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Intestinal Microbiota and Susceptibility to Viral Infections: Role of Probiotics

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1 INTRODUCTION

Intestinal viral pathogens have an enormous social and economic impact worldwide. Gastrointestinal viruses are excreted with the feces and are transmitted by the orofecal route, by direct contact with infected individuals or by contact or consumption of water or contaminated food. Intestinal viral infections coursing with diarrhea are commonly treated by oral or parenteral rehydrating therapies, although alternative treatments such as those based on probiotics are gaining interest for both the treatment and prevention of infectious diarrheas. Probiotics are live bacteria that generally belong to the lactic acid bacteria group or to the bifidobacteria, but also include some bacilli or yeasts. In general, these microorganisms are common members of the intestinal microbiota but they are also employed in the manufacturing of many fermented foods. Numerous reports have identified several bacterial probiotic strains as useful tools for the treatment of intestinal disorders and there are many clinical studies indicating that specific probiotic strains, such as *Lactobacillus rhamnosus* GG (LGG) or *Bifidobacterium* Bb12, have a positive impact on gastroenteritis. Several animal and clinical trials with these and other strains have documented a reduced severity and duration of diarrhea after oral intake. Most of the positive effects are believed to be based on immunoregulation, which gives way to enhanced cellular immune responses or higher levels of virus-specific immunoglobulins. However, other mechanisms exist for explaining the antagonistic effect of probiotics against viral infections, which include a direct interaction of viruses with bacteria and the blocking of specific viral receptors in target cells.

Evidence obtained from viruses that do not replicate in the gastrointestinal tract and, more recently, from gastrointestinal viruses, demonstrate that some viruses take advantage of microbiota-triggered processes or microbiota-derived substances for their replication. However, in other cases viral infection is inhibited by mechanisms dependent on the presence of the intestinal microbiota. This is achieved by direct or indirect effects that are generally related to immunoregulation (Pfeiffer and Sonnenburg, 2011; Wilks et al., 2013). This dual role of the microbiota can be exploited, together with the use of probiotics, for developing new therapeutic strategies to fight against viral infections.

2 GASTROINTESTINAL VIRUSES

A great variety of viruses target the gastrointestinal tract, although not all of them cause gastroenteritis. The most important enteric viruses correspond to rotaviruses (RV, *Reoviridae* family), noroviruses (NoV, *Caliciviridae* family), enteric adenoviruses (*Adenoviridae* family), astroviruses (*Astroviridae* family), and enteroviruses (*Picornaviridae* family) (Buesa and Rodríguez Díaz, 2006). NoV and RV are by far the most relevant viral groups responsible for gastroenteritis and due to their relevance and to the existence of some clinical trials with probiotics, they will be the focus of this chapter. However, at some locations enteric adenoviruses of defined subtypes are the second agents responsible for infantile gastroenteritis and astroviruses may be responsible of up to 9% of the viral diarrhea incidence in developed and developing countries. One member of the kuboviruses (enteroviruses) is responsible for gastroenteritis outbreaks due to the consumption of contaminated food (Aichi virus). Finally, an important representative of enteroviruses, the poliovirus, replicates at the intestine, being primarily asymptomatic, although it finally can target the nervous system resulting in poliomyelitis. With the exception of poliovirus, data on the interaction of human gastrointestinal viruses different than RV and NoV with the microbiota or

probiotics are still lacking. Due to the importance of RV and NoV in viral gastroenteritis in humans, these two virus groups will be discussed in more detail.

RV are segmented double-stranded RNA viruses belonging to the *Reoviridae* family that infect mature enterocytes. RV infection courses with diarrhea and they are the main etiological cause of severe gastroenteritis in children. Most children are infected by these viruses during childhood until the age of 5 years, when most of the population is seroconverted and infection becomes asymptomatic. Dehydration caused by RV is responsible of approximately 500,000 deaths per year, mostly in developing countries where deficient medical care exists. However, the incidence is also high in developed countries and they are responsible for high infant morbidity. Dehydration caused by RV diarrhea can be severe and also lead to many hospitalizations in these countries, resulting in high social costs. Based on the antigenicity of the capsid VP6 protein, seven RV serogroups have been defined, but serogroup A is the most important from a public health perspective. The RV 70-nm icosahedral capsid is formed by three protein shells that protect 11 segments of genetic material. Three major structural and nonstructural proteins (NSPs) are of interest in epidemiological studies and vaccine development against group A RV: the NSP4, which represents the first viral enterotoxin described and is involved in the physiopathology of RV infection (E genotypes 1-14), and the structural proteins VP7 (G genotypes 1-27) and VP4 (P genotypes from 1 to 37). These two last proteins are of special interest due to their capacity to elicit neutralizing antibodies. Currently, several anti-RV vaccines are marketed (Rotateq, Rotarix) that cover different serotypes. However, RV represent one of the viral intestinal pathogens where more intense research has been carried out in the use of probiotics aimed at prevention and alleviation of diarrhea symptoms and duration. This is of special interest in developing countries, where the impact of RV diarrhea is high and the probiotic therapy and prophylaxis could be envisaged as a treatment. This has many advantages due to easier application compared to vaccination (reduction in the need of cold chain in some cases, and in the need of specialized personnel for application), resulting in a more economic and feasible alternative.

It is not surprising that, owing to the importance of RV infection in children, a great proportion of the research in the field of the relationship between probiotics and viral intestinal pathogens has been carried out with RV. However, while RV only cause diarrhea in children until 5 years of age and their infections are generally asymptomatic in adults, other important pathogens, such as the NoV, infect all age groups.

NoV are members of the *Caliciviridae* family that infect the small intestine of mammals and are responsible for the majority of sporadic gastroenteritis and gastroenteritis outbreaks transmitted by food and water (contaminated drinking water and recreational swimming water) worldwide. The incubation time ranges from 15 to 48 h, producing gastroenteritis for 12 to 60 h from the beginning of the symptoms. NoVs infections course with a self-limited diarrhea, abdominal pain, vomiting, and fever, but in particular cases it can lead to severe dehydration and death. It is estimated that 50% of gastroenteritis linked to the consumption of contaminated foods is due to the presence of NoV, for which these viruses represent an important public health problem and produce big economical expenses due to nosocomial infections, work absenteeism, infections in restaurants, and so forth. However, these estimations are difficult because in most cases of infectious diarrhea, the identification of the pathogen is not achieved. This is the case for NoV, where there is a lack of simple and rapid tools for diagnosis. Nowadays, the most employed technique for the detection of NoV presence is the determination of the NoV RNA by RT-PCR or RT-qPCR, which requires nucleic acid extraction from stools and the use of a technique requiring specialized personnel and equipment. In addition, the high genetic variability of these viruses complicates their detection and means that the incidence of NoV infection is probably underestimated. Contrarily to RV, where effective vaccines carrying attenuated viruses from various genotypes are marketed, nowadays no effective vaccine against NoV has been developed.

Like other members of the *Caliciviridae* family, NoV has a linear single-stranded RNA genome of positive polarity of about 7 kb surrounded by a major capsid protein (VP1) of about 500 amino acids. Their genetic variability is very high and up to five genogroups have been defined to date (GI to GV) with multiple genotypes and strains. Members of the same genogroup differ between 45% and 65% in their capsid genes, while differences within the same genotype are between 14% and 44%. Genogroups GI (with 8 genotypes, GI.1 to GI.8) and GII (with 19 genotypes, GII.1 to GII.19) are the main NoV causing diarrhea in humans and, among them, the genotype GII.4 is currently the most extended genotype causing outbreaks around the world.

Contrary to RV, which are able to replicate in several cell types in vitro and where small animal models able to reproduce the infection and symptoms exist, research on the human NoV field has been hampered by the lack of in vitro or in vivo models for the study of viral pathogenicity. Notwithstanding this, the latest discoveries point to lymphocytes as targets for in vitro infections and replication of human NoV (Jones et al., 2014; see below). The fact that until now the only source of human NoV were the feces of infected individuals means that most of the knowledge related to immunogenicity and binding to cellular receptors of human NoV came from the study of the so-called viral-like particles (VLP), which consist in viral particles devoid of their genetic material resulting from the spontaneous auto-assembling of the VP1 protein. VLP preserve similar antigenicity and attachment properties compared to complete viruses and they are generally obtained by recombinant baculovirus infection in insect cells (Jiang et al., 1992). Another important model for human NoV study

are the so-called P-particles. It is known that the C-terminal portion of the VP1 protein, of about 300 amino acids (protruding domain or P domain), is able to form dimers and autoassembles into subviral particles of icosahedral symmetry called P-particles, which are 20 nm in diameter and consist in 12 P-domain dimers. Noroviral P-particles retain the capacity to bind the receptors, which are specific for each NoV genotype and they are able to induce the production of blocking antibodies. This makes them, together with their easy production in prokaryotic host like *Escherichia coli*, useful tools for the study of binding specificity and in vaccine development (Tan et al., 2008). The third model for NoV is constituted by murine NoV (MNoV, genogroup GV) or feline calicivirus. MNoV are able to in vitro infect B cells and macrophages and produce asymptomatic infections in mice with concomitant viral shedding in feces. These kinds of NoV surrogates constitute the almost exclusive alternative (with the exception of the infection in volunteers) for the study of inactivation of NoV after different food treatments. However, the susceptibility of MNoV to different temperatures, environmental, or food processing conditions is totally different from those of human NoV, which questions the utility of this approach (Richards, 2012).

For cellular attachment, NoV recognize glycoconjugates present at the cell surface that are part of the ABH system of human histo-blood group antigens (HBGA) and Lewis antigens (*a*, *b*, *x*, and *y*), which are present in the saliva, gastroduodenal mucosa, and erythrocytes. Differences in the ABH system among individuals have an impact on the susceptibility to infection because each NoV genotype displays a different binding profile (Huang et al., 2005). Additionally, the secretor status is also important and individuals with mutations in the FUT-2 gene, which encodes the fucosyltransferase involved in H-antigen synthesis in secretions, lack ABH antigens in saliva and epithelial cells and are less susceptible to NoV infections. However, NoV cellular tropism is not known and it is not clear whether the binding to HBGA on cell surfaces is only aiding in cellular attachment or if it is also involved in the subsequent infection process.

3 MICROBIOTA OF THE GASTROINTESTINAL TRACT AND VIRUS SUSCEPTIBILITY

The gastrointestinal tract constitutes one of the most complex ecosystems with a huge bacterial population that at some locations can reach up to 10^{12} bacteria per gram of content. More than 1000 species have been described as being part of this ecological niche. Despite this complexity, the phylogenetic diversity is limited and most of the bacterial species present belong to the phyla *Bacteroidetes* and *Firmicutes* (Blottiere et al., 2013). The bacterial composition is dependent on the individual and is affected by many factors such as the genetics, diet, and health status. However, a core microbiome is shared between individuals, which probably reflects the important physiological and immune functions attributed to the microbiota. Imbalances in its composition, referred to as dysbiosis, have been linked to a series of pathologies that include inflammatory bowel diseases, obesity, or diabetes, among others (Collado et al., 2009; de Moreno de LeBlanc and LeBlanc, 2014). The intestinal immune system has to remain hyporesponsive to the endogenous microbiota and a complex cross talk is established between the microbiota and epithelium that assures this and, at the same time, prepares the immune system to respond against microbial pathogens. The host immune system is able to recognize bacterial-derived products (cell wall components such as peptidoglycan or lipopolysaccharide (LPS) or bacterial DNA) via specialized receptors such as the toll-like receptors (TLR). This triggers a series of signaling events that end up in the maturation and differentiation of epithelial and immune cells and in the maintenance of the intestinal barrier (Gill and Prasad, 2008).

Viruses that infect the gastrointestinal mucosa have co-evolved with the gut microbial population for which it is not surprising that, as occurred with bacterial pathogens, some aspects of virus infectivity may be modulated by the presence of this microbiota. The first evidence linking the microbiota to viral susceptibility was derived from the use of two models that, with their limitations, have been extensively employed in these studies: germ-free and antibiotic-treated animals (usually mice). The first model has the advantage of being completely deprived of microbiota and therefore, the role of intestinal bacteria in viral infectivity can be easily studied. Furthermore, the effects of individual bacterial strains (probiotics or commensals) or more complex or defined microbiota can be studied by inoculating and colonizing the germ-free animals. However, these animals present some disadvantages due to their immature immune system and altered intestinal physiology. The second model, treating animals with antibiotic cocktails, is a more simple and economical way to get rid of the microbiota; however, this approach also has some limitations. Sensitivity to antibiotics differs between bacteria and even using broad-spectrum antibiotics, a complete depletion of the microbiota cannot be assured.

Earlier evidence of the participation of the intestinal microbiota in viral susceptibility was obtained from animal viruses or viruses that do not target the gastrointestinal tract. The replication of dengue virus in its vector, the mosquito *Aedes aegypti*, is increased in the gut of antibiotic-treated insects. In this system, the microbiota was shown to control the expression of antimicrobial peptides regulated by pathways mediated by TLR signaling (Xi et al., 2008). It is hypothesized that the mosquito microbiota enhances the antiviral response by basal stimulation of the immune system. A second example of a role of the microbiota in controlling viral replication is the case of the influenza virus. Germ-free or antibiotic-treated animals infected with this virus show exacerbated disease symptoms and decreased specific IgG production, for which it is

established that the microbiota is needed to develop an adequate antiviral response. In this case, an indirect mechanism is proposed by which the microbiota activates the inflammasome and induces the migration of immune cells from the lung to lymph nodes to prime specific T-cell responses (Ichinohe et al., 2011).

Contrary to these two latter cases, microbiota may also participate in processes that result in an enhancement of viral infectivity. Germ-free mice are less susceptible to develop leukemia induced by the murine leukemia virus (Isaak et al., 1988). It is postulated that this resistance to infection is the result of a diminished population of target immune cells for viral replication in germ-free mice. In another example, the mouse mammary tumor virus (MMTV), which is transmitted to the offspring through the milk, uses a mechanism based on the microbiota to evade the immune system. MMTV virions are able to trigger TLR4 by means of a bacterial product, LPS. This results in the production of immunosuppressive cytokine IL-10, which blocks the antiviral response (Kane et al., 2011). In this case, MMTV takes advantage of the mechanisms that induce tolerance to the indigenous microbiota to evade the immune response. As will be discussed later, evidence on the participation of the intestinal microbiota and their derived components in virus infectivity has been finally obtained for human viruses that infect the gastrointestinal tract producing or not gastroenteritis: polioviruses, NoV, and RV. In these cases, a positive effect of the microbiota in viral infection was found. In principle, this seems contradictory with the protective effects against diarrhea that are usually attributed to probiotics (also members of the microbiota), evidencing that distinct mechanisms initiated by intestinal bacteria modulate the infectivity.

4 SUGGESTED ANTAGONISTIC MECHANISMS OF PROBIOTICS AGAINST INTESTINAL VIRAL PATHOGENS

The antagonistic effect of probiotics has been exhaustively studied for microbial pathogens such as *Clostridium*, *Helicobacter*, *Salmonella*, or enteropathogen *E. coli* and the inhibitory mechanisms have been elucidated in several cases (Lievin-Le Moal and Servin, 2014). It is believed that most of these mechanisms also apply to the inhibition or viral infection.

Probiotics can interfere with the viral cycle by acting at several steps, interfering with some aspect of viral replication or pathogenesis, or protecting the mucosal barrier function (summarized in Figures 62.1 and 62.2). Also, immunomodulation at the level of innate and adaptive responses has emerged as a major mechanism for antiviral effects. By means of several mechanisms that are still poorly understood, probiotics can modulate specific host pathways and affect the synthesis of

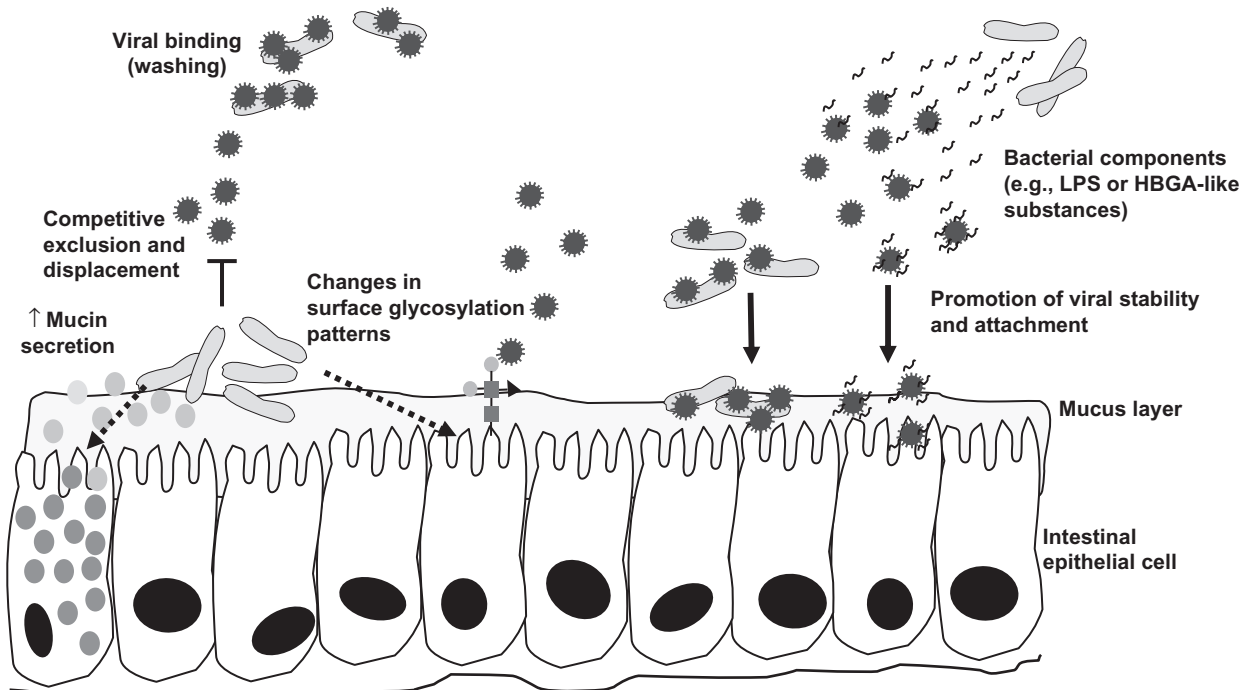


FIGURE 62.1 Possible interactions of probiotics/intestinal microbiota with enteric viruses. Effects on viral accessibility and attachment to target cells. The intestinal microbiota can promote or inhibit viral infection through several mechanisms: (i) influencing mucus production or the glycosylation state of surface glycoproteins, which in some cases are the target for viral recognition; (ii) by competitive exclusion or displacement from attachment to viral receptors; and (iii) bacteria can promote virus retention to the cell surface or structural components derived from them are needed for the virus to be infective.

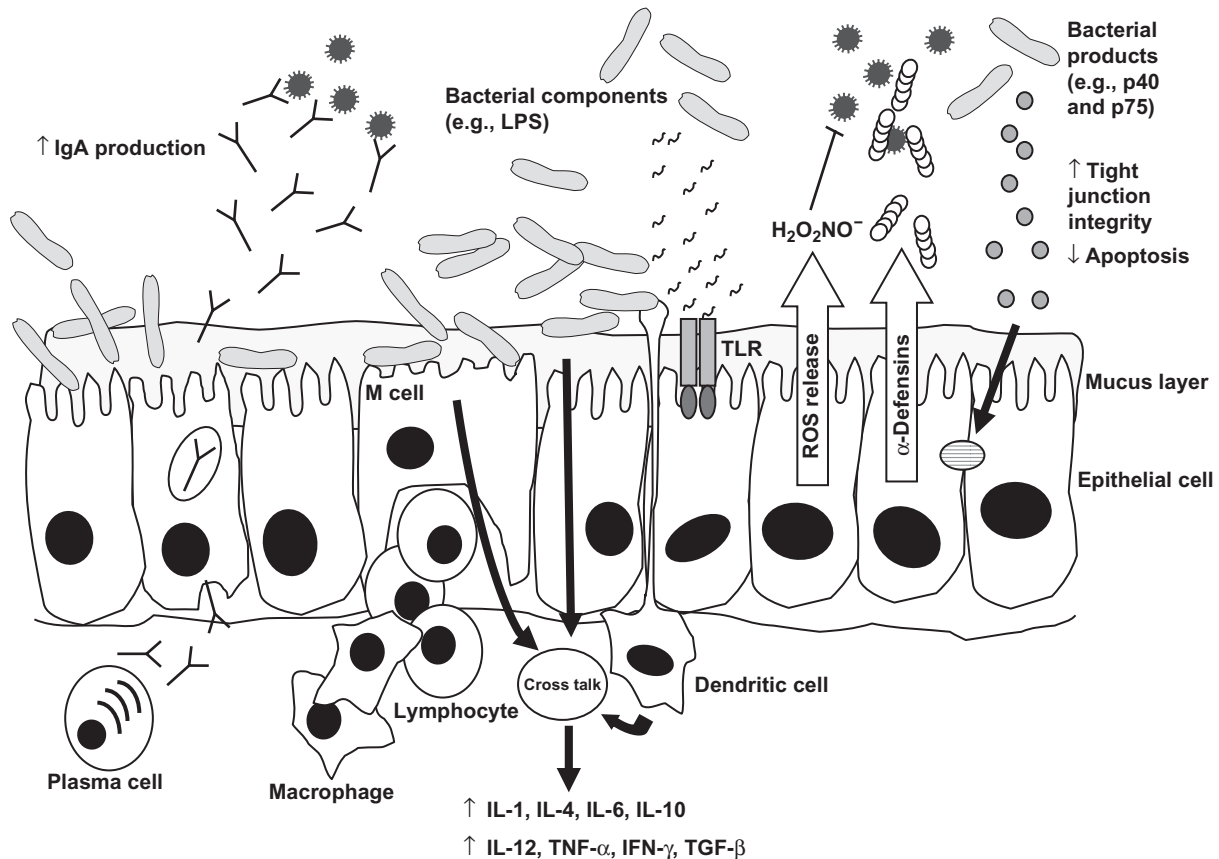


FIGURE 62.2 Effects on viral infectivity triggered by the cross talk probiotic/microbiota-host. Probiotics and the microbiota may interfere with viral replication at different levels. They can induce the synthesis of compounds with antiviral activity by epithelial cells. The cross talk established between epithelial/immune cells enhances epithelial barrier functions and innate as well as adaptive immune responses. In specific cases, this cross talk can be exploited by some viruses to escape the immune response. Modified from Rodriguez-Diaz and Monedero (2012).

many cytokines (e.g., IL-1, IL-2, IL-4, IL-6, IL-10, IL-12, IFN- γ , and TNF- α). This has as a consequence a series of modulatory effects on immune cells: increased cytotoxic and phagocytic capacity of NK cells or macrophages and immune cell (T and B lymphocytes) proliferation and differentiation. This last effect results in increased antibody responses (Gill and Prasad, 2008). Thus, the adjuvant effect of probiotics has been documented for vaccination against several viral pathogens such as hepatitis B, poliovirus, or influenza, giving rise to increased IgG and IgA titers. In a similar way, consumption of fermented milk containing LGG was demonstrated to be effective in the promotion of specific IgA secreting cells and increased plasma IgA titers in RV vaccination and during RV infection (Kaila et al., 1992) and the same effect has been evidenced in different animal models.

Some studies have suggested that specific probiotics are able to synthesize antiviral substances, although their nature is still unknown (Botic et al., 2007; Seo et al., 2010). The many organic acids produced by fermentation of carbohydrates by lactobacilli or bifidobacteria have microbicide activity per se, and it is established through the use of in vitro models that these acids are able to inhibit infectivity for some viruses. This suggests that the acid environment may reduce viral infection. However, the fact that enteric viruses have evolved to replicate in the gastrointestinal niche and are therefore adapted to thrive in this environment may limit this effect. Production of virucide substances can also be induced in the host cells by probiotics. Reactive oxygen species production such as H₂O₂ or are NO⁻ were increased by LGG and *Lactobacillus casei* Shirota and strains of *Lactobacillus pentosus*, *Lactobacillus plantarum*, and *Lactobacillus fermentum* up to 50% depending on the combination of strain and cell line. This results in diminished or complete abolishment of the disruption of in vitro culture monolayers by RV infection (Maragkoudakis et al., 2010). However, other experimental models argue against this mode of action. Infection by RV in cultured cells promotes chloride excretion linked to an increase in reactive oxygen species and mediated by the viral enterotoxin NSP4. This effect can be inhibited by supernatants of the probiotic yeasts *Saccharomyces boulardii*, which prevents RV-induced oxidative stress (Buccigrossi et al., 2014). As this probiotic yeast has proven beneficial effects on RV diarrhea, these results provide new clues on the anti-RV effects of probiotics.

Other antiviral molecules whose production is induced in response to the microbiota are defensins. These peptides have a strong antibacterial activity, but it has been described that alpha-defensin binds to human adenovirus limiting viral replication in vitro by preventing virus uncoating in the cell entry process. However, the relevance of this phenomenon in vivo has to be established (Wilson et al., 2013).

The relationship between probiotics and mucus synthesis has been proven in some cases. LGG and *L. plantarum* 299v are able to increase mucin secretion in cultured cells by upregulating the expression of MUC-2 and MUC-3 genes. The thick mucus layer (compared to the width of the epithelial layer) represents a first line of defense against infection and it is composed of mucins and other glycoproteins that interact with the microbiota and in some cases represents a first anchor site and a source of nutrients for many enteric bacteria. At the same time, its dynamic renewal and the movements linked to the transit through the gastrointestinal tract means that it constitutes, besides a physical barrier limiting access to epithelial cells, a way to entrap and exclude viral particles promoting their elimination in the feces. Lactobacilli and bifidobacteria also possess surface factors that promote their binding to intestinal mucus or components of the extracellular matrix. Some of these proteins are extracellular factors, such as the surface layer proteins (SlpA) or specialized proteins for mucosal binding: the mucus binding pili structures that are found in some lactobacilli (e.g., LGG and some bifidobacteria). In other occasions, adhesion factors consist of “moonlighting proteins,” which are generally cytoplasmic proteins that are targeted to the surface by still unclear export mechanisms (chaperones, glycolytic enzymes, etc.) and that in some cases mediate in the interaction with the mucosal surface in strains of *L. plantarum*, *L. casei*, *Lactobacillus reuteri*, or *Lactobacillus johnsonii*. These adhesion factors possess lectin-like activity, help in the colonization process of these bacteria, and may participate in the competitive exclusion and displacement of virus from target cells. Apart from this, probiotic strains have been shown to directly bind viruses. Thus, LGG or *Bifidobacterium lactis* Bb12, the two strains in which efficacy on viral diarrheas has been better documented, display a remarkable binding ability to RV (Salminen et al., 2010). Other probiotics such as *L. casei* BL23 or *E. coli* Nissle 1917 are able to bind viral particles (Rubio-del-Campo et al., 2014). The nature of the molecules that promote this binding is not known. Many virus receptors consist of glycosylated proteins present at the cell surfaces. It is thus anticipated that these probiotic strains express on their surfaces glycosylated proteins or other polymeric substances of carbohydrate nature that can be mimicking viral receptors. As will be discussed later, this already has been reported for other enteric strains, which possess sugar structures at the surface resembling HBGA and are able to interact with NoV VLP.

Probiotics may contribute to the maintenance of the integrity of the intestinal epithelial barrier that is compromised during intestinal viral infections. Viral diarrheas comprise several mechanisms that result in an increase of secretion of water and electrolytes and in the paracellular epithelial permeability. In addition, epithelial damage and apoptosis occur. Several probiotic strains have demonstrated their capacity to counteract these effects via distinct mechanisms. Therefore, through the maintenance of intestinal homeostasis probiotics may help to keep the intestinal barrier integrity. LGG secrete soluble factors to the culture medium that are protein members of the machinery for cell wall biosynthesis and turnover (endolysins p40 and p75). These proteins have been shown to act on the Akt and PI3-K signaling pathways through activation of the epidermal growth factor receptor (Wang et al., 2014). This results in protection of the epithelium both in vitro and in vivo from apoptosis triggered by inflammatory cytokines like TNF- α or IFN- γ , oxidative damage, or chemically induced inflammation. Also, low-molecular-weight peptides released by LGG induce cytoprotective heat-shock proteins (HSP25 and HSP72). All this results in apoptosis resistance and maintenance of the epithelial structure by increasing the expression of proteins of the tight junctions, such as zonula occludens-1, occludin, or claudin (Seth et al., 2008). LGG p40 also increases MUC-2 expression in mice in vivo, leading to an increased mucus production and thickening of the intestinal mucus layer, which as described before may protect against infection (Wang et al., 2014). Other characterized proteic factors may contribute to maintain the intestinal homeostasis by playing a role in the host-bacterium cross talk. The cell wall-anchored proteinase from *L. casei*, PrtP, an enzyme required for milk casein degradation, has proven to play a role in intestinal homeostasis by selective degradation of proinflammatory cytokines (von Schillde et al., 2012). The serpin homologues secreted by bifidobacterial species, which are specific inhibitors of proteases such as the human neutrophil elastase, are susceptible to reduce tissue damage at sites of intestinal inflammation (Ivanov et al., 2006).

5 ROTAVIRUSES, NOROVIRUSES, AND THE INTESTINAL MICROBIOTA

Due to the lack of simple models for the study of the interactions between RV, NoV, and the intestinal microbiota/probiotics, very few studies exist on this matter. Different strains of lactobacilli have been shown to bind on their surfaces P-particles of human NoV from genotypes GI.1 and GII.4. Competitive experiments using lactobacilli and GII.4 P-particles showed that the presence of the bacteria inhibited the binding of the subviral particles to intestinal epithelial HT-29 cells (Rubio-del-Campo et al., 2014). These observations also would be in agreement with previous reports showing that specific strains of *Lactobacillus brevis*, *Lactobacillus gasseri*, *Lactobacillus salivarius*, and *Lactobacillus acidophilus* isolated from

feces display on their surfaces lectin-like activities that reside in their S-layer proteins and are able to bind types A, B, and H HBGAs. These data suggest two types of mechanisms by which probiotics may interfere with binding of NoV to their target cells. First, probiotics may inhibit viral binding by competitive exclusion by blocking viral receptors and second, they can bind viruses on the surface promoting their elimination in feces and limiting viral access to target cells.

An intestinal isolate of *Enterobacter* sp. presents on its surface polymeric substances resembling A-type HBGA and it is able to bind NoV VLP of the GI.1 and GII.6 genotypes, which have binding capacity to this HBGA (Miura et al., 2013). The presence of very close serological relationships to A, B, and H HBGA is present in about 10% of Gram-negative enterobacteria belonging to *E. coli*, *Salmonella*, *Citrobacter*, *Proteus*, *Klebsiella*, *Pseudomonas*, and *Serratia*, which explains the presence of immunoglobulins against nonself HBGA in humans. The existence of this HBGA activity in bacteria is generally dependent on the expression of different glycosyltransferases that are encoded in O-antigen synthesis gene clusters and that are responsible for the incorporation of the polysaccharide moieties in LPS of Gram-negative bacteria.

A recent breakthrough on NoV research is represented by the finding that, similar to murine NoV, human NoV can infect lymphocytes (Jones et al., 2014). Since the discovery of human NoV 40 years ago, multiple attempts to reproduce infection in in vitro cell culture systems or adequate small animal models failed. Recently, it has been demonstrated that human NoV infection can take place in vitro by using B cells as hosts and that this infectivity is linked to the intestinal microbiota (Jones et al., 2014). Stocks of NoV isolated from feces and filtered (devoid of any accompanying bacteria) are much less infective than unfiltered NoV preparations. It was also shown that enteric bacteria, such as *Enterobacter cloacae*, that express H-type HBGA on its surface, enhances GII.4 NoV attachment and infection in target cells in a dose-dependent manner (Jones et al., 2014). The same effect was observed by using synthetic H-type HBGA. However, an *E. coli* strain lacking HBGA activity or its LPS does not show any effect. In agreement with these findings, MNoV infection in antibiotic-treated mice is reduced compared to mice with normal intestinal microbiota. Microbiota ablation with antibiotics also prevented persistent MNoV infection in mice. This effect was dependent on the IFN- γ receptor (Baldrige et al., 2015), which again suggests that intestinal microbiota promotes MNoV persistence and that components of the innate immune system counteract it. Although additional mechanisms derived from the microbiota cannot be excluded, the interaction of NoV with the intestinal bacteria seems a crucial factor for a productive attachment of the virus to target cells. Biopsies analysis of individuals infected with NoV and infections using tissue explants pointed to intestinal epithelial cells as targets for human NoV replication, but it is now evident that these viruses may present different cellular tropisms. Similarly, it has been shown that in vivo infection by MNoV in mice targets B lymphocytes from intestinal Peyer's patches. Whether these results, which have been obtained with the GII.4 genotype, could be extended to other NoV genotypes needs to be explored. In any case, these new findings open the door to a kind of research on NoV biology that has been elusive in past years. The mechanism on how NoV use HBGA molecules derived from the microbiota for infectivity is not known. Earlier studies evidenced that the in vitro binding of NoV VLPs to several HBGA was promoted by uncharacterized stool components and the participation of antibodies or proteins in this process was discarded, as the effects were not sensitive to temperature (Harrington et al., 2004). This raised the hypothesis that the microbiota or lipidic or polysaccharide substances derived from it are participating in the process. Similar to human NoV and MNoV, it has been demonstrated that antibiotic treatment in mice or the use of germ-free mice reduced RV infection in more than 40% (Uchiyama et al., 2014). A more persistent anti-RV response in terms of specific IgA secreting cells, linked to a reduced severity and diarrhea duration, is also found in animals with depleted microbiota. Therefore, the two virus groups responsible for the majority of gastroenteritis rely on the intestinal microbiota for infectivity.

Another research study on the participation of the intestinal microbiota in the infectivity of enteric viruses has revealed that other microbial-derived products are also important for viral pathogenesis. Human polioviruses can infect transgenic mice that express the poliovirus receptor (PVR). In this model, ablation of the intestinal microbiota by antibiotic treatment or the use of germ-free mice resulted in reduced viral infectivity. It has been demonstrated that the presence of bacterial strains enhanced viral binding to PVR and infectivity and that the bacterial component LPS was responsible for this (Kuss et al., 2011). The VP1 capsid protein of the poliovirus virion binds LPS and this binding stabilizes the virion making it more heat-stable and bleach-resistant (Robinson et al., 2014). A specific point mutation has been characterized in poliovirus VP1 (T99K) that presents a reduced interaction with LPS and rendered less infective viruses. Therefore, two different mechanisms mediated by the microbiota are involved in the enhancement of poliovirus infectivity: virion stability and cell attachment, but attachment requires significantly less LPS than stabilization (Robinson et al., 2014). Only the polysaccharide moiety of LPS is needed for this, as detoxified LPS in which the lipid A portion is removed retains the stabilization ability to poliovirus. The polysaccharide chitin (long polymer of *N*-acetylglucosamine, GlcNAc) and the peptidoglycan component of the bacterial cell wall (a polymer carrying GlcNAc) have similar stabilizing characteristics. In contrast, monomeric GlcNAc or chitohexaose (six GlcNAc residues) lack this effect, for which it is postulated that polysaccharides with more than six GlcNAc residues are required (Robinson et al., 2014).

The relevant role of the microbiota in viral infectivity can be viewed as a “double-edged sword,” and it can have important implications in the use of probiotics. Some in vitro experiments have revealed that specific probiotic strains may impact bacterial pathogen adhesion. Thus, LGG enhanced adhesion of *E. coli* serotype O157 to human intestinal mucus by 50% (Lee et al., 2003) and other *L. rhamnosus* strains have similar effects (300% increment in adhesion) of *Salmonella typhimurium* (Tuomola et al., 1999). Also, *Enterococcus faecium* strains of veterinary use improved the adhesion of *Campylobacter jejuni* by 200% (Rinkinen et al., 2003). Therefore, it seems that specific combinations of probiotic-pathogen may represent a potential risk by increasing the interaction of the pathogen with the mucosa. The same situation was observed when *L. casei* BL23 and *E. coli* Nissle 1917 were assayed for inhibition of GII.4 P-particles binding to HT-29 cells (Rubio-del-Campo et al., 2014). Exclusion and displacement experiments show that bacteria enhance the retention of the subviral particles to the cell surface, suggesting the possibility that in some specific cases viruses can take advantage of the intestinal microbiota or particular bacteria to reach the target cells. According to the results of infectivity of human GII.4 NoV in B cells (Jones et al., 2014), this situation is probably also encountered in vivo. Whether specific probiotics can block viral attachment or, conversely, they contribute to its cellular surface binding needs to be explored.

RV and NoV use oligosaccharides from glycoconjugates on cellular surfaces as receptors for attachment and entry. RV recognize sialic acid (*N*-acetyl-neuraminic acid) residues as a first step for cellular entry. As already mentioned, NoV display binding specificities toward α 1,2-fucosylated carbohydrates and α 2,3-sialylated carbohydrates, which form part of HBGA expressed at mucosal surfaces. Many bacterial species also bind to glycosylated surface proteins via their adhesins or can take advantage of glycoconjugates present in the mucosa as a carbon source. Susceptibility to NoV infections has been related to HBGA types but the individual secretor status, determined by the presence of the H antigen in secretions and epithelia and linked to the FUT-2 gene, is of special relevance. It has been shown that the antibody prevalence and titer to NoV was dependent on the secretor status rather than the blood group (Larsson et al., 2006). Thus, NoV infection depends on the gut microbiota and the secretor status, but could it be possible that the gut microbiota was also dependent on the secretor status? Recent studies have shown exactly this (Wacklin et al., 2011, 2014): human gut microbiota (HGM) composition varies depending on the FUT-2 genotype. Individuals defective in FUT-2 are less susceptible to NoV infections and at the same time have less diverse intestinal microbiota and presented marked diminished numbers of *Bifidobacterium bifidum*, *Bifidobacterium adolescentis*, and *Bifidobacterium catenulatum/pseudocatenulatum* (Wacklin et al., 2011). This establishes a link between the secretor status and NoV susceptibility through the microbiota. Interestingly, specific members of the microbiota such as *Bacteroides thetaiotaomicron* can modulate the fucosylation state of surface glycans of enterocytes by regulating the host fucosylation pathways (Bry et al., 1996). This species and a strain of *L. casei* secrete soluble factors to the culture supernatant that increase the surface galactose content in enterocyte cells, limiting RV infection (Varyukhina et al., 2011). Thus, in the complex gut ecosystem, viral infectivity studies have to consider many aspects that include host genetic background, glycobiology, and gut microbiota.

6 EFFICACY OF PROBIOTICS AGAINST ENTERIC VIRUSES IN IN VITRO MODELS, ANIMAL, AND CLINICAL TRIALS

We recently reviewed the scientific data describing evidence of protection conferred by probiotics against intestinal viral infections in vitro, in laboratory animal studies, and in clinical trials (Rodriguez-Diaz and Monedero, 2012). In the present section, we intend to update that data to track the new evidence published since 2011.

Due to their importance and to the existence of proper in vitro and in vivo models, most of the evidence of the effectiveness of probiotics has been obtained with RV as the viral model. In the same way, RV was the selected pathogen in most of the clinical trials where the efficacy of probiotics on viral pathogens has been investigated. This is due to the great prevalence of these viruses in the infantile population, even after the introduction of RV vaccines, and to the existence of diagnostic tools for their detection. Fortunately, new research on probiotics and NoV, the other viruses responsible for the great majority of gastroenteritis, is being published with the use of NoV surrogates in vitro, and at least in one clinical trial in humans. New data has also been presented with respect to other viruses from the intestinal tract, as the transmissible gastroenteritis coronavirus (TGC). Probiotics have also proved to be effective against other viruses, including respiratory viruses (Al Kassaa et al., 2014), but they will not be discussed in this chapter.

6.1 Efficacy of Probiotics: In Vitro Data

The in vitro models are of special interest to study not only the ability of probiotics to block viral infection but also to unravel the mechanisms of such inhibition at the cellular level. It is now well established that RV (as well as NoV) utilize oligosaccharides in the surface of the cells in the first steps of viral infection as receptors and co-receptors.

Varyukhina and collaborators have shown that glycan-modifying bacteria such as the probiotic bacteria *L. casei* DN114001 and the commensal bacteria *Bacteroides thetaiotaomicron* are able to inhibit RV infection in the mucus secretor human cell line HT-29-MTX through the modification of the glycan surface of the cells. This modification was made through a secreted soluble factor of the bacteria that proved to have the same effect as the utilization of a galactosyltransferase from bovine milk (Varyukhina et al., 2011). A novel mechanism for anti-RV activity has been recently described for the probiotic yeast *S. boulardii*. The authors of the work investigated the role of oxidative stress (reactive oxygen species and oxidized and reduced glutathione) in the chloride secretion induced by RV and its toxin NSP4 in human epithelial cells. Both the antioxidant N-acetylcysteine and the supernatants of *S. boulardii* cultures were able to prevent RV and NSP4 chloride secretion via reduction of the oxidative stress. The proposed mechanism of action then will be the production of unidentified soluble metabolites by *S. boulardii* that prevent oxidative stress (Buccigrossi et al., 2014). In vitro studies have also been utilized recently to evaluate novel probiotic strains, while these efforts are of interest to characterize the potential as probiotics of new bacterial isolates, few clues are reported on how probiotics exert their function. A new probiotic bacterium, *Bifidobacterium longum* subsp. *infantis* CECT7210, showed anti-RV activity in HT-29 and MA-104 cells (Munoz et al., 2011). Similarly, three new bacteria isolated from feces of breast-fed infants (*Lactobacillus paracasei* CNCM I-4034, *B. breve* CNCM I-4035, and *L. rhamnosus* CNCM I-4036) were analyzed for their anti-RV activity in HT-29 cells. The supernatants from *L. rhamnosus* CNCM I-4036 and *L. paracasei* CNCM I-4034 proved to have strain-dependent antiviral activity, while *B. breve* CNCM I-4035 did not have any anti-RV activity (Munoz-Quezada et al., 2013).

Despite their great importance for health, human NoV have been difficult to cultivate in an in vitro cell culture system, which impaired their study, including the investigation of potential probiotics against them. Recently, different NoV surrogates have been utilized to study the anti-NV activity of different bacterial strains. Lee and collaborators reported the inactivation of feline calicivirus and murine NoV during the fermentation of *Dongchimi*, a Korean vegetal fermented product. In this case, no evidence of the mode of action or the bacteria responsible of the effect was obtained, but this represents the first report shedding some light on NoV inactivation by fermented products (Lee et al., 2012). More recently, the feline calicivirus was also utilized as a human NoV surrogate to study the antiviral activity of the probiotic strain *Lactococcus lactis* subsp. *lactis* LM0230. The authors show that the preincubation of the virus with the bacterial cells as well as with the filtrated culture media reduced the viral infection (Aboubakr et al., 2014). Perhaps the most utilized human NoV surrogates for structural, functional, and antigenic studies have been the NoV VLP and NoV P-particles. These last surrogates were utilized to assay the ability of several probiotic and nonprobiotic bacteria from intestinal or food origin to bind human NoV and to study whether that binding produced any impairment in the NoV-host interaction. Interestingly, only when the bacteria (*L. casei* BL23 or *E. coli* Nissle 1917) and NoV P-particles (GI.1 genotype) were coadministered to HT-29 cells, a reduction of NoV P-particles binding to cells was found. When either NoV P-particles or probiotic bacteria were preincubated with the cells, an increase in the NoV particles binding to HT-29 was observed (Rubio-del-Campo et al., 2014). As already discussed, this surprising enhancement effect of probiotics on NoV attachment has been recently corroborated by the establishment of a human NoV cell culture system with the participation of gut microbiota (Jones et al., 2014).

The TGC is a virus of great importance on pig farms as it produces acute diarrhea in pigs and can cause a high mortality in newborn piglets. Putative probiotic bacteria *Lactobacillus* spp. (probio 37 and probio 38) isolated from porcine gastrointestinal tract was shown to have anti-TGC activity in vitro. The filtrated supernatants of both isolates were able to reduce TGC infectivity in ST cells (Kumar et al., 2010). Similarly, the probiotic *E. faecium* NCIMB 10415 strain was able to inhibit TGC virus infection and to increase ST cells viability. In this case, four protocols were studied, including pretreatment of cells, competition, postinfection treatment, and cell-free preincubation. In all four cases, an antiviral dose-response effect was observed (Chai et al., 2013).

6.2 Animal Models

Similar to the in vitro studies, the animal models have been utilized not only to study the efficacy of probiotics but mostly to obtain mechanistic information on the conferred protection. In this case, all the new data has been obtained for RV in three different animal models including rats, mice, and pigs. On top of the study of single probiotic strains and pathogenic RV, the effect of HGM transplantation and the use of vaccine strains of RV have also been addressed recently. Most of the results point to the modulation of the host immune system as the main mechanism of how probiotics help the organisms to fight against RV diarrhea.

Ventola and collaborators utilized the newborn rat model to study the effectiveness of live or dead LGG against simian RV SA11 strain. While a reduction of diarrhea was not observed, the authors found that both live and dead probiotics possessed beneficial effects for the rat pups in terms of prevention of the reduction of body weight losses and prevention of colon swelling compared to the infected control group (Ventola et al., 2012).

The adult mice model was utilized to confirm the anti-RV properties of the probiotic bacterial strain *B. longum* subsp. *infantis* CECT7210. The authors found a delay of rotavirus shedding at 48 h postinfection and a lower antigen concentration at 7 days from infection, thus confirming their previous in vitro results (Munoz et al., 2011). More interesting are the results obtained with the newborn mice model where reduction of diarrhea can result in a better understanding of the anti-RV effect of probiotics. The probiotic strain *L. gasseri* SBT2055 was heat inactivated and utilized to feed fertilized adult female mice. The dams were subsequently orally immunized with RV SA11 strain. After birth, the pups of immunized females were challenged with RV and the diarrhea and IgA recorded. This study showed a reduction in diarrhea in the pups from the group of mice that had been fed with STB2055 associated to a higher production of IgA in the immunized dams that also received the probiotic (Kadooka et al., 2012). Two different strains of *L. reuteri* (DSM 17938 and ATCC PTA 6475) were also utilized in a neonatal mice model. Newborn mice were infected with homologous RV murine strain EC and subsequently treated or not with probiotics. Both strains were able to reduce the duration of diarrhea. The authors could explain the beneficial effect of probiotics through various mechanisms, which included a suppression of proinflammatory molecules such as macrophage inflammatory protein-1 α and IL-1 β , but also a reduction in IL-7, IL-10, IL-12, and IFN- γ was observed, together with increased anti-RV specific antibodies. The administration of probiotics also improved intestinal histopathology and enhanced intestinal microbiome richness and phylogenetic diversity (Preidis et al., 2012). Different doses and times of administration of LGG were assayed also in newborn mice to evaluate different interventions with these probiotic bacteria. The authors found that the most efficient intervention was the pretreating of the pups with the higher doses of bacteria. The shortening in diarrhea and reduction of epithelium vacuolation in the jejunum could be explained by an increase of anti-RV IgA and IFN- γ (Zhang et al., 2013).

The gnotobiotic (gn) piglet model has been utilized in several studies in recent years to study how modulation of immune responses by probiotics can help in fighting rotavirus infections. In this sense, Azevedo and coworkers showed that a combination of *L. acidophilus* and *L. reuteri* can modulate cytokine responses in gn pigs infected with human RV, suggesting a regulatory role of lactic acid bacteria in maintaining gut homeostasis (Azevedo et al., 2012). The gn piglet model also has been utilized to study the effect of different dosages of the proinflammatory *L. acidophilus* NCFM used as an adjuvant of human RV vaccine. Interestingly, a low dose of NCFM increased IFN- γ T-cell response and downregulated TGF- β and IL-10 production compared to a high dose. These results are highly relevant because the same probiotic bacteria at different dosages can have opposite effects and either promote or suppress IFN- γ producing T cell or Treg cell immune responses (Liu et al., 2014; Wen et al., 2012). A combination of two probiotics (LGG and *B. lactis* Bb12) were utilized in combination or not with milk colostrum (col/milk) and the immune response to a human RV attenuated vaccine (AttHRV) studied. The complexity of the study design makes it difficult to draw conclusions other than in words of the authors: “col/milk components (soluble mediators) affect initial probiotic colonization, and together, they modulate neonatal antibody responses to oral AttHRV vaccine in complex ways” (Chattha et al., 2013). In a subsequent study, the authors evaluated the same probiotics in the gn piglet model in response to the AttHRV and to a virulent human RV strain. Similar to what was observed with different doses of probiotics, the modulation of immunological responses mediated by LGG- and Bb12-colonized gn piglets to the virulent RV differed from the responses to the vaccine strain (Chattha et al., 2014). The combination of LGG and Bb12 also modulated the innate immune response to RV in the gn piglet model. The authors showed that the combination of vaccination and colonization by LGG and Bb12 completely abolished diarrhea after virulent RV challenge through immunomaturation as reflected by increased frequencies of CD4, SWC3a, CD11R1, and MHCII expressing mononuclear cells (MNCs) and conventional dendritic cells in intestinal tissues and blood post-challenge (Vlasova et al., 2013). Gn pigs were also utilized to study the mechanism of LGG protection conferred to RV. The authors show that LGG is able to protect ileal epithelium from virulent human rotavirus injuries. Oral treatment with this strain increased the integrity of epithelium through the compensatory expression of α -catenin and β -catenin; tight junction proteins occludin, claudin-3, and claudin-4; and leak protein claudin-2. LGG also increased mucin production and maintained the serum levels of the proinflammatory cytokine TGF- β (Liu et al., 2013). LGG has also been shown to protect against RV challenge in the gn piglet model through a novel mechanism reducing the autophagy induced by RV induction, as the treatment with LGG reduced the autophagy markers ATG16L1 and Beclin-1 and autophagy regulator mTOR preventing virus-induced tissue damage (Wu et al., 2014).

Interesting results have been obtained in models in which transplantation of HGM to the gn pigs has been carried out. It can be argued that the lack of microbiota in the gn piglet model may interfere with the obtained results, especially if the same probiotic strains are to be used in humans that are obviously not depleted of their microbiota. Two recent investigations address this issue with the use of transplantation models of HGM into gn piglets. The very well-characterized LGG strain was utilized in both gn pigs and HGM transplanted gn pigs. As expected, the HGM group differed from the gn pigs in their response to LGG treatment. The HGM group had an enhanced Th1 cellular immunity, but LGG treatment did not affect antibody responses in comparison to gn piglets that also responded with increased antibody response to RV.

The authors argued that a higher dose of LGG might be needed to overtake the influence of the transplanted microbiota to achieve the immunostimulatory effect in the HGM pigs (Wen et al., 2014). In their effort to validate the transplanted HGM piglet model, the same research group showed that both LGG and virulent human RV modulate the transplanted HGM similarly to what happens in human natural gut microbiota, suggesting that the HGM pig model is valuable for testing the response of microbiota to probiotic interventions (Zhang et al., 2014).

6.3 Clinical Trials

While the *in vitro* studies and animal models are very valuable tools for the characterization of new putative probiotics and for the elucidation of their underlying mechanisms of action, none of them can substitute for clinical trials to study the safety and efficacy of a probiotic intervention in humans. Since our last revision, a variety of clinical trials have been published with different probiotics and results. Again, the variability in administration protocols and study designs makes it difficult to obtain general conclusions. While all the published interventions have not detected any safety issues related to the administered probiotics, some of them were shown to be inefficient for the treatment of RV-induced diarrhea. Also, for the first time the use of probiotics has been addressed to NoV-induced diarrhea in humans. An open-case controlled study was conducted to assay the efficacy of *L. casei* Shirota against NoV in the elderly. The study enrolled 77 adults with an average age of 84 years. The authors found that while daily consumption of milk fermented with the probiotic did not prevent the diarrhea produced by NoV, a reduction in the mean duration of fever was observed (Nagata et al., 2011). Several clinical trials have been performed in the last few years studying the effect of several probiotics against RV in the infant population. *S. boulardii* was shown to be efficient against RV diarrhea in infants in a double-blinded, placebo-controlled study in Brazil. The study included 182 infants and 57% were confirmed to be positive for rotavirus by commercial ELISA tests. The infants that received the probiotic yeast (200 mg/day for 5 days) after the onset of diarrhea had reduced diarrhea duration, thus proving its efficiency (Correa et al., 2011). Another randomized double-blind, placebo-controlled clinical trial was performed to study the effectiveness of *Bacillus coagulans* against RV diarrhea in children in Kolkata, India. In this case, the authors did not find any beneficial effects conferred by this probiotic strain in their study population (Dutta et al., 2011). Also, a randomized double-blind, placebo-controlled clinical trial involving 106 infants (6–48 months of age) using *L. reuteri* DSM 17938 as the probiotic strain did not show any beneficial effect in RV-produced diarrhea (Wanke and Szajewska, 2012). The efficacy against RV of *B. lactis* and *S. boulardii* was assayed in a prospective randomized clinical trial with 75 children. Both treatments resulted in a shortened diarrhea duration compared to the nonprobiotic group (Erdogan et al., 2012). A novel probiotic combination called BIO-THREE, composed of three different bacteria (strains of *Enterococcus faecalis*, *Clostridium butyricum*, and *Bacillus mesentericus*), was tested against RV- and *Salmonella*-produced diarrhea in a single center open-label controlled clinical trial. The trial involved 159 patients from 3 months to 14 years of age (42 confirmed to be RV positive). The results showed a reduction in diarrhea duration while there was no effect in diarrhea severity (Huang et al., 2013). LGG is one of the better-characterized probiotics *in vitro*, *in vivo*, and in clinical trials. In 2014, Aggarwal and collaborators showed the efficacy of LGG in the reduction of the duration of diarrhea in an open-case controlled study in India enrolling 200 children with RV and non-RV diarrhea (Aggarwal et al., 2014). More interestingly, Sindhu and collaborators performed a randomized double-blinded, placebo-controlled trial in children ($n=124$) infected with RV and *Cryptosporidium*, a protozoan parasite. Children infected with RV that also received LGG exhibited fewer repeated diarrheal episodes and impaired intestinal function after the intervention. This group also had significant increased IgG levels postintervention (Sindhu et al., 2014). This is one of the few studies in humans that provides a mechanistic explanation of probiotics mechanism of action against viral diarrhea.

Unfortunately, a unification of study designs with the different tested probiotics does not exist to date. Furthermore, all the studies were conducted in a single center and with a relatively small number of patients. The European Society for Pediatric Gastroenterology, Hepatology and Nutrition and the European Society for Pediatric Infectious Diseases have recently published their “Evidence-Based Guidelines for the Management of Acute Gastroenteritis in Children in Europe.” In this guide, only two probiotic strains (LGG and *S. boulardii*) are strongly recommended, always in combination with oral rehydration. Interestingly, the recommendation is accompanied with “moderate to low quality of evidence” in the reduction of the duration and intensity of the symptoms (Guarino et al., 2014), indicating that more scientific evidence is needed to warrant the beneficial activity of probiotics to fight viral diarrhea in humans.

7 CONCLUSIONS

Immunomodulation has emerged as the most plausible mechanism by which probiotics exert their antiviral effects in the gastrointestinal tract. The scientific data about mechanisms are still scarce but clinical trials support the use of specific strains

to alleviate diarrhea symptoms and reduce its duration, mostly in children. However, standardized treatment protocols (strains, doses, and frequencies) are still needed. The dual role of microbiota in the susceptibility to viral infections (viral pathogens can benefit from the microbiota for infection or they can be inhibited) may constitute the basis for new antiviral therapies. The knowledge about the virus/host/microbiota interactions should be extended and provide new information on what specific bacteria are playing a role and what are the interactions exploited by the viruses. It is known that probiotics can modulate the composition of the microbiota. Consequently, it can be envisaged that the levels of different bacteria could be manipulated or the interaction mechanisms disturbed by the use of probiotics or other dietary interventions.

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