

LGG[®]
Summatim

Health effects of LGG[®]



LGG® Summatim
Third, updated edition
Abbreviated version of the original book.

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Preface

Since 1983, when *Lactobacillus rhamnosus* GG (ATCC 53103) was isolated from the intestinal tract of a healthy human, a significant body of evidence has accumulated on its health effects. Research groups around the world have been intrigued by this probiotic strain, which has led to a wide range of clinical studies being conducted on humans as well as on interesting results from experimental studies. *L. rhamnosus* GG is still the most studied probiotic worldwide, even though the probiotic market has expanded enormously over the past 20 years. The

primary focus of studies done on *L. rhamnosus* GG has always been immunity in its different forms.

The publication of LGG® Summatim originates from the need to gather the extensive scientific evidence on *L. rhamnosus* GG to an easily approachable form. The general introduction on gastrointestinal microbiota and intestinal barrier function in the beginning of this book offers some background information that helps to understand for example the mechanisms discussed later in the book.

1. Introduction

1.1 Gastrointestinal microbiota

The gastrointestinal tract is populated by bacteria, archaea, yeasts and fungi, which are generally referred to as the gastrointestinal microbiota. The fact that the adult gastrointestinal microbiota weighs approximately 1,5 kilograms and there is over 10 times the amount of microbial cells in the gut than there are somatic cells in the human body illustrate the magnitude of the microbial community residing in the gastrointestinal tract. Especially the bacterial community in the gut is exceptionally diverse, consisting of members of nine phyla, of which *Firmicutes*, *Bacteroidetes* and *Actinobacteria* are dominant. The differences in the bacterial levels throughout the gastrointestinal tract are substantial, differentiating from less than 10^4 cfu/g in the stomach to 10^{11} – 10^{12} cfu/g in the colon.

Since the application of culture-independent molecular ecological studies based on ribosomal RNA (rRNA) sequences during the past decade, improved possibilities to study the microbiota have become apparent. New technologies, such as barcoded pyrosequencing and phylogenetic fingerprinting using DNA microarrays offer detailed analysis of multiple samples in a relatively short time, thereby creating wider possibilities for assessing the composition of the human microbiota. So far there have been several attempts to describe the normal human microbiota, but this has proven to be a dif-

The gastrointestinal microbiota is the largest immunological organ of the body.

ficult task because of the large differences in the microbial composition between individuals. Even the number of bacterial species in the gastrointestinal microbiota remains controversial, estimates ranging from 400 to 1000 phylotypes, which roughly corresponds to the number of bacterial species. (For reviews, see e.g. Palmer et al., 2007; Rajilic-Stojanovic et al., 2007; Zoetendal et al., 2008; Leser and Molbak, 2009)

Even though the knowledge of the composition of normal gastrointestinal microbiota remains incomplete, it is known that the microbiota has several important functions in maintaining the health of the host. The basis for the beneficial health effects is the symbiotic relationship between the gastrointestinal microbiota and the host. The role of the host is to provide a stable environment and nutrients for the microbiota whereas the microbiota has a significant role in maturation of the gastrointestinal tract, providing the host with nutritional contributions and protecting the host from harmful microbes. In addition, it has also been stated that the gastrointestinal microbiota is the largest immunological organ of the body as it has an important role in the maturation and maintenance of the immune system. The host

microbiota also contributes to cell proliferation and energy harvest, as well as having a large catalytic potential, leading to formation of metabolites which may be either harmful or beneficial to the health of the host. Composition of the gastrointestinal microbiota has so far been linked to several diseases including irritable bowel syndrome, inflammatory bowel diseases, numerous cancer

types and obesity. However, more studies which utilise novel methods to study the microbiota are still needed to assess the role of the microbiota in the health of the host. (For reviews, see e.g. Blaut and Clavel, 2007; Palmer et al., 2007; Zoetendal et al., 2008; Leser and Molbak, 2009)

In conclusion,

human gastrointestinal microbiota provides the host with nutrients and protects it from harmful microbes. The knowledge of the composition of the microbiota has increased significantly during the past decade due to the application of new molecular methods. In recent years, several microbiota-disease-linkages have been assessed in experimental and clinical studies, but more research is needed to confirm the role of the host microbiota in health and disease.

1.2 Intestinal barrier function

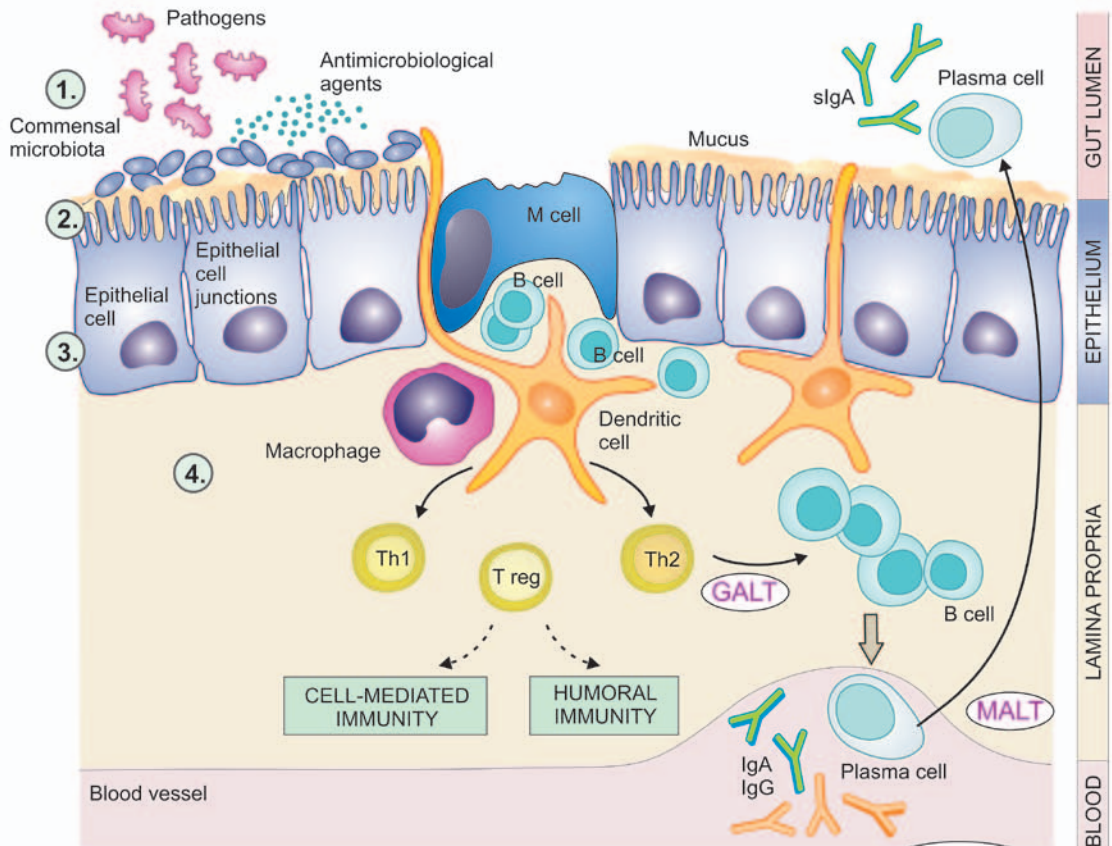
The fact that the mucosal surface of the gastrointestinal tract is the largest body surface (300–400 m²) in contact with the external environment illustrates its significance in protecting the health of the host. The gastrointestinal tract is constantly exposed to a large variety of environmental antigens, microbes and pathogens, and therefore the protection of the host against pathogenic invasion or exaggerated inflammatory response is essential. In addition, the gastrointestinal tract is the primary site for digestion as well as water and electrolyte exchange. To be able to optimally perform both these tasks, highly sophisticated functioning of the intestinal wall is needed. The intestinal barrier function can be described as the sum of the protective barriers that together are in charge of the optimal functioning of the intestinal wall: the commensal microbiota, mucus layer, intestinal epithelium and intestinal immune system. The key elements of the intestinal barrier function are presented in Figure 1. (For reviews, see e.g. Lievin-Le Moal and Servin, 2006; Schenk and Mueller, 2008)

Even though the intestinal epithelium is only a single-cell layer lining the gut lumen, it creates a crucially important physical and chemical barrier in the gut. As a physical barrier, the intestinal epithelium protects the host from harmful intraluminal entities, such as foreign antigens, micro-organisms and their toxins, while it also acts as a selectively permeable filter, permitting the absorption of vital nutrients, electrolytes and water. Three components, desmosomes, adherens junctions and tight junctions, are in charge of the integrity of the intestinal epithelium. Selective permeability is mediated through transcellular and paracellular pathways, which are controlled by membrane pumps, ion channels and

tight junctions, adapting the permeability according to physiological needs. The intestinal epithelium also provides a chemical barrier against pathogens, as it is covered by mucus-producing cells and antimicrobial molecules. (For reviews, see e.g. Lievin-Le Moal and Servin, 2006; Magalhaes et al. 2007; Groschwitz and Hogan, 2009).

The mucosal surface of the gastrointestinal tract is the largest body surface in contact with the external environment.

The gastrointestinal microbiota has an important role in the colonisation resistance of the intestine, preventing exogenous microbes both harmful and harmless from establishing themselves permanently in the digestive tract. The commensal microbiota competes with the pathogens and with chemical (e.g. gastric acid, bile acids, enzymes), biological (e.g. peristalsis) and immunological factors that also exacerbate the living conditions of intruder microbes, the adhesion and colonisation of most of the pathogens can be inhibited. In addition to taking part in the colonisation resistance, the host microbiota also optimises the functioning of the intestinal epithelium. The epithelium and the microbiota are involved in a continuous crosstalk, and the microbiota is responsible for e.g. spurring the epithelial cell turnover and increasing the number of certain cell types (e.g. secretory goblet cells) in the epithelium. Secretory goblet cells are in charge of another important function of the intestinal epithelial cells – the production of mucins. Mucins are responsible for the viscosity of the intestinal



Illustrator: Soie Latini

- Key elements of the intestinal barrier function and the main role of each element:**
- 1. Commensal microbiota**
Colonisation resistance through e.g. production of antimicrobial agents.
 - 2. Mucus layer**
Protection of the intestinal epithelium
 - 3. Intestinal epithelium**
Integrity and selective permeability of the intestinal wall
 - 4. Intestinal immune system**
First line defence against infectious agents

Figure 1. Key elements of the intestinal barrier function.

mucus layer, which acts as a medium for protection, lubrication, and transport between the luminal contents and the epithelial lining. In addition, intestinal mucus provides nutrients for intestinal bacteria, therefore having an important role in supporting the ability of the bacteria to survive and multiply. (For reviews, see e.g. Deplancke and Gaskins, 2001; Lievin-Le Moal and Servin, 2006; Leser and Molbak, 2009)

In addition to physical and chemical barriers created by the epithelium, microbiota and mucus layer, the immunological defence is an integral part of the intestinal barrier function. It has been estimated that over two-thirds of all lymphocytes of the human body are located in the intestinal epithelial and sub-epithelial layers. The mucosal immunity has the difficult task of maintaining the balance between the capacity for protective immune responses against infectious agents and the ability

to tolerate the load of antigens present in the intestinal lumen. Mucosa-associated lymphoid tissue (MALT) has a significant role in inducing immune responses against pathogens as well as inducing tolerance against antigens present in the gut lumen. Gut associated lymphoid tissue (GALT) is generally divided into inductive and effector sites. Antigens of the mucosal lumen are collected on inductive sites and that is also where an immune response is induced. Effector sites on the other hand are the sites where immune cells differentiate and exert their function (a cellular response mediated by T cells, a local humoral response by B cells). The mucosal immune system acts as the first-line defence and reduces the need for systemic immunity, and is therefore crucially important to the health of the host. (For reviews, see e.g. Cummings et al., 2004; Magalhaes et al., 2007; Schenk and Mueller, 2008)

In summary,

the protective functions of intestinal microbiota, intestinal epithelium, mucus layer covering the epithelium and the intestinal immune system can together be referred to as the intestinal barrier function. The intestinal barrier works both as a physical and chemical barrier and as an immunological first-line defence to optimise the protection of the host from harmful intraluminal entities.

2. Health effects of LGG®

Infectious diseases, including oral, respiratory and gastrointestinal infections, are a major health problem among otherwise healthy populations. They put a great health burden on patients and their families and an enormous economic burden on society in terms of consultations with doctors, direct medical costs and the indirect costs of missed days from work. It has been estimated that in an otherwise healthy population, children suffer from approximately 5 to 10 respiratory infections per year while the prevalence in adults is on average, from 1 to 5 respiratory infections in a year. In children, acute respiratory infections, inclusive of their most prevalent complication, otitis media, account for 80% of all infectious diseases diagnosed in general practice. Viral gastroenteritis is the second most common infection. Daycare attendance is known to be a major risk factor for infections in children. In the elderly respiratory infections account for approximately half and gastroenteritis for one-third of all infections.

Mucosal epithelial surfaces cover an enormous area of the gastrointestinal and respiratory tract and serve as primary ports of entry for most infectious agents. In the gastrointestinal tract, the host is protected against harmful micro-organisms by physical and chemical barriers created by the gastrointestinal epithelium. An intact intestinal epithelium with a normal indigenous microbiota creates a barrier, known as colonization resistance, against pathogens. Normal microbiota can promote

colonisation resistance by competing for nutrients or available adhesion sites on the mucosa, as well as by producing metabolic and regulatory substances. Bacterial adhesion to host cells or mucosal surfaces is always the fundamental first step in the disease process, and therefore interruption of the pathogen adhesion could reduce the risk of infection of the host. Probiotic bacteria generally come from species that belong to the normal intestinal microbiota. Thus, they adapt as a part of the normal indigenous microbiota. On the other hand, the adhesion of probiotics to mucosal surfaces is one of the main properties by which they can prevent the attachment of pathogens. Other mechanisms are e.g. enhancement of immune response, strengthening of the mucosal barrier, or suppressing intestinal inflammation.

“The ILSI Europe Nutrition and Immunity in Man” Task Force published its workshop report on markers to measure immunomodulation in human nutrition intervention studies in 2005 (Albers et al., 2005). The report described several markers and methods to measure the immune response but also admitted that, in healthy adults, small decreases or increases in single selected markers may not be clinically significant. The report also scored immune function markers for their usefulness. The highly suitable markers were identified to be 1) vaccine-specific serum antibody production and 2) delayed-type hypersensitivity response after local antigen application to measure systemic immune functions

in vivo. In mucosal immune markers, and especially to describe gut-associated immune function, the highest scores went to vaccine-specific sIgA and non-specific sIgA in saliva and stool immunoglobulins. The workshop report also concluded that “the real test of the efficacy of a food or food component that claims to improve immune function is a change in the incidence of infectious episodes or the severity or duration of symptoms of infection as this is the outcome of greatest clinical significance”. When this cannot be confirmed, the measurement of a change in one or more aspects of immune function may provide information on the likely mechanism of the dietary intervention.

L. rhamnosus GG is one of the most researched probiotic strains in the world.

Lactobacillus rhamnosus GG (ATCC 53103, LGG®) is one of the most researched probiotic strains in the world. It has been studied extensively in humans and experimental animals for a wide variety of uses. *L. rhamnosus* GG was isolated from an adult human in 1983, and it has a safe history of use in food products since 1990. The strain has most of the characteristics generally proposed for a good probiotic strain, including excellent survival in and transient colonisation of the gastrointestinal tract, which is based on its adhesion capacity to intestinal mucus and epithelial cells. Successful recovery of the strain in stool samples has made dose-response studies possible and enabled the evaluation of effective dosing using food products. *L. rhamnosus* GG has also been found to have many beneficial health effects.

2.1 LGG® supports immunity

LGG® decreases incidence of gastrointestinal infections

Probiotics has been mostly studied in the prevention and treatment of gastrointestinal infections, e.g. diarrhoea. Most of the studies have been conducted in children in which the studies consistently show a statistically significant benefit seen as a reduction of the duration of diarrhoea with well-identified probiotic strains like *L. rhamnosus* GG.

In a study conducted in Poland children hospitalised for other reasons than gastrointestinal problems were randomised to get *L. rhamnosus* GG (6×10^9 cfu, twice a day) or the placebo product during their hospital stay (Szajewska et al., 2001). Study nurses recorded the incidences of diarrhoea and the number of loose or watery stools. Rotavirus antigens were analysed in stool samples during a diarrhoeal period. The administration of *L. rhamnosus* GG reduced the incidence of acute diarrhoea (33.3% vs. 6.7%, placebo and *L. rhamnosus* GG, respectively, $p=0.002$). Although there were no statistically significant differences between the groups in the prevalence of rotavirus antigen in stool samples (27.8% vs. 20%, placebo and *L. rhamnosus* GG, respectively), children in the *L. rhamnosus* GG group had less gastroenteritis due to rotavirus (16.7% vs. 2.2%, placebo and *L. rhamnosus* GG, respectively, $p=0.02$). (Figure 2)

Another study was conducted in children living in suburban areas in Peru (Oberhelman et al., 1999). There the *L. rhamnosus* GG (3.7×10^{10} cfu/day) or placebo doses were delivered in capsules, the content of which was mixed with liquid cherry gelatine before the administration. The study products were delivered directly to the

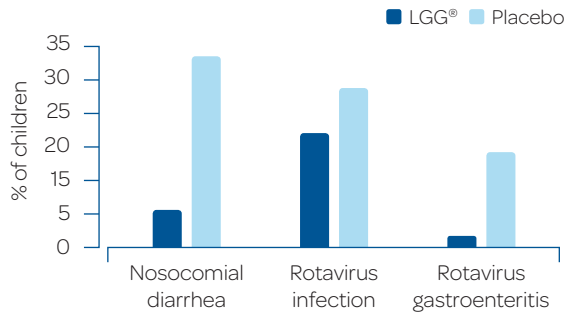


Figure 2. Efficacy of *L. rhamnosus* GG (10^9 cfu/day) in prevention of nosocomial diarrhoea in infants (Szajewska et al., 2001).

homes of children six days a week. The incidence of acute diarrhoea was lower in the *L. rhamnosus* GG group, compared to the placebo (5.21 vs. 6.2 episodes/child/year, $p=0.028$). Furthermore, there were few adenovirus infections in the *L. rhamnosus* GG group, compared to the placebo group (8 vs. 19, $p=0.03$). The duration of administration was 15 months, suggesting that long-term consumption of the probiotic strain does not eliminate the beneficial effect.

In a study conducted in Indian children with acute watery diarrhoea children were randomised to receive either only oral rehydration solution (ORS, $n=185$), ORS+ *L. rhamnosus* GG powder containing 10^{10} colony forming units (cfu) ($n=188$), or ORS+ *L. rhamnosus* GG powder containing 10^{12} cfu ($n=186$) twice daily for a minimum period of 7 days or until diarrhoea stopped along with correction of dehydration (Basu et al., 2009). Both the doses of *L. rhamnosus* GG (10^{10} and 10^{12} cfu) were equally effective to decrease the frequency and duration of diarrhoea and reduction in hospital stay in patients of acute watery diarrhoea of *L. rhamnosus* GG used (Basu et al., 2009). In another study in 684 children with acute

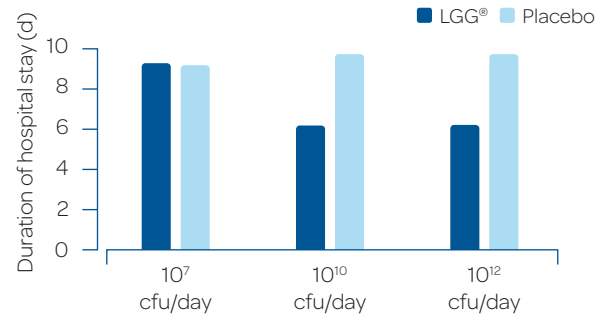
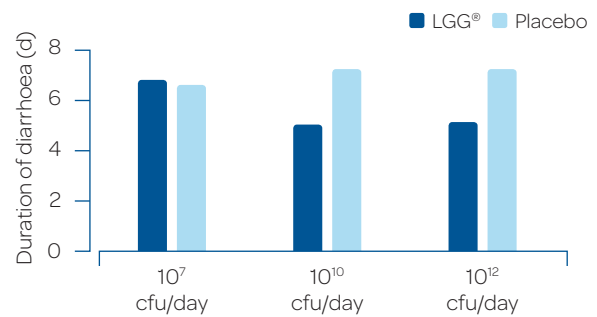


Figure 3. Effect of the dose of *L. rhamnosus* GG on the duration of acute watery diarrhoea and hospital stay in children (Basu et al., 2007 and 2009).

watery diarrhoea in India however, *L. rhamnosus* GG with a lower dose of 10^7 cfu had no effect on the duration or frequency of diarrhoea or to the length of the hospital stay (Basu et al., 2007b). (Figure 3) In a case of persistent diarrhoea in 235 children in India, *L. rhamnosus* GG (10^7 cfu) again significantly decreased the frequency and duration of diarrhoea (Basu et al., 2007a).

Children aged 3-36 months ($n=571$) visiting a family paediatrician for acute diarrhoea were randomly allocated to receive oral rehydration solution (control group) or ORS with *L. rhamnosus* GG (10^9 cfu); *Saccharomyces boulardii* (10^9 cfu); *Bacillus clauseni* (10^9 cfu); mix of *L. delbrueckii* var *bulgaricus* (10^9 cfu), *Streptococcus*

thermophilus (10^9 cfu), *L. acidophilus* (10^9 cfu) and *Bifidobacterium bifidum* (10^8 cfu); or *Enterococcus faecium* SF68 (10^7 cfu) (Canani et al., 2007). Median duration of diarrhoea was significantly shorter ($p < 0.001$) in children who received *L. rhamnosus* GG (78.5 hours) and the mix of four bacterial strains (70.0 hours) than in children who received oral rehydration solution alone (115.0 hours). One day after the first probiotic administration, the daily number of stools was significantly lower ($p < 0.001$) in children who received *L. rhamnosus* GG and in those who received the probiotic mix than in the other groups. The remaining preparations did not affect primary outcomes.

An Italian study (Guarino et al., 1997) and the European multicenter study (Guandalini et al., 2000) showed a significant effect of *L. rhamnosus* GG both in rotavirus infections and in cases where the cause of the diarrhoea was unknown. Similarly, in a study performed in Petroskoi (Russia), the difference was significantly in favour of the *L. rhamnosus* GG group, even though only 27% of the patients had rotavirus diarrhoea. About a fifth had diarrhoea caused by known bacteria and in about half the cases the aetiology was unknown (Shornikova et al., 1997). Therefore, it seems that *L. rhamnosus* GG is effective not only in rotavirus diarrhoea but also in some infections where the aetiology is unknown.

The results of risk reduction studies on acute diarrhoea are also consistent with results of a published strain-specific meta-analysis on the shortening effect of *L. rhamnosus* GG on the duration of acute diarrhoea in children (Szajewska et al., 2007). Eight randomised, controlled trials (RCT) with 988 participants were included in the study, mainly in children hospitalised due to dehydration. Administration of *L. rhamnosus* GG significantly reduced the duration of acute diarrhoea, compared to

Administration of *L. rhamnosus* GG can significantly reduce the duration of acute diarrhoea.

placebo (seven RCTs, 876 infants, weighted mean difference, WMD -1.1 days (95% confidence interval, CI -1.9 to -0.3), particularly of rotavirus etiology (WMD -2.1 days, 95% CI -3.6 to -0.6), risk of diarrhoea >7 days (one RTC, $n=287$, relative risk 0.25, 95% CI 0.09-0.75). Studies using fermented milk products with *L. rhamnosus* GG were excluded, but the authors commented that these studies on fermented milk products confirm the results of the meta-analysis.

There are two studies of the effect of *L. rhamnosus* GG administration on the incidence of acute diarrhoea in adult travellers. In the first study *L. rhamnosus* GG (2×10^9 cfu/day) or placebo sachets were delivered to 820 volunteer tourists before their trip to a high diarrhoeal risk destination (Oksanen et al., 1990). A study doctor was available in the destination and the data was collected by a questionnaire that was returned during the home flight. The probiotic administration showed a reduction in the incidence of acute diarrhoea only in one of the two destinations (24% vs. 40%, $p=0.04$ for one week's trip), but not in the total population (41% vs. 46%, n.s.). In the destination with the non-significant effect, the age distribution was not even between the groups: the age was higher in the placebo group, potentially influencing the results. In the second study on traveller's diarrhoea the risk of diarrhoea varied, depending on the destination (Hilton et al., 1997). Adult participants ($n=245$) were randomised to get *L. rhamnosus* GG (2×10^9 cfu/day) or placebo. The incidence of acute diarrhoea was lower in the *L. rhamnosus* GG group, compared to placebo (3.9% vs. 7.4%, $p=0.05$).

In conclusion,

L. rhamnosus GG has consistently been shown to reduce the risk of acute diarrhoea in children. Studies also show that *L. rhamnosus* GG is able to reduce the duration of acute diarrhoea on average by one day in children hospitalized due to dehydration.

LGG® decreases incidence of antibiotic associated diarrhoea

Possibly the most common indication for the clinical use of probiotics is their ability to prevent the side-effects of antimicrobial treatments, such as diarrhoea and abdominal pain as well as disturbances in gastrointestinal microbiota. The administration of antimicrobial agents disturbs the ecological balance between the host and the microbiota (Sullivan et al., 2001). Antibiotics also interfere with the metabolism of the microbiota, for instance, by impeding the formation of short-chain fatty acids in the colon. Probiotics are therefore well-suited for maintaining or re-establishing the balance of the gastrointestinal microbiota. Also, a well-balanced microbiota prevents the establishment of resistant microbial strains.

The effect of *L. rhamnosus* GG taken in a capsule form has been proved to reduce the side effects of antibiotics in children. In a randomised, double-blind, placebo-controlled study, common acute infections in 188 children were treated by commonly used antibiotics, through the care of family physicians (Vanderhoof et al., 1999). Half the patients received 1-2 *L. rhamnosus* GG capsules (1×10^{10} cfu) once a day, the other half re-

ceived identical placebo capsules without the bacteria (one capsule for children <12 kg, two capsules for those >12 kg). Any gastrointestinal complaints were monitored via telephone interviews. Significantly less diarrhoea and daily defecations were reported in the *L. rhamnosus* GG group than in the control group. Furthermore, the stools were more solid and the study group had less abdominal pain than the placebo group (Figure 4). *L. rhamnosus* GG did not cause any side effects in this or in other studies.

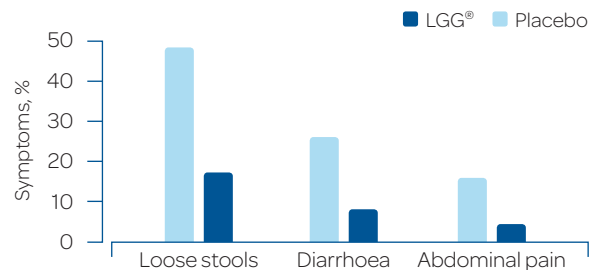


Figure 4. Effect of *L. rhamnosus* GG on intestinal symptoms caused by antibiotics in children (Vanderhoof et al 1999).

Another study was conducted in Finland with children prescribed oral antibiotics for the treatment of acute respiratory infections (Arvola et al., 1999). The children were randomised to receive either one placebo

(n=58) or one *L. rhamnosus* GG (n=61) capsule twice a day (2×10^{10} cfu). The parents kept a daily symptom diary at home and recorded stool frequency and consistency. In cases of diarrhoea, stool samples were analysed for adenovirus, rotavirus, calicivirus and astrovirus as well as for *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, *Clostridia difficile*, *Staphylococcus aureus* and yeasts. Within two weeks of antimicrobial treatment the incidence of diarrhoea was 5% in the *L. rhamnosus* GG group and 16% in the placebo group ($p=0.05$).

In a small study with adult volunteers *L. rhamnosus* GG reduced significantly diarrhoea caused by erythromycin as well as somewhat reducing abdominal pain (Siitonen et al., 1990). In the study, volunteers took a *L. rhamnosus* GG -fermented milk product or a placebo yoghurt (post-pasteurised yoghurt without the living bacteria) in the morning and evening, half an hour after they had taken an antibiotic.

Armuzzi et al. studied the effect of *L. rhamnosus* GG on gastrointestinal discomfort caused by the antibiotic treatment of *H. pylori* (2001a, 2001b). In a pilot study (Armuzzi et al., 2001b) 120 asymptomatic volunteers carrying *H. pylori* were randomised to the eradication therapy with pantoprazole, clarithromycin and tinidazole for one week or the same regimen supplemented with *L. rhamnosus* GG (6×10^9 cfu/sachet) for two weeks. *L. rhamnosus* GG was taken 2 h after breakfast and dinner, mixed with water. Bloating, diarrhoea and taste disturbances were the most frequent side effects during the eradication week and were significantly reduced in the *L. rhamnosus* GG group. The same pattern was observed throughout the follow-up period. The overall assessment of treatment tolerability showed a significant trend in favour of the *L. rhamnosus* GG -supplemented group ($p=0.03$).

In another, double-blinded, placebo-controlled study, 60 healthy asymptomatic *H. pylori* positive volunteers were randomised to one week eradication therapy with *L. rhamnosus* GG (6×10^9 cfu/sachet) for two weeks, or to the same regimen with a placebo preparation (Armuzzi et al., 2001a). Again, diarrhoea, nausea and taste disturbances were significantly reduced in the *L. rhamnosus* GG group compared to the placebo (RR=0.1, 0.3 and 0.5 respectively). An overall assessment of treatment tolerability showed a significant difference in favour of the *L. rhamnosus* GG group ($p=0.04$). There was no difference between the groups in the success of the eradication of *H. pylori* (in both studies it was about 80%), but supplementation with *L. rhamnosus* GG helped to improve the tolerability of the antibiotics. In a very similar design Cremonini et al. (2002) showed also beneficial effect by preventing AAD (Figure 5).

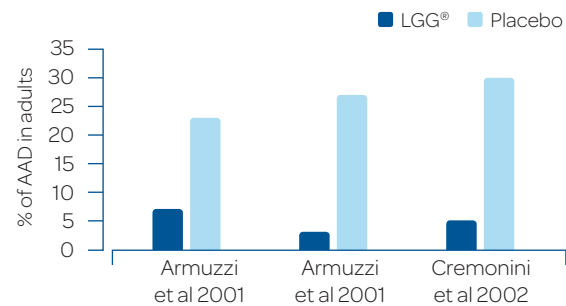


Figure 5. Efficacy of *L. rhamnosus* GG in prevention of antibiotic associated diarrhoea (AAD) in adults.

Recently, in a randomised, double-blind, placebo-controlled trial, 83 *H. pylori* infected children were randomised to receive either *L. rhamnosus* GG or placebo during 7-day intervention (Szajewska et al., 2009). Again, there was no difference in eradication rate between two groups but the risk of therapy-related diarrhoea seemed

to be lower in the *L. rhamnosus* GG group than in the placebo group (6% vs. 20%).

A randomised, double-blinded, placebo-controlled study was performed with 267 initially hospitalised adult patients treated with intravenous or oral antibiotics for a presumed or proven infection (cellulites, pneumonia, urinary tract infection and pyelonephritis) (Thomas et al., 2001). The main groups of antibiotics were β -lactams (cephalosporins 60%, penicillin 27%) and fluoroquinolones (39%). *L. rhamnosus* GG (1×10^{10} cfu) or placebo capsules were given twice a day. The *L. rhamnosus* GG intervention had no effect either on the incidence or on the duration of mild or severe diarrhoea.

Broad-spectrum antibiotics, especially for immunocompromised patients, can cause serious D-lactic acidosis due to the intestinal lactobacilli producing D-lactic acid. *Lactobacillus* GG produces L-lactic acid and has been successfully used to treat one such case (Gavazzi et al., 2001).

Although *L. rhamnosus* GG is susceptible to the most common antibiotics, it has been shown to survive in the intestines during antibiotic treatment in most test subjects. The survival of *L. rhamnosus* GG can be explained by the antibiotic and bacterial preparations being taken at different times, and possibly by the lower antibiotic level in the bowel than in the blood stream.

To sum up,

L. rhamnosus GG supplementation reduces antimicrobial treatment-related side effects such as abdominal pain and prevents clinically significantly antibiotic-associated diarrhoea. Antibiotic and probiotic preparation should be taken at different times.

LGG® decreases incidence of respiratory infections

There is increasing evidence that probiotics may offer protection against respiratory infections and common cold. The effect of *L. rhamnosus* GG in decreasing respiratory infections has been studied in three studies in children and one in adults training for a marathon.

One study was conducted in healthy children attending daycare at local daycare centres (Hatakka et al.,

2001). Altogether, 513 children from 18 daycare centres in Helsinki, Finland, were randomised to the *L. rhamnosus* GG or placebo groups, to consume milk enriched with *L. rhamnosus* GG ($1-2 \times 10^8$ cfu/day) or standard milk at their day-care meals five days a week, for seven months. The randomisation was done separately to children less than three years and to those of three years and older. There was no statistically significant difference in the children's age in the groups, but when the ages were compared inside the age groups (year-by-year level),

the *L. rhamnosus* GG group contained more children of older age. Thus, also age-adjusted results were analysed. Although the number of days with illness symptoms reported by parents was not statistically significantly lower in the *L. rhamnosus* GG group, the absence due to any illness from daycare was less frequent (4.9 vs. 5.8 days/child, $p=0.03$; age-adjusted $p=0.09$) (Figure 6). Also, children in the *L. rhamnosus* GG group had one-week longer time without respiratory symptoms from the beginning of the study (4 wk vs. 5 wk, $p=0.03$). Although there was no difference in respiratory symptoms reported by parents, children in the *L. rhamnosus* GG group had fewer respiratory infections with complications (e.g. otitis media) as diagnosed by physicians (relative difference between the groups -17%) and they needed less antibiotics to treat respiratory tract infections (relative reduction -19%).

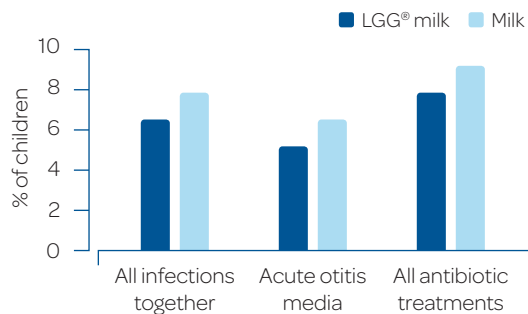


Figure 6. Effect of *L. rhamnosus* GG milk on infections, acute otitis media and antibiotic treatments in daycare children (Hatakka et al., 2001).

In another randomised, double-blind, placebo controlled study at a daycare centre in Croatia, children who received 100 ml fermented milk product with *L. rhamnosus* GG (10^9 cfu)($n=139$) had a significantly less res-

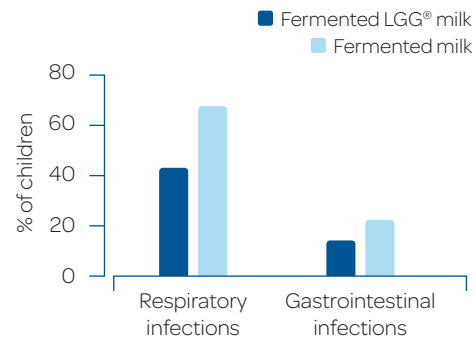


Figure 7. Effect of *L. rhamnosus* GG fermented milk on infections in daycare children (Hojsak et al., 2009).

piratory tract infections compared to the placebo group ($n=142$) (43.2% vs 67.6%, $p<0.001$) (Figure 7) (Hojsak et al., 2009). Children receiving placebo had more gastrointestinal infections but difference was not statistically significant (22.5% vs 14.4%, $p=0.079$) and there was no difference in absence from the daycare centre ($p=0.069$). In a paediatric hospital, the effect of *L. rhamnosus* GG was studied in preventing nosocomial infections (Hojsak et al., 2010). In this randomised, double-blind, placebo-controlled study 742 children were randomly allocated

Children who received *L. rhamnosus* GG containing milk product had less infections.

to receive 100 ml fermented milk product containing *L. rhamnosus* GG (10^9 cfu) or the same product without bacteria. In the *L. rhamnosus* GG group there was a significantly reduced risk of gastrointestinal infections (relative risk [RR] 0.4, 95% confidence interval [CI] 0.25–0.7, number needed to treat [NNT] 15, 95% CI 9–34) and res-

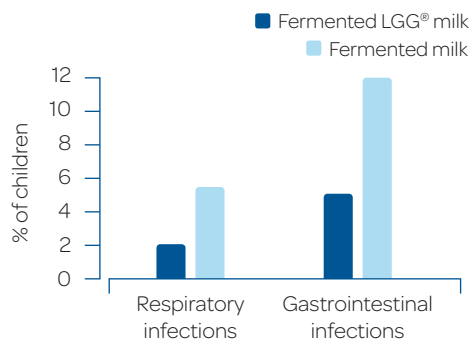


Figure 8. Effect of *L. rhamnosus* GG fermented milk on infections in hospitalised children (Hojsak et al., 2010).

piratory tract infections (RR 0.38, 95% CI 0.18–0.85, NNT 30, 95% CI 16–159) compared to the placebo (Figure 8).

One study has been conducted in healthy adults (n=119) who were training for a marathon run in the summer (Kekkonen et al., 2007). They were randomised to consume *L. rhamnosus* GG milk drink (4×10^{10} cfu/day) or a similar placebo drink (without probiotics) during their training period (3 months) and 4 weeks after the run. There was neither an effect on the number of healthy days, duration or number of upper respiratory tract infections or on gastrointestinal infections or their durations during the training period. However, the duration of gastrointestinal symptoms were shorter in the *L. rhamnosus* GG group during the two weeks after the marathon (1 vs. 2.3 days, $p=0.046$).

To summarise,

in children *L. rhamnosus* GG seems to be effective in decreasing respiratory infections, but in adults the data is insufficient to draw any conclusions.

LGG® enhances antibody formation during viral infection

The enhancing effect of *L. rhamnosus* GG on antibody production in connection with natural infections has been studied in rotavirus-induced diarrhoea (Kaila et al., 1995; Kaila et al., 1992; Majamaa et al., 1995). These studies were conducted in children coming to the hospital due to acute diarrhoea and dehydration. They were randomised to receive either *L. rhamnosus* GG or placebo, after an oral rehydration. *L. rhamnosus* GG or placebo was given in the form of fermented milk (Kaila et al., 1992) or freeze-dried powder (Kaila et al., 1995; Majamaa et al., 1995) during their hospital stay. The blood samples were collected at admission and 8 days after that, and 3-4 weeks after hospitalisation as well. The duration of diarrhoea in the hospital was significantly shorter in the *L. rhamnosus* GG groups (about one day), and there was a significant increase in the number of total immunoglobulin secreting cells at the acute phase of infection in all Ig classes in all of these studies. Significantly higher levels of B lymphocytes producing the IgA antibody against the rotavirus antigen were observed in the blood of children who received *L. rhamnosus* GG than in those receiving the placebo. The increase in the antigen-specific IgA producing cells was detected after three weeks from the infection, but not in the acute phase, indicating the period needed for the maturation of the lymphocytes. In two of the studies, the level of rotavirus-specific IgA response was increased also on sera (Kaila et al., 1995; Majamaa et al., 1995). The results indicate that the antigen-specific response is “too slow” to help in the recovery of rotavirus diarrhoea, but the non-specific immunoglobulin response may be an important mechanism – also in reducing the risk or symptoms of a disease. This was

noticed in the study by (Szajewska et al., 2001). In this study children had as many rotavirus infections both in the *L. rhamnosus* GG and the placebo groups, but they had gastroenteritis less frequently due to rotavirus in the *L. rhamnosus* GG group (16.7% vs. 2.2%, $p=0.02$). In addition, better antigen-specific immune response may enhance the protection against re-infections.

LGG® may increase vaccine antibody responses

There are indications that *L. rhamnosus* GG could increase the vaccine-specific antibodies after vaccinations. The first study was conducted in infants, who were given *L. rhamnosus* GG or placebo powder for five days together with an oral rotavirus vaccine (Isolauri et al., 1995). There was a significantly more frequent rise in rotavirus-specific antibody secreting cell response in IgM-class, compared to the placebo (79% vs. 29%, $p=0.02$), and a higher level of vaccine-specific IgM secreting cells in the *L. rhamnosus* GG group, compared to placebo ($p=0.02$). Seroconversion in IgA class was more frequent in the *L. rhamnosus* GG group than with the placebo ($p=0.05$), and a tendency to higher IgA and IgG antibodies in sera was detected in subjects who were given *L. rhamnosus* GG in connection with the vaccination ($p=0.10$).

An enhanced production of antibodies against an oral vaccine was also shown in adults, who were given *L. rhamnosus* GG -fermented milk or placebo milk before and during the poliovirus vaccination (de Vrese et al., 2005). *L. rhamnosus* GG significantly increased the poliovirus neutralizing antibody titers (polio serotype 1, $p=0.048$, and serotype 2, $p=0.014$, serotype 3, $p=n.s.$) and the formation of poliovirus-specific IgA in serum (polio serotype 1, $p=0.36$, serotype 2, $p=0.02$, serotype

L. rhamnosus GG may increase vaccine-specific antibodies after vaccination.

3, $p=0.076$), and had a tendency to increase the formation of IgG in serum (serotype 1, $p=0.083$, serotype 2, $p=0.291$, serotype 3, $p=0.211$). The results of this study show that the boosting effects of *L. rhamnosus* GG are not limited to antibody formation in infections that take place in gastrointestinal tract only, but may have an influence in the body as a whole.

The third vaccination study was conducted with adult volunteers (He et al., 2000). They were randomised to receive *L. rhamnosus* GG or placebo powder for seven days for boosting the oral *Salmonella typhi* vaccine. All subjects responded well to the vaccine, but no significant differences were observed in the numbers of IgA-, IgG- and IgM-secreting cells among the groups. A tendency was observed for more volunteers given *L. rhamnosus* GG to evince a high number of vaccine-specific antibody-secreting cells in the IgA class, but the difference was not statistically significant. No humoral response was measured.

Effect on permeability

Chronic non-steroidal anti-inflammatory drugs destroy gastrointestinal mucosa, leading to ulceration. The protective effect of fermented milk drinks on indometacin-induced alterations of mucosal permeability has been studied. The fermented milk drinks contained live or heat-inactivated strains of *L. rhamnosus* GG, *L. helveticus* and *L. acidophilus* ($>10^7$ cfu/g each) (Gotteland et al., 2001). Four gastrointestinal permeability tests were car-

ried out in randomised order on 16 healthy adults: 1) basal, 2) after indometacin, 3) after indometacin when the fermented milk drink with living bacteria was consumed for five days, 4) after indometacin when the fermented milk drink with heat-inactivated bacteria was consumed for five days. Gastric permeability was measured by sucrose urinary excretion, and intestinal permeability by lactulose/mannitole excretion. Indometacin significantly increased both gastric and intestinal permeability. The fermented milk with live *L. rhamnosus* GG significantly reduced abnormal gastric permeability, but not the intestinal permeability induced by indometacin. The drink with the heat-inactivated bacteria had no effect.

In 3–5 year-old Malawian children ($n=164$) the effect of *L. rhamnosus* GG on intestinal function and permeability was studied (Galpin et al., 2005). *L. rhamnosus* GG had no effect on the intestinal function or integrity measured by urinary sucrose excretion and lactulose/mannitole excretion. Also in children with short-bowel syndrome ($n=21$), *L. rhamnosus* GG had no effect on intestinal permeability measured by lactulose/mannitole test (Sentongo et al., 2008). However, in an open label pilot study with children with Crohn's disease ($n=4$), daily *L. rhamnosus* GG consumption for six months significantly decreased intestinal permeability (Gupta et al., 2000).

Modulation of other markers of immune function

In duodenal mucosa biopsies of adults, *L. rhamnosus* GG affected mainly genes involved in immune response and inflammation (TGF- β , TNF family members, cytokines, nitric oxide synthase 1, defensin alpha1), apoptosis, cell growth and cell differentiation (cyclins, caspases, oncogenes), cell signalling (ICAMs and integrins), cell adhesion (cadherins), signal transcription and transduction

indicating that *L. rhamnosus* GG is able to modulate mucosal immune responses in the intestinal mucosa (Di Caro et al., 2005). The administration of *L. rhamnosus* GG has also resulted in systemic changes in immune function. In healthy adults, *L. rhamnosus* GG appeared to have anti-inflammatory effects reflected as a decrease in inflammatory mediators such as sensitive CRP, inflammatory cytokines and inflammatory lipid-derived mediators, namely lysophosphatidylcholines and sphingomyelins (Kekkonen et al., 2008b, Kekkonen et al., 2008c). Also, in another study with healthy adults, *L. rhamnosus* GG has decreased the production of proinflammatory cytokines and increased anti-inflammatory *ex vivo* cytokines production by PBMC (Schultz et al., 2003) as well as in patients with Crohn's disease (Braat et al., 2004). In a human intervention, *L. rhamnosus* GG enhanced significantly the formation of the phagocytic receptors CR1, CR3, FcγRIII and FcγR in neutrophil blood cells in

healthy adults but suppressed the response of milk-hypersensitive adults during a milk challenge (Pelto et al., 1998). It was concluded that probiotic bacteria appear to modulate the non-specific immune response differently in healthy subjects and hypersensitive subjects by immunostimulation in healthy and by down-regulation in the hypersensitive ones (Pelto et al., 1998).

In allergic children *L. rhamnosus* GG seem to modulate immune response differently. In allergy-prone children, the induction of Th1-type immune response and low-grade inflammation measured by sensitive CRP in serum by *L. rhamnosus* GG has been proposed as an action mechanism for the prevention of atopic diseases (Marschan et al., 2008; Viljanen et al., 2005b). In addition in allergic children, *L. rhamnosus* GG may alleviate intestinal inflammation and increase faecal IgA (Viljanen et al., 2005a).

To sum up,

L. rhamnosus GG is able to enhance antibody production as well as increase the vaccine-specific antibodies after vaccination. *L. rhamnosus* GG may also normalise intestinal permeability, but more studies should be done to confirm the results. *L. rhamnosus* GG modulates mucosal immune responses in the intestinal mucosa and thereby results in systemic changes to the immune function such as decrease in inflammatory mediators.

2.2 Other areas

Allergy

The prevalence of atopic diseases has been increasing in Western countries and today these conditions comprise the most common chronic disease of childhood. The factors contributing to atopic diseases are aberrant barrier functions of the skin epithelium and gut mucosa and dysregulation of the immune response to environmental antigens (Isolauri et al., 2008). In recent years probiotics have been investigated with the target to counteract the immunological and gut mucosal barrier dysfunction associated with allergic disease.

A randomised, placebo-controlled study on children with an atopic eczema with allergy to milk showed that the intensity and extension of the rash and subjective symptoms decreased significantly faster when their milk elimination diet contained *L. rhamnosus* GG (Majamaa and Isolauri, 1997). The intestinal inflammation was measured using the cytokine content of their stools. TNF- α was found to fall significantly more rapidly in the *L. rhamnosus* GG group compared to the placebo, indicating a faster recovery from inflammation. In another clinical study, *L. rhamnosus* GG was given to infants that manifested atopic eczema during exclusive breastfeeding and had no exposure to any infant food or substitute formula (Isolauri et al., 2000). They were weaned to a probiotic (*L. rhamnosus* GG or bifidobacteria) -supplemented extensively hydrolysed whey protein formula or to the same formula, without probiotics. After two months, the atopic eczema was significantly improved in the probiotic groups as compared to the placebo. In addition, the inflammatory eosinophilic protein X in urine decreased, and anti-inflammatory TGF- β in the serum

increased, indicating again faster recovery from inflammation. In other studies with infants suffering from atopic dermatitis, *L. rhamnosus* GG has had however no effect on the severity scoring of atopic dermatitis (Brouwer et al., 2006; Folster-Holst et al., 2006; Gruber et al., 2007).

To further study if probiotic bacteria reduce symptoms of the atopic eczema/dermatitis syndrome (AEDS) in food-allergic infants and whether or not there are differences in probiotic preparations, a follow-up study with 230 infants was conducted (Viljanen et al., 2005c). The infants were randomised in a double-blinded manner, to receive *L. rhamnosus* GG, a mixture of four probiotic strains (*L. rhamnosus* GG, *L. rhamnosus* LC705, *Bifidobacterium breve* 99 and *Propionibacterium shermanii* ssp. *freudenreichii* JS), or a placebo for 4 weeks. In the whole group, the mean Severity Scoring of Atopic Dermatitis (SCORAD) (at baseline 32.5) decreased by 65% but with no immediate differences between treatment groups or at 4 weeks after the treatment. Similarly, no treatment differences were observed in infants with CMA. In IgE-sensitised infants, however, the *L. rhamnosus* GG group showed a greater reduction in SCORAD than did the placebo group, -26.1 vs -19.8 (p=0.036). It was concluded that treatment with *L. rhamnosus* GG may alleviate AEDS symptoms in IgE-sensitised infants but not in non-IgE-sensitised infants. In the same study, it was shown that after treatment, faecal IgA levels tended to be higher in probiotic groups than in the placebo group (*L. rhamnosus* GG vs. placebo, p=0.064; MIX vs. placebo, p=0.064), and faecal antitrypsin decreased in the *L. rhamnosus* GG group, but not in other treatment groups indicating that *L. rhamnosus* GG may alleviate intestinal inflammation in infants with AEDS and CMA (Viljanen et al., 2005a). Probiotics also modulated sys-

temic immune responses differently, as *L. rhamnosus* GG raised IFN- γ production of PBMC in infants with CMA and IgE-associated dermatitis thus providing beneficial Th1 immunomodulatory signals whereas MIX increased the secretion of IL-4 (Pohjavuori et al., 2004). In infants with IgE-associated AEDS, treatment with *L. rhamnosus* GG induced higher C-reactive protein levels than in the placebo group ($p=0.021$) (Viljanen et al., 2005b). Concomitantly, IL-6 levels increased after treatment with *L. rhamnosus* GG ($p=0.023$), but not with MIX or the placebo. However, the use of MIX induced an increase in plasma IL-10 levels ($p=0.016$) (Viljanen et al., 2005b). It was concluded that probiotics induced systemically detectable low-grade inflammation, which might explain the clinical effects of probiotics in AEDS and CMA (Viljanen et al., 2005b). In later analysis it was shown that infants receiving probiotic bacteria had higher plasma levels of CRP ($p=0.008$), total IgA ($p=0.016$), total IgE ($p=0.047$) and IL-10 ($p=0.002$) than infants in the placebo group (Marschan et al., 2008). The increased plasma CRP level at age 6 months was associated with a decreased risk of eczema [odds ratio (OR) 0.41 [95% confidence interval (CI) 0.17-0.99], $p=0.046$], as well as with a decreased risk of allergic disease [OR 0.38 (95% CI 0.16-0.87), $p=0.023$] at age 2 years when adjusted with probiotic use. The association of CRP with a decreased risk of eczema at 2 years of age in allergy-prone children supports the view that chronic, low-grade inflammation provides protection against eczema, emphasising the role of chronic microbial exposure as an immune modulator giving protection against allergy (Marschan et al., 2008).

To evaluate if the development of allergic diseases can be prevented in early infancy by modulating the intestinal microflora with probiotic bacteria, a group of families at high risk of allergy was selected and 159 moth-

ers were randomised to receive two *L. rhamnosus* GG (10^{10} cfu) or placebo capsules daily for 2-4 weeks before the expected date of the birth (Kalliomaki et al., 2001). After the birth, either the breastfeeding mother or the infant consumed the bacteria for six months. The children were clinically examined at the age of two and prevalence of atopic eczema was 23% in the *L. rhamnosus* GG group and 46% in the placebo group (Kalliomaki et al., 2001). This result was confirmed also in the four and seven years follow-up (Figure 9) (Kalliomaki et al., 2003; Kalliomaki et al., 2007). In the seven year follow-up in accordance with Cox regression, the risk of eczema was significantly reduced in the *L. rhamnosus* GG group compared with the placebo group (odds ratio, 0.58; 95% CI, 0.35-0.94; $p=0.027$) (Kalliomaki et al., 2007). In the two-year follow-up it was shown that *L. rhamnosus* GG intervention had no significant effects on the composition or quantity of gut microbiota (Rinne et al., 2006). However, it was shown that immunoglobulin secreting cells were higher in the children whose mothers received *L. rhamnosus* GG and the numbers correlated with sCD14 in colostrum, suggesting that probiotics during breastfeeding may positively influence gut immunity (Rinne et al.,

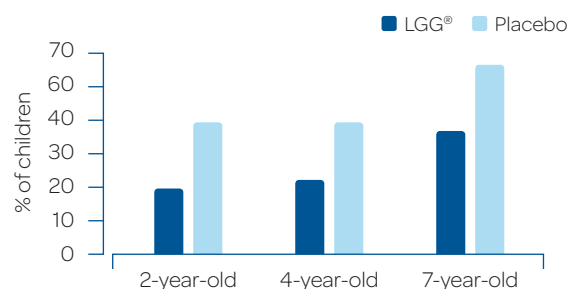


Figure 9. Proportion of 2-, 4- and 7-year-old children with atopic eczema (%) in a follow-up study with *L. rhamnosus* GG (Kalliomäki et al., 2001, 2003 and 2007).

L. rhamnosus GG significantly reduce the risk of atopic eczema.

2005). In addition, *L. rhamnosus* GG increased the level of anti-inflammatory TGF- β 2 in breast milk significantly compared to the placebo group (Rautava et al., 2002). In the four-year follow-up, it was also concluded that the perinatal administration of probiotics was safe as it did not influence the height of the weight-for-height of the children (Laitinen et al., 2005). Contrary to the results of the study by Kalliomäki et al., in a similar clinical setting with mothers and children having high risk of atopic disease, the supplementation of *L. rhamnosus* GG (10^9 cfu) had no effect on the prevalence of atopic dermatitis at the age of 2 compared with the placebo (28% vs 27.3%) (Kopp et al., 2008).

Intervention with probiotics in the treatment of food allergy of in small children has provided somewhat promising results, and a similar effect was studied in teenagers and young adults which were allergic to birch pollen and apple (Helin et al., 2002). An open oral chal-

lenge test with apple was done in the beginning, during and end of the birch pollen season. The patients were randomly assigned to receive either *L. rhamnosus* GG or a placebo 2,5 months before the pollen season, 1 month during the pollen season and 2 months after the season. *L. rhamnosus* GG did not alleviate the symptoms or reduce the medication during of after the 2 subsequent months (Helin et al., 2002). However, after 5.5 months, rBet v1 and rMal d1 specific IgA levels had increased from the baseline in the *L. rhamnosus* GG compared with the placebo ($p=0.02$). rBet v1 specific IgE serum levels did not differ between the groups. In the *L. rhamnosus* GG group, rBet v1 specific IgE levels correlated positively with the stimulated total IgA ($p=0.04$) and IgG ($p=0.003$) in saliva. In the placebo group, rBet v1 specific IgE levels correlated negatively with stimulated rBet v1 and rMal d1 IgA levels ($p=0.009$ for both) and IgG ($p=0.02$ and $p=0.03$, respectively). It was concluded that *L. rhamnosus* GG showed immunostimulating effects on oral mucosa seen as increased allergen specific IgA levels in saliva (Pirainen et al., 2008).

To summarise,

L. rhamnosus GG may alleviate the symptoms of atopic eczema and alleviate the intestinal inflammation. *L. rhamnosus* GG may also decrease the risk of atopic diseases as in a seven-year follow-up study children who received *L. rhamnosus* GG had less atopic eczema.

Inflammatory bowel diseases

There are several chronic intestinal diseases without known aetiology, such as Crohn's disease, ulcerative colitis and pouchitis. They are collectively referred as inflammatory bowel diseases (IBD) that have very diverse features but also share several similarities with respect to pathomechanisms and clinical course (Strober et al., 2007). In addition to the genetic background and autoimmune nature of the disease, the role of intestinal microbiota in the initiation and progression of these diseases is also speculated (Sartor 2008). IBD is thought to be caused by an aggressive immune response to luminal bacteria and is characterised by a Th-1 type cytokine pattern.

Effects of *L. rhamnosus* GG® have been studied in four separate trials on Crohn's disease patients (Gubta et al., 2003, Prantera et al., 2002, Schultz et al., 2004, Bousvaros et al., 2005). Overall, *L. rhamnosus* GG seems to be ineffective in maintaining or prolonging remission or preventing recurrence after surgically induced remis-

sion. The only positive results have been obtained with children with mildly to moderately active Crohn's disease. The results of this pilot, open label study showed a significant improvement in clinical activity and improved intestinal permeability (Gubta et al., 2003).

L. rhamnosus GG has shown preliminary positive effect in maintaining remission in pouchitis (Gosselink et al., 2004). Also results from an open-label pilot study in the treatment of refractory "pouchitis" with capsules filled with *L. rhamnosus* GG and fructooligosaccharide report a beneficial effect in pouchitis as an adjunct therapy to antibiotics (Friedman et al., 2002). With regard to the induction of remission in pouchitis, *L. rhamnosus* GG was ineffective in three-month intervention (Kuisma et al., 2003).

In one open study, 187 subjects with inactive ulcerative colitis were randomised to receive *L. rhamnosus* GG for one year. *L. rhamnosus* GG indicated the same efficacy as mesalazine on the maintenance of remission in ulcerative colitis patients (Zocco et al., 2006).

To conclude,

L. rhamnosus GG has shown promising preliminary results in the maintaining remission in ulcerative colitis, but more clinical studies are needed to clarify the results.

Gastrointestinal discomfort

There are three studies on treatment of gastrointestinal symptoms, such as irritable bowel syndrome (IBS), with *L. rhamnosus* GG. The first pilot study was made with enterocoated tablets (O'Sullivan and O'Morain, 2000).

Symptoms were recorded in daily diaries and by periodic questionnaires. *L. rhamnosus* GG intake did not have significant effects on the symptoms. The study group consisted of patients with bloating as the main symptom. It was noted, however, that there tended to be a reduction in the number of unformed bowel motions with *L. rhamnosus* GG treatment for patients with diarrhoea (O'Sullivan and O'Morain 2000).

On the contrary, in a double-blind, randomised, controlled trial with 104 children who fulfilled the Rome II criteria for IBS, functional abdominal pain or functional dyspepsia were given *L. rhamnosus* GG or a placebo. By the end of the intervention, frequency of abdominal pain was significantly alleviated in the *L. rhamnosus* GG group compared to the placebo group (Gawronska et al., 2007). However, in another study of children with IBS (64 participants), *L. rhamnosus* GG had no effect on relieving abdominal pain but appeared to lower incidence of perceived abdominal distension (Bausserman et al., 2005). Treatment success occurred in 25% of the *L. rhamnosus* GG group compared to 9.6% in the placebo group ($p=0.03$).

To conclude,

L. rhamnosus GG may relieve some types of symptoms of IBS, but more studies are needed to confirm and specify the effects.

Oral health

Lactobacilli are common bacteria in the oral cavity but are generally regarded as potentially cariogenic, growing together with *Streptococcus mutans*. However, *in vitro* studies have shown that *L. rhamnosus* GG fermentates sucrose and lactose slowly or not at all (Saxelin, 1997) and suppresses the growth of the *Streptococcus mutans*-group streptococci, which are the indicator bacteria of dental caries (Meurman et al., 1995). During the past decade, these *in vitro* findings have led to several clinical

studies conducted on the oral health effect of *L. rhamnosus* GG, including studies on the risk factors of dental caries, oral *Candida* prevalence and hyposalivation.

The long-term effect of *L. rhamnosus* GG on the risk of caries in children was studied in a randomised, placebo-controlled study at 18 daycare centres in Finland (Näse et al., 2001). Five days a week for seven months, the children were given pasteurised milk that contained *L. rhamnosus* GG ($5-10 \times 10^5$ cfu/ml) or standard milk as a placebo with all meals consumed at the daycare centres. Children's oral health was recorded at baseline and at

the end of the 7-month intervention. Risk of dental caries was assessed based on decayed, missing or filled teeth (dmft), initial caries and *Streptococcus mutans* counts in saliva-dental plaque samples. The results showed

a smaller risk of dental caries in the *L. rhamnosus* GG group, the risk of dental caries being 44% (OR=0.56, p=0.01) lower in the *L. rhamnosus* GG group compared to the placebo group.

In conclusion,

L. rhamnosus GG has shown potential in reducing the risk of dental caries.

Rheumatoid arthritis

Probiotic therapy in rheumatoid arthritis patients is an interesting area of research, where *L. rhamnosus* GG has shown positive preliminary results. Over 10 years ago, Malin et al. (1996, 1997) showed that in children with chronic arthritis, short-term consumption (10 days) of *L. rhamnosus* GG can normalise high urease enzyme activity in stools, which indicates an imbalance in the intestinal microbiota. These results first suggested that *L. rhamnosus* GG may have a beneficial effect in the treatment of chronic arthritis patients. More recently, the long-term effects of *L. rhamnosus* GG in patients

with rheumatoid arthritis were studied in a double-blind, placebo-controlled, randomised study (Hatakka et al., 2003). At the end of the one-year study, a tendency towards a reduction in the number of swollen and tender joints in the *L. rhamnosus* GG group was shown as compared to the placebo group. Arthritic activity tended to decrease more in the *L. rhamnosus* GG group compared to the placebo, and the patients in the *L. rhamnosus* GG group also needed less medication for rheumatoid arthritis. Due to the limited number of patients, the results were not statistically significant, but the tendency towards a beneficial impact was clear.

To conclude,

L. rhamnosus GG has shown promising preliminary results in the treatment of rheumatoid arthritis, but more clinical studies are needed to confirm these findings.

Cystic fibroses

There are not many studies on the treatment of cystic fibroses with probiotics. *L. rhamnosus* GG has shown preliminary positive effects in three clinical trials. In a pilot study, Bruzesse et al. (2004) found that *L. rhamnosus* GG alleviated intestinal inflammation in children with cystic fibroses: specifically, the concentration of calprotectin and nitric oxide decreased after four weeks' administration compared to the control group. A further preliminary study from the same Italian group found that taking *L. rhamnosus* GG bacteria daily for six months sig-

nificantly reduced the number of pulmonary infections and hospital admissions (Bruzesse et al., 2007). This study was randomised, placebo-controlled, cross-over study with children mean age 13.2 years. The results of this study suggest that *L. rhamnosus* GG may delay respiratory impairment and a relationship exists between intestinal and pulmonary inflammation. Recently Pina et al. (2008) showed in a pilot study improvement of intestinal functions by *L. rhamnosus* GG administration twice daily for two weeks. Thirteen of twenty cystic fibroses patients (81.3%) had improved stool appearance and intestinal comfort.

To summarise,

preliminary studies on the treatment of cystic fibroses with *L. rhamnosus* GG are positive but larger clinical studies are needed before any definite conclusion can be made.

2.3 Mechanism behind the effects

Experimental and *in vitro* studies explain the mechanisms of action and support the understanding of how *L. rhamnosus* GG is able to modulate immune response and thus reduce both gastrointestinal and respiratory infections (Figure 10). Studies demonstrate that the administration of *L. rhamnosus* GG in animal experiments reduced the growth of challenged pathogen in the intestinal tract, reduced the translocation of intestinal bacteria to tissues and internal organs, and enhanced the immune response of animals (Hudault et al., 1997; Lee et al., 2000; Naaber et al., 1998; Sherman et al., 2004; Wagner et al., 2000; Wagner et al., 1997a). Enhanced immune response partly explains the improvement/normalisation of the mucosal barrier that is shown to reduce bacterial translocation to tissues (Kirjavainen et al., 1999; Negretti et al., 1997; Wagner et al., 2000; Wagner et al., 1997b). One potential mechanism to reduce bacterial translocation by *L. rhamnosus* GG is that it normalises a disrupted mucosal barrier. Both rotavirus and cow milk increased absorption of intact protein through small bowel mucosa, but the concomitant administration of *L. rhamnosus* GG counteracted the permeability disturbance (Isolauri et al., 1993a; Isolauri et al., 1993b). In addition, the number of antibody-secreting cells increased (Isolauri et al., 1993a; Isolauri et al., 1993b). *In vitro* and experimental studies also indicate that *L. rhamnosus* GG is able to secrete specific proteins that have an influence on the integrity of mucosal cells as well as their apoptosis-proliferation rate (Mack et al., 2003; Mack et al., 1999; Seth et al., 2008; Tao et al., 2006; Yan et al., 2007). The administration of *L. rhamnosus* GG in monoassociated rats showed increased production of epithelial cells in both the small and large intestine as measured by the

Presence of the pili structure in L. rhamnosus GG may be essential for the adhesion to human intestinal mucus and it may also provide explanations for the immune stimulating effects.

mitotic index, number of crypt cells, depth of the crypts and the height of villi and the number of epithelial cells in villi (Banasaz et al., 2002). Although *L. rhamnosus* GG was shown to attach to intestinal epithelial cells of mono-associated mice, the inability of the strain to degrade the intestinal mucus was also demonstrated, which is an important safety aspect (Ruseler-van Embden et al., 1995).

In primary human leukocytes *L. rhamnosus* GG has induced the expression and production of the proinflammatory Th-1-type cytokines TNF- α , IL-1 β , IL-6 and IL-18 in peripheral blood mononuclear cells, but not the Th-2 type cytokine IL-4 and relatively little IL-10 (Kekkonen et al., 2008a; Miettinen et al., 1998; Miettinen et al., 1996). *L. rhamnosus* GG has also activated the transcription factor NF- κ B, which is the central activator of innate immune response, and the Toll-like receptors TLR1 and TLR2, which mediate bacterial recognition and cellular signalling (Miettinen et al., 2000). *L. rhamnosus* GG is recognised by the TLR2 receptor (Miettinen et al., 2008) and thus is able to induce a cascade of immunological events in the gut epithelial cells and/or antigen-presenting cells. *L. rhamnosus* GG may modulate systemic immune responses through epithelial cells (Lopez et al., 2008; Wallace et al., 2003; Zhang et al., 2005) as well as through underlying professional antigen-presenting cells such as macrophages and dendritic cells (Latvala

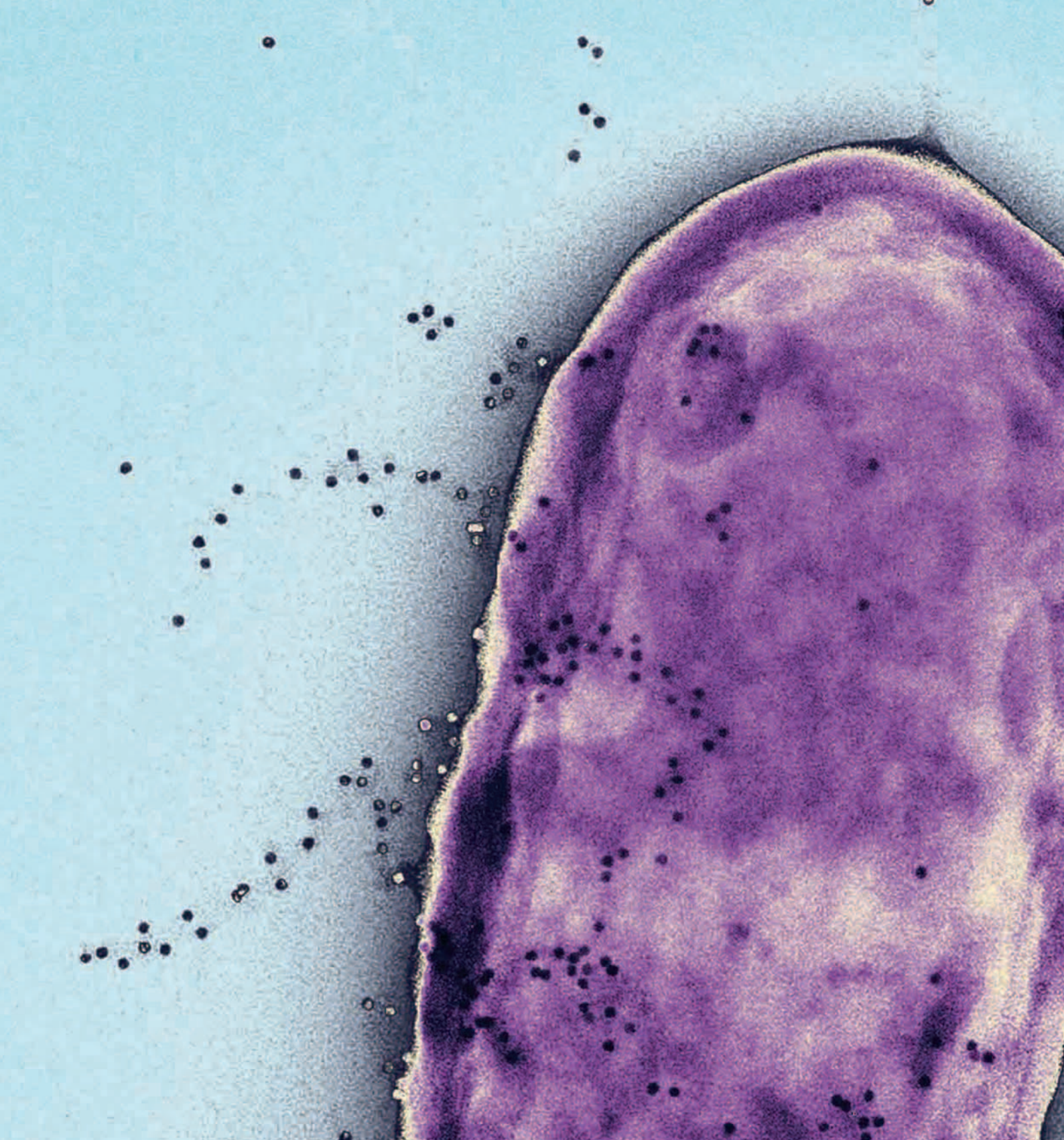
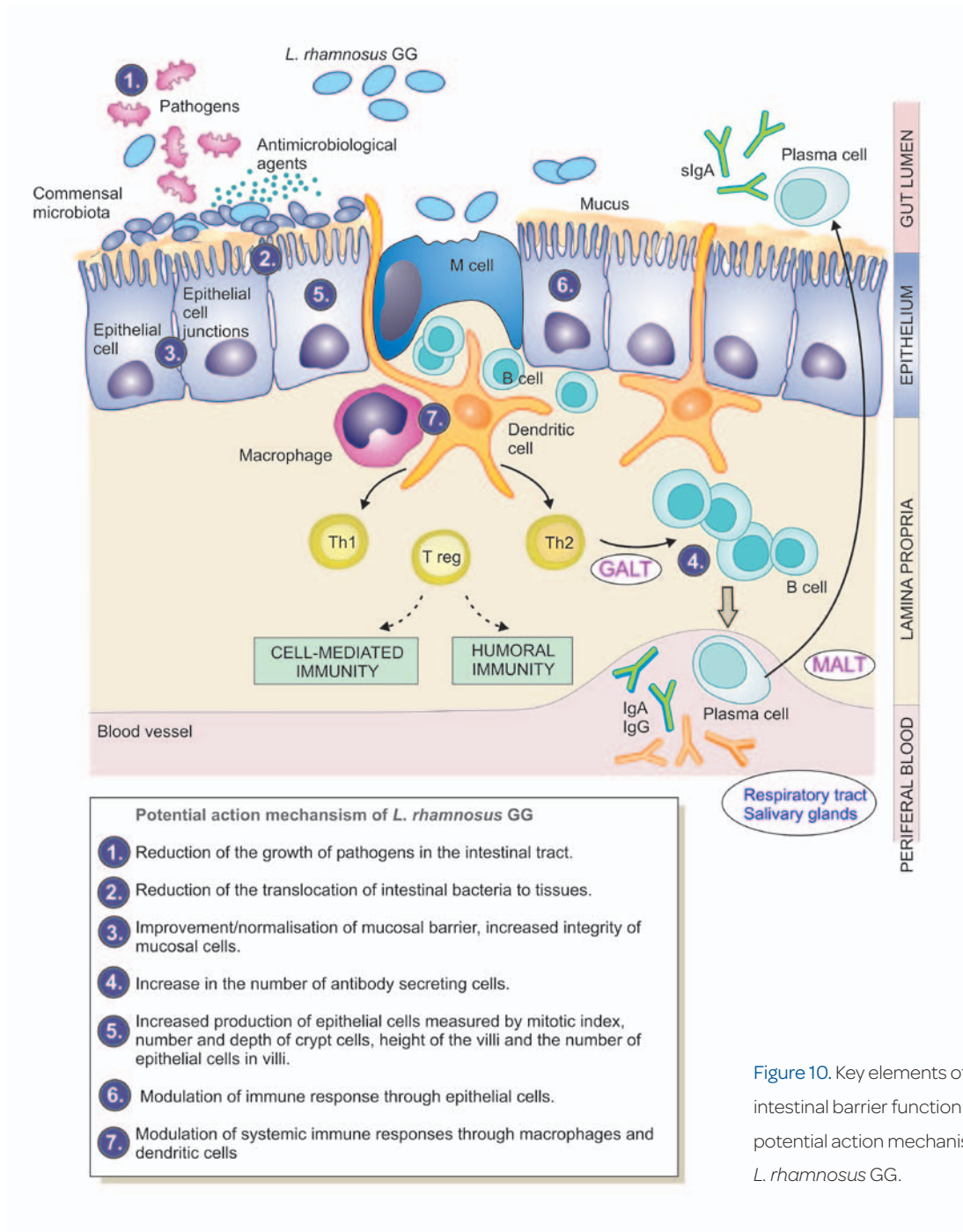




Figure 11. *L. rhamnosus* GG and pili structures. Represented with the kind permission of Antoni Hendrikxs, Matti Kankainen & Willem M. de Vos - Utrecht, Helsinki and Wageningen University.



Illustrator: Sole Latini

Figure 10. Key elements of the intestinal barrier function and potential action mechanism of *L. rhamnosus* GG.

et al., 2008; Miettinen et al., 2000; Veckman et al., 2003; Veckman et al., 2004) and thereby exerting systemic immunomodulatory effects.

The chromosomal DNA of *L. rhamnosus* GG was shown to contain a specific, strongly immunogenic structure that stimulated both the murine and human lymphocytes *in vitro* (Iliev et al., 2005), and in a mice model suppressed OVA-specific IgE production *in vivo* (Iliev et al., 2008). These results indicate that not only live *L. rhamnosus* GG but also its structural components may have immunomodulatory effects. To explore the biological function of *L. rhamnosus* GG, its genome

has now been sequenced as one of the first probiotics. Its 3.0 Mbp genome sequence has been compared to a similarly sized *L. rhamnosus* Lc705 genome (Kankainen et al., 2009). The study revealed a previously unreported observation of a pili structure, a proteinaceous surface-exposed polymeric protein structure, in probiotic lactobacilli (Figure 11). It was concluded that the presence of the pili structure may be essential for the adhesion to human intestinal mucus and it may also provide explanations for the immunity-stimulating effects for *L. rhamnosus* GG as pili from Gram-positive pathogens have brought about immunostimulatory effects.

In conclusion,

although experimental and *in vitro* studies cannot be directly extrapolated to humans, these studies indicate that *L. rhamnosus* GG may suppress the intestinal growth of a pathogen and reduce their translocation to blood circulation and organs, enhance both systemic and local immune responses, and stabilise mucosal barrier during an infection, thus reducing infections and improving the recovery.

3. Dose and matrix

When discussing the health benefits of probiotics, it is essential to take into consideration the dosage of the probiotic strain. Until recent years, it was generally considered that the product matrix in which the probiotic is consumed (e.g. milk-based product vs. capsule) is of high significance when considering the adequate daily dose of the probiotic. Therefore it was held that all final product forms need to be tested in clinical trials. Nowadays, however, repeating clinical studies with the same outcome parameters but with different product forms is not regarded as necessary if a sufficient daily dose for the health benefit in question is known and survival of the probiotic strain in different food matrixes is adequately documented. Recovery of the probiotic strain in faecal samples after consumption of the probiotic in various product matrixes can be considered adequate documentation of the survival and persistence of the probiotic in the gastrointestinal tract – and furthermore, proof of similar health benefits for the host.

An adequate dose of *L. rhamnosus* GG, independent on the product form, can be included in a balanced daily diet. *L. rhamnosus* GG can reach its target site in the gastrointestinal tract; it is able to survive through the gastrointestinal tract and adhere to the intestinal mucus and epithelial cells, as demonstrated in biopsy samples taken during and after consumption (Alander et al., 1999). It has been demonstrated that *L. rhamnosus* GG can be recovered in faecal samples when consumed

*Sufficient daily dose of *L. rhamnosus* GG to evoke health effects is between 10^8 and 10^{10} cfu/day, depending on the outcome parameter considered.*

in many types of products, e.g. in yoghurt, cheese or capsule form (Saxelin et al., 2010) as well as in enterocoated tablets or fermented milks (Saxelin et al., 1993).

In addition to the dosage of the probiotic strain, it is important to consider the purpose of use, as there are implications that the required daily dose is dependent on the health condition considered. The lowest daily dose of *L. rhamnosus* GG shown to have health-promoting impact is 1×10^8 cfu/day. The health-promoting impact of this dose was shown in a study which addressed respiratory infections (Hatakka et al., 2001) and dental caries (Näse et al., 2001) in children consuming *L. rhamnosus* GG -enriched milk as well as in a study which addressed persistent diarrhoea in children who consumed oral rehydration solution (ORS) containing *L. rhamnosus* GG powder (Basu et al., 2007a). Also, a relatively low dose of *L. rhamnosus* GG (6×10^9 cfu/day in powder form suspended in milk or formula) has demonstrated efficacy in mild diarrhoea not requiring hospital admission (Guarino et al., 1997). However, similar daily doses (1×10^8 cfu suspended to ORS or 1×10^9 cfu suspended to any liquid or semi-solid food, respectively) were shown to be in-

sufficient in the treatment of acute diarrhoea (Basu et al., 2007b; Misra et al., 2009). These findings suggest that the sufficient daily dose is dependent on the outcome parameter considered. It has also been demonstrated that a significantly higher dose of *L. rhamnosus*

GG (2×10^{12} cfu/day, dissolved in ORS), administered to children with acute watery diarrhoea, does not result in extra health benefits compared to the “regular” dose of 2×10^{10} cfu/day, as both doses were shown to be equally effective (Basu et al., 2009).

In conclusion,

when discussing the health benefits of *L. rhamnosus* GG, it is important to consider the daily dose of the probiotic and the outcome parameter considered. The lowest daily dose of *L. rhamnosus* GG shown to have health-promoting impacts in clinical studies is 1×10^8 cfu/day. However, more dose-response studies are needed to confirm the optimal daily dose in various health conditions affecting adults and children.

4. Safety aspects

The safety of *L. rhamnosus* GG has been more extensively studied than the safety of any other probiotic bacterium. *L. rhamnosus* GG (isolated from adult human) has a safe history of use in foods and it has been used since 1990. There are no risk groups for *L. rhamnosus* GG probiotic. The safety of *L. rhamnosus* GG has been repeatedly documented in experimental and clinical studies. *L. rhamnosus* GG has been administered in numerous clinical trials in healthy and sick people in various age groups (aged, adults, children, infants and premature infants) without any adverse effects (Dani et al., 2002; Salminen et al., 2004). Large epidemiological studies showed that rapidly increasing consumption of the strain did not increase the incidence of *Lactobacillus* or *L. rhamnosus* isolates in blood culture samples (Salminen et al., 2002) and no risk groups of immunocompromised patients could be identified (Salminen et al., 2004). A recent guideline document by European paediatric associations recommends the use of *L. rhamnosus* GG in diarrhoeal diseases in children (Guarino et al. 2008). A European medical expert group additionally concludes in its report that probiotics are safe, and that

The safety of L. rhamnosus GG has been more extensively studied than the safety of any other probiotic bacterium.

based on current knowledge, there is no need to restrict their use in any consumer groups (Floch et al. 2008).

Lactobacillus rhamnosus has achieved Qualified Presumption of Safety (QPS) –status from EFSA’s Scientific Committee and according to the U.S. Food and Drug administration (FDA) the use of *L. rhamnosus* GG in infant formulas contains no risk (<http://www.cfsan.fda.gov/~rdb/opagras1.html>). No health risks of *L. rhamnosus* GG are not known even if consumed to excess.

L. rhamnosus GG is sensitive to most antibiotics in clinical use. *L. rhamnosus* GG, as all *L. rhamnosus* strains are resistant to vancomycin, but the resistance genes are distinct from transferable genes (which are generally situated in a plasmid) and are situated in the chromosome. *L. rhamnosus* GG does not carry plasmids that could spread transferable genes (Tynkkynen et al., 1998).

To summarise,

the safety of *L. rhamnosus* GG has been more extensively studied than the safety of any other probiotic bacterium. *L. rhamnosus* GG has a safe history of use in foods and the safety of *L. rhamnosus* GG has been repeatedly documented in experimental and clinical studies.

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