R1^{-}$ cells, whereas treatment with this bacterium is associated with increased levels of IFN-γ in $CD172a^{-}CD11R1^{low}$ cells [48]. Then, the direct exposure of porcine APCs to *L. jensenii* TL2937 in the absence of inflammatory signals activates $CD172a^{+}$ APCs and causes them to become phenotypically and functionally mature and to display tolerogenic properties [48]. Treatment of APCs with *L. jensenii* TL2937 also results in differential modulation of the production of pro- and anti-inflammatory cytokines in response to ETEC challenge. The differential effects of the TL2937 strain in each PPs APC population persist because increased production of IFN-γ is observed in $CD172a^{-}CD11R1^{low}$ cells and improved synthesis of IL-10 is detected in $CD172a^{+}CD11R1^{high}$ and $CD172a^{+}CD11R1^{-}$ cells. Moreover, of the six negative regulators of TLRs tested, SIGIRR, A20, and IRAK-M mRNA expression was up-regulated in $CD172a^{+}$ cells stimulated with *L. jensenii* TL2937 [48].

In a recent study a co-culture system with a PIE cell monolayer and immunocompetent cells from swine PPs was used to model an *in vitro* PPs culture system [85]. A significant upregulation of proinflammatory cytokines was observed in PIE cells co-cultured with PPs APCs and challenged with ETEC. These results showed that PIE cells did not responded differently to TLR4 activation when co-cultured with APCs. Moreover, the pretreatment of PIE cells with *L. jensenii* TL2937 reduced proinflammatory cytokines in response to ETEC that this effect was related to upregulation of the same three TLR negative regulators: A20, Bcl-3, and MKP-1 as in PIE cell monocultures [85]. On the contrary, the indirect effect of *L. jensenii* TL2937 on APCs in co-cultures was completely different to those observed in APCs monocultures. In PIE-APCs co-cultures, no modifications in the levels of TGF-β in $CD172a^{+}CD11R1^{-}$ and $CD172a^{+}CD11R1^{high}$ cells or levels of IFN-γ in $CD172a^{-}CD11R1^{low}$ cells were observed. However, increased levels of IL-10 were found in $CD172a^{+}$ cells co-cultured with PIE cells. In addition, no modification in SIGIRR, A20 or IRAK-M expression was observed in those cells. Notably, Bcl-3 expression was upregulated in APCs cells co-cultured with PIE cells [85]. These results indicated that the response of PPs APCs to *L. jensenii* TL2937 is significantly modified when the stimulus is mediated indirectly through IECs. Considering the capacity of *L. jensenii* TL2937 to functionally modulate the response of PIE cells and porcine APCs, it was hypothetized that this strain would significantly impact on piglets' immune heath. The *in vivo* experiments in pigs indicate that *L. jensenii* TL2937 is able to improve immunity and regulate excessive inflammation [85]. These effects seem to be related to the complex secretion of cytokines induced by the probiotic strain in the gut. *L. jensenii* TL2937 could strongly induced secretion of IL-10 and IFN-γ that would be related to the beneficial effects achieved by the immunobiotic strain.

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PROBIOTICS AND *CITROBACTER RODENTIUM* INFECTION

Citrobacter rodentium is a Gram-negative enteric bacterium that is a natural pathogen of mice. In its natural host, *C. rodentium* causes colonic epithelial hyperplasia accompanied by mild diarrhea. Its principal importance, however, is that infection of mice with *C. rodentium* provides a convenient small animal model to investigate the molecular and cellular pathogenesis of infections with the human pathogens, enteropathogenic *Escherichia coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC). This is because all three pathogens produce virtually indistinguishable attaching and effacing lesions in the intestinal epithelium, due to the fact that they carry the locus for enterocyte effacement, a highly conserved pathogenicity island, which is required for the development of these lesions [86]. Therefore, the mouse model of infection with *C. rodentium* has proved invaluable in elucidating key features of the pathogenesis of infections with attaching and effacing enterobacteria in general. Moreover, this model allows the investigation of the cellular and molecular mechanisms involved in host-protective immunity and bacterial-induced intestinal inflammation. Intestinal infection with *C. rodentium* induces a strong local Th17 response in the colon. Although this inflammatory immune response helps to clear the pathogen, it also induces inflammation-associated pathology in the gut and thus, has to be tightly controlled [87].

Some laboratories have used this mouse model to evaluate the efficacy of probiotic bacteria to beneficially modulate the response to attaching and effacing enterobacteria [88] (Table 3) evaluated in adult and infant mice the capacity of *L. acidophilus* NCFM to protect against *C. rodentium* infection. The study found that the preventive administration of the NCFM strain significantly enhanced host defense against enteric bacterial infection and attenuated bacteria-mediated colitis. Probiotic treatment was associated with a decrease in *C. rodentium* colonization and translocation, and increase in its clearance. *L. acidophilus* NCFM was able to improve intestinal IgA secretion, stimulated regulatory cytokine expression in the colon (TGF-β and IL-10) and reduced pro-inflammatory cytokine expression (TNF-α, IL-6, and IL-12) and myeloperoxidase activity. Later, a key role in the immunoregulatory effect of the NCFM strain was attributed to DCs in infant mice [89]. DC isolation and adoptive transfer was used to examine their function in probiotic activity. Authors demonstrated that when mice were adoptively transferred with *L. acidophilus* NCFM-primed DCs instead of the oral consumption of the probiotic strain, there was a similar effect on fecal *C. rodentium* counts, IgA levels, and colonic histopathology, as well as cytokine levels after intestinal bacterial infection. Similarly, [90] first studied in adult mice the capacity of a mixture of *L. rhamnosus* strain R0011 and *L. helveticus* strain R0052 (Lacidofil) to improve resistance against *C. rodentium* infection. The work showed that probiotic treatment was able to modulate mucosal inflammation, reduce apoptosis in the colon, and improve IFN-γ production. These changes allowed mice that received viable probiotics to remain healthy after *C. rodentium* challenge. Later, the same probiotic treatment was evaluated in 14 days-age neonatal mice [91]. *C. rodentium* infection caused weight loss and death indicating that neonatal mice are

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highly susceptible to infection. Probiotic treatment was able to significantly improve survival of neonatal mice, by reducing weight loss, colonic epithelial cell hyperplasia, and mucosal barrier dysfunction. Those probiotic effects were observed in *C. rodentium*–infected wild-type mice, but not in *rag1*$^{-/-}$ animals, indicating a key role of T cells in reducing the adverse sequelae of neonatal enteric infection. Recently, an important role was attributed to T-regulatory cells in the beneficial effect induced by the *L. rhamnosus* R0011 and *L. helveticus* R0052 mixture [92]. In both, adult and neonatal mice it was demonstrated that this probiotic treatment reduced pro-inflammatory cytokine expression (TNF-α and IL-17) while promoted transcription of IL-10 and FOXP3, and increased follicular T-regulatory cells. Those studies clearly indicate that probiotics can be used as effective tools to beneficially modulate the balance of pro- and anti-inflammatory components during *C. rodentium* infection, which is translated in an improved resistance to the infection, even in neonatal hosts. In support to this statement, it was recently reported that a probiotic *L. acidophilus* strain promotes host-protective immunity and attenuate *C. rodentium*-induced intestinal inflammation by modulating NF-κB pathway [93]. The study showed that the improved host defense against *C. rodentium* infection correlated with enhanced colonic IL-10 and TGF-β expression and inhibition of NF-κB pathway in probiotic-treated 3 days-age neonatal mice.

Table 3: Probiotics and *Citrobacter rodentium* infection.

Strain	Viability	Mice	Route	Challenge	Protective effect	Immunoregulatory effect	Ref
***Bacillus subtilis* 3610**	Viable	Adult mice	Oral	*Citrobacter rodentium*	-Prevention of pathogen-associated intestinal disease.	-Protection is TLR4 dependent, with requirement of myeloid cells.	[102]
***Lactobacillus rhamnosus* strain R0011 and *Lactobacillus helveticus* strain R0052**	Viable	Neonatal and adult mice	Oral	*Citrobacter rodentium*	-Amelioration of barrier dysfunction, epithelial hyperplasia, and binding of the pathogen to host colonocytes. -Normalization of fecal microbiota.	-Reduction of pro-inflammatory cytokine expression (TNF-α and IL-17) -Promotion of transcription of IL-10 and FOXP3. -Increment of follicular T-regulatory cells.	[92]
***Bifidobacterium breve* UCC2003**	Viable	Adult mice	Oral	*Citrobacter rodentium*	-Reduction of pathogen colonization in the gut.	-Evasion of adaptive B-cell responses due to exopolysaccharide (EPS) production.	[94]
Lactobacillus acidophilus	Viable	Neonatal mice	Oral	*Citrobacter rodentium*	-Reduction of bacterial colonization. -Decrease of intestinal inflammation.	-Enhancement of colonic IL-10 and TGF-β expression. -Modulation of NF-kB pathway.	[93]
Mixture of *Lactobacillus rhamnosus* strain R0011 and *Lactobacillus helveticus* strain R0052	Viable	Neonatal mice	Oral	*Citrobacter rodentium*	-Promotion of survival. -Amelioration of weight loss, colonic epithelial cell hyperplasia, and mucosal barrier dysfunction.	-Requirement of T cells in reducing the adverse sequelae of neonatal enteric infection.	[91]

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***Lactobacillus acidophilus* NCFM**	Viable	Infant mice	Oral	*Citrobacter rodentium*	-Reduction of susceptibility of infection. -Attenuation of pathogen associated colonic pathology. -Lower pathogen output in the fecal pellets.	-Protective immune response by stimulating the host to produce higher IgA in the intestinal lumen and enhance protective bacterial-antigen-specific immune responses. -Up-regulation of DCs co-stimulatory molecules.	[89]
Mixture of *Lactobacillus rhamnosus* strain R0011 and *Lactobacillus acidophilus* strain R0052 (Lacidofil)	Viable	Adult mice	Oral	*Citrobacter rodentium*	-Inhibition of pathogen growth *in vitro*. -Decrease of pathogen colonization of the colon. -Reduction in colonic hyperplasia, epithelial cell damage, number of apoptotic colonocytes, and colitis in infected mice.	-Increased IFN-γ production by splenocytes. -Increase IL-10 levels. -Change from a primarily proinflammatory Th1 cell response to a more balanced Th1/Thr (regulatory) host immune response.	[90]
***Lactobacillus acidophilus* NCFM**	Viable	Infant and adult mice	Oral	*Citrobacter rodentium*	-Decrease of pathogen colonization and translocation. -Increase of pathogen clearance. -Attenuation of bacteria-mediated colitis.	-Suppression of colonic mieloperoxidase activity and down regulation of pro-inflammatory cytokine expressions (TNF-α, IL-6, and IL-12). -Stimulation of regulatory cytokine expression (TGF-β, IL-10) in the colon, and induction of intestinal IgA secretion.	[88]

In an *in vivo* mice model, the administration of *Bifidobacterium breve* UCC2003 prior to *Citrobacter rodentium* challenge significantly reduced the total bacterial load during infection. The production of surface exopolysaccharide/capsule (EPS) by *B. breve* UCC2003 would provide tolerance to the adverse environmental conditions from the gastrointestinal tract and, the mechanism by which *B. breve* UCC2003 reduces pathogen levels would be because of total *B. breve* load and the ability to form biofilms [94]. Moreover, the authors observed the adaptive response influenced by surface EPS and impairment in immune cell trafficking of B cells and the number of innate cellular populations [94]. The critical chemo-attractants were evaluated by IFN-γ–, TNF-α–, and IL-12–positive T cells, and IL-12–positive B cells, which were significantly attenuated in mice treated with *B. breve* UCC2003 (EPS+). Besides, it was observed significantly lower numbers of splenic plasma cells and decreased antibody responses in mice treated with *B. breve* UCC2003 (EPS+). Also, using a B-cell–deficient mouse strain, it was confirmed that *B. breve* UCC2003 surface EPS is crucial for persistence within the murine host, through subversion of B-cell responses, similar to that used by many pathogenic bacteria [94].

CONCLUSIONS

The field of application for probiotics has increased significantly in the past decades. The mechanisms of action of probiotics are better understood now, thanks to detailed cellular and molecular *in vitro* and *in vivo* studies. As discussed in this work, research suggests that probiotics

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are able to decrease the risk or duration of gastrointestinal infection symptoms, such as those produced by *Salmonella*, pathogenic *Escherichia coli* or *Citrobacter rodentium*. It has been clearly demonstrated that probiotics can act directly against pathogenic bacteria by producing antimicrobial agents, or by competing for nutrients. They can also be effective against intestinal pathogens by interacting with the host, either by reinforcing the function of the epithelium barrier or by modifying the immune system response. As reviewed by Lebeer *et al.* [95], the final conclusion of works that have studied the molecular mechanism of probiotic immunomodulatory activities in the gastrointestinal tract is that: *"their effect depends on the combination of distinct microbial-associated molecular patterns (MAMPs) that interact with various pattern recognition receptors (PRRs) and the associated co-receptors that fine tune signaling, as well as on the quantity and quality of these MAMPs. Therefore, host-probiotic interactions are not univocal but involve complex interactions among various microbial molecules, host receptors, and adaptor molecules"*. These interactions of probiotics' MAMPs with the PRRs expressed in intestinal epithelial cells and antigen-presenting cells beneficially modulate immune responses in the gastrointestinal tract and improve the resistance against pathogens (Figure 2). It seems that both, the improvement of specific cellular and humoral immune responses together with an efficient regulation of pathogen-induced inflammation are necessary to achieve full protection against gastrointestinal infectious diseases. Probiotic strains able to modulate effector and regulatory mucosal immune responses can reduce pathogen loads and protect against inflammatory tissue damage. This expanding knowledge about the cellular and molecular effects of beneficial probiotic bacteria in innate and adaptive mucosal immune system will allow the possibility of new treatments for improving health not only in humans but also in animals.

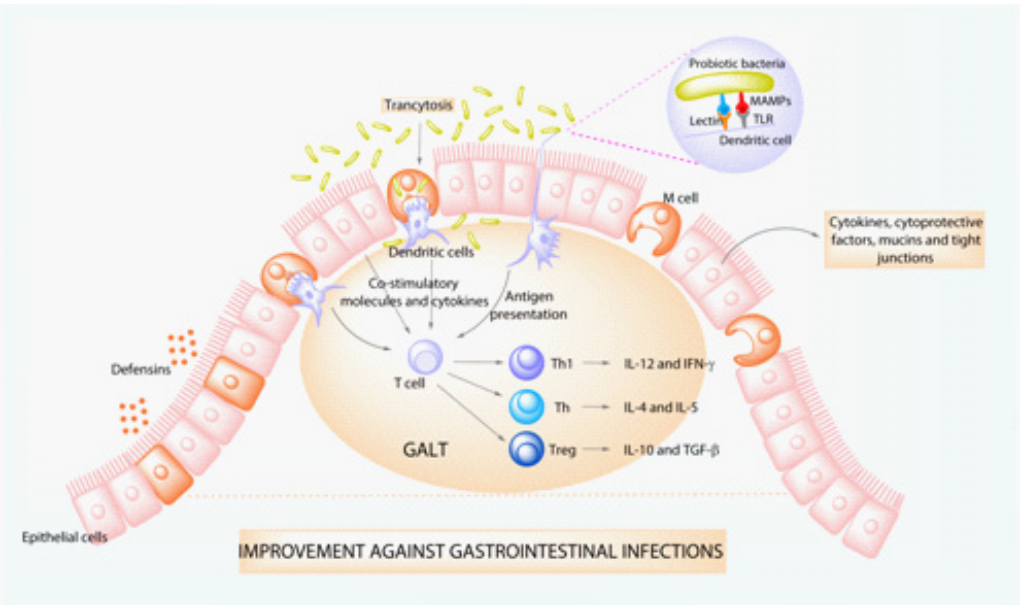

Figure 2: Proposed mechanisms for the immunoregulatory activities of probiotics.

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