

The role of intestinal microbiota and the immune system

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Abstract. – BACKGROUND: The human gut is an ecosystem consisting of a great number of commensal bacteria living in symbiosis with the host. Several data confirm that gut microbiota is engaged in a dynamic interaction with the intestinal innate and adaptive immune system, affecting different aspects of its development and function.

AIM: To review the immunological functions of gut microbiota and improve knowledge of its therapeutic implications for several intestinal and extra-intestinal diseases associated to dysregulation of the immune system.

METHODS: Significant articles were identified by literature search and selected based on content, including atopic diseases, inflammatory bowel diseases and treatment of these conditions with probiotics.

RESULTS: Accumulating evidence indicates that intestinal microflora has protective, metabolic, trophic and immunological functions and is able to establish a “cross-talk” with the immune component of mucosal immunity, comprising cellular and soluble elements. When one or more steps in this fine interaction fail, autoimmune or auto-inflammatory diseases may occur. Furthermore, it results from the data that probiotics, used for the treatment of the diseases caused by the dysregulation of the immune system, can have a beneficial effect by different mechanisms.

CONCLUSIONS: Gut microbiota interacts with both innate and adaptive immune system, playing a pivotal role in maintenance and disruption of gut immune quiescence. A cross talk between the mucosal immune system and endogenous microflora favours a mutual growth, survival and inflammatory control of the intestinal ecosystem. Based on these evidences, probiotics can be used as an ecological therapy in the treatment of immune diseases.

Key Words:

Gut microbiota, Intestinal immune system, Atopic diseases, Inflammatory bowel disease, Probiotics.

Introduction

The intestinal microbiota is composed of 10^{13-14} microorganisms (Figure 1), with at least 100 times as many genes as our genome, the microbiome. Its composition is individual-specific, ranges among individuals and also within the same individual during life. Many factors can influence the gut flora composition, as diet, age, medications, illness, stress and lifestyle. The gastrointestinal (GI) tract contains both “friendly” bugs, such as Gram-positive *Lactobacilli* and *Bifidobacteria* dominate (> 85% of total bacteria), and potential pathogenic bacteria, coexisting in a complex symbiosis. Several evidences show that intestinal microbiota supports energy metabolism and immune and trophic functions in the host. Furthermore, the concept of a “super-organism” has emerged to reflect the physiologic importance of mutually advantageous bidirectional host-microbe interactions in the gut. When this equilibrium is altered several gastrointestinal and extraintestinal diseases can occur.

Gut Microbiota and Intestinal Mucosa Development

Intestinal microflora has protective, metabolic, trophic and immunological functions. In fact, together with digestive enzymes, mucins, peristalsis, epithelial barrier with tight junctions, microbiota belongs to the so called non-immune component of mucosal immunity and is able to establish a “cross-talk” with the immune component of mucosal immunity, comprising cellular and soluble elements¹. Commensal microbiota can profoundly influence the development of the gut mucosal immune system and be crucial in preventing exogenous pathogen intrusion, both by direct interaction with pathogenic bacteria and by stimulation of the immune system. Comparative

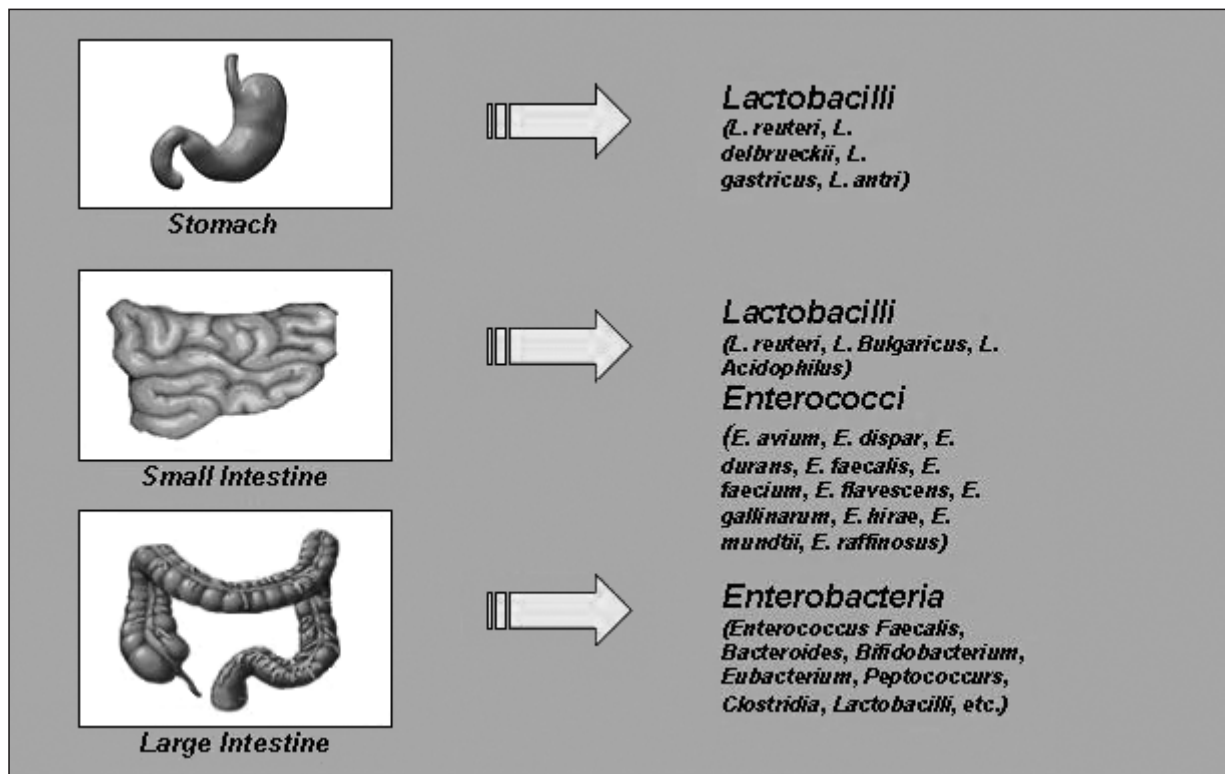


Figure 1. GUT microbiota composition and distribution.

studies in germ-free and conventional animals have established that the intestinal microflora is essential for the development and function of the mucosal immune system especially during early life, a process important to overall immunity in adults². Different studies over the time have demonstrated that the development of the ultra-structure of the intestinal mucosa is dependent on luminal bacteria. For example, villus capillaries in germ-free mice develop poorly during weaning and remain poorly developed until adulthood, indicating a microbial contribution in angiogenesis of villus-core³⁻⁵. Germ-free animals show defects in the development of gut-associated lymphoid tissue (GALT) and antibody production, fewer cellular lymphoid follicles (Peyer's patches), reduced cellular lamina propria, and fewer plasma cells in germinal center of the mesenteric lymph node (MLNs) compared with animals under conventional conditions⁶⁻⁸. The microbiota also contributes to the development of intra-epithelial lymphocytes (IEL).

Gut Microbiota and Innate Immune System

A cross talk between the mucosal innate immune system and endogenous microflora

favours a mutual growth, survival and inflammatory control of the intestinal ecosystem. A typical feature of innate immunity is the ability of distinguishing between potentially pathogenic microbial components and harmless antigens by "pattern recognition receptors" (PRRs) and among those toll-like receptors (TLRs) enable mammalian cells to recognize conserved characteristic molecules present on microorganisms and described as pathogen-associated molecular patterns (PAMPs)⁹⁻¹¹. Since these molecules e.g. lipopolysaccharides, peptidoglycans, flagellin, formylated peptides and others, are present also on commensal bacteria it seems more precise to call them microbe-associated molecular patterns (MAMPs). In mammals TLRs are present on macrophages, neutrophils, dendritic cells (DCs), intestinal epithelial cells (ECs) and other cells belonging to innate immune system. Over the time, researches have led different studies demonstrating that microbiota can regulate the intestinal innate immune system by modulating TLRs expression on immunosensory cells surface through MAMPs; recognition of microbes leads to activation of nuclear factor-kappa B (NF-κB) signaling pathway and consequently

triggers cytokine production, up-regulation of co-stimulatory molecules on antigen presenting cells, leading to activation of T cells. Thus, innate immunity is tightly linked to adaptive immunity⁹⁻¹¹.

In physiological conditions, complex and poorly understood cell interactions regulate in the gut responses to food antigens and to antigens of the normal microflora in close proximity to a large population of rapidly renewing ECs, specialized IELs and other immunologically active cells present in the mucosa. Cells of innate immunity are able to produce cytokines essential for inflammatory reactions as well as factors critical for the subsequent initiation of specific immunity. The contact with bacteria and their components through the PRRs on their surface initiates innate immunity responses¹¹. So far, the expression and modulation of TLRs on DCs (which represent the link between innate and adaptive immunity) and on ECs play an active role during interaction with external environment and in regulating the mucosal immune responses.

ECs are perhaps the most important part of the innate defense mechanism of mucosal surfaces. It has been recently shown that ECs are directly involved in various immune processes, in addition to their absorptive, digestive and secretory functions. For example, they show a peculiar capacity of transporting secretory immunoglobulins, produced by plasma cells in lamina propria, to the lumen, by binding these to a receptor of polymeric immunoglobulins (pIgR)¹². There is also strong evidence that ECs can present antigens¹³. ECs express numerous molecules involved in antigen presentation: transplantation antigens class I, both classic and non-classic (HLQ A-C, HLA E, CD1d, MIC-A/MICB), transplantation antigens class II. ECs can allow the interaction with other cells of the immune system and participate to the inflammatory response to microbial invasion. Human intestinal ECs express an important lipopolysaccharide-binding molecule CD14 that, together with TLRs, can maintain the intricate balance between the self and the environment in the gut¹⁴. Furthermore, these cells release soluble form of CD14 which may be implicated in shaping the interaction between the mucosal immune system and gut bacteria.

Another mechanism for microbiota's immunomodulation is the capacity of regulating the production of the mucins from intestinal goblet cells. Mucins may directly limit intestinal infec-

tions by adhering to pathogens^{15,16} and protect against acid gastric and duodenal secretions. The mucus also provide a medium in which bacterial-derived metabolites with signaling functions are secreted and concentrated. Thus, the mucus layer may promote mutualism by keeping bacteria at bay and restricting overt immune stimulation while facilitating host-commensal or commensal-commensal cross-talk through the diffusion of bacterial products.

Gut Microbiota and Adaptive Immune System

An important immune compartment in the bowel is the lamina propria, where is present a large number of macrophages, DCs, T cells, and IgA-secreting B cells. Acquired immune response is primarily imprinted in GALT (Peyer's patches (PPs) and MLNs)¹⁷⁻¹⁹. Indeed, regulatory cells are also present in the lamina propria where they maintain tolerance to foods and self-antigens. In the lamina propria are also present resident CD4, CD8 and B cells, while some CD8 lymphocytes migrate to tip of the villous, where they became IELs^{20,21}. The B cells in the lamina propria are activated and become IgA-producing plasma cells. The IgA is transported across the epithelial layer and secreted in the gut lumen. Antigens sampled in the lamina propria are taken up by DCs and transported via draining lymphatic vessels to the MLNs and secondarily to the gut lymphoid tissue (PPs), to response to gut antigens. The important feature is the presence of specialized ECs, called microfold, or M cells. The M-cells sample the antigens in the gut lumen and transport bacteria to professional antigen-presenting-cells, such as DCs on their basolateral surface²².

The larger part of the bacteria are killed by macrophages, while those transferred by M-cells to DCs can survive for several days. In healthy individuals DCs sample the antigens and induce T-cells unresponsiveness, probably by stimulating balanced differentiation of naïve T cells into either effectors cells (Th1, Th2, Th17) or regulatory T cells (Tr1, Th3), to maintain tolerance toward commensal and food antigens. Specialized DCs in the lamina propria can sample luminal antigens directly through extended cellular processes that penetrate in the lumen. DCs containing antigens and expressing CD103 migrate to lymphoid tissue, where interact with naïve lymphocytes to generate primed effectors lymphocytes. DCs containing alive bacteria migrate

to the MLNs and induce IgA-producing plasma cells. After the antigens is cleared, most effector cells have died leaving a cohort of long-lived memory cells that can rapidly increase immunity on reencounter with their cognate antigen. Once an antigen has been detected by DCs, it is processed in its endoplasmic reticulum to form small peptides that can be presented on MHC class II molecules to lymphocytes in GALT. Most of DCs reside in the lamina propria and migrate via afferent lymphatic to GALT. Entry of both DCs and lymphocytes into GALT is dependent on specific adhesion molecules (L-selectin, CCR7 and L-selectin ligand, CCL21 interaction). DCs direct the lymphocytes location where the antigen is most likely encountered by imprinting tissue specificity. DCs also determine the nature of immune response: early production of IL-2 is important for the development of an IFN- δ -secreting Th1 response; Th17 response is driven by IL-1, IL-6 and IL-23. Interaction with immature DCs or the presence of IL-10 and TGF-beta can lead to induction of regulatory T cells with an anti-inflammatory response. Lymphocytes priming in the lymphoid tissue change adhesion molecules expressions and lose the ability to enter lymphoid tissue by down regulation of CCR7 and L-selectin expression, while gaining expression of new adhesion molecules that direct migration to peripheral tissue. This essential feature is called "homing".

When one or more steps in this complex system fail, autoimmune or auto-inflammatory diseases may occur.

Gut Microbiota and Atopic Diseases

The prevalence of allergic diseases (such as eczema, food allergy, hay fever and asthma) has been increasing worldwide during the past 40 years, especially in the western countries and among children²³. Although genetic susceptibility plays a pivotal role in atopy changes, the prevalence of these diseases has been much faster than any possible shift in genetic constitution. Therefore, accumulating evidence indicates that environmental factors in the gut, such as commensal bacteria, may play an important role in the maintenance and disruption of gut immune quiescence²⁴. The development of the intestinal microflora starts immediately after birth and depends on bacteria from the mother and the surrounding environment; in particular, type of delivery (vaginal or caesarian), breast-feeding, diet and country of birth influence the infant gut mi-

crobiota²⁵⁻²⁹ and this explains the remarkable inter-individual variability observed in the infant gut bacteria during the first months of life³⁰. Many studies suggest that the intestinal microflora induces maturation of host immunity and a "distortion" in any of its functions could potentially contribute to the development of allergic diseases. This distortion comes from environmental changes associated with western lifestyles, including dietary changes, antibiotic use and other medications, such as antacids, proton pump inhibitors, and non-steroidal anti-inflammatory drugs. In 1989, Strachan introduced the "*hygiene hypothesis*", which states that a lack of microbial exposure during childhood results in a perturbation in gut microbiota composition and in aberrant immune responses to innocuous antigens later in the life^{31,32} with development of atopic diseases, defined as chronic inflammatory disorders caused by aberrant T-helper 2 (Th2)-type immune responses against common innocuous environmental antigens (allergens) in susceptible individual³³ (Figure 2). The initial immunological explanation for this hypothesis was a lack of microbial antigen-induced immune deviation from the Th2 cytokine profile to a Th1-type profile, resulting in the development of enhanced Th2-cell responses to allergens³⁴⁻³⁶. However, this explanation does not take into account that the prevalence of Th1-associated diseases, such as Crohn's disease, type 1 diabetes and multiple sclerosis, was also increasing and that chronic parasitic worm (helminth) infections inducing strong Th2 responses and high IgE levels are not associated with an increased risk of allergy³⁷. An alternative interpretation conceives anti-inflammatory immune responses to be of fundamental importance in the development of mucosal and systemic tolerance³⁸. These immunosuppressive mechanisms are managed by regulatory T-cell classes (Treg cells) controlling through interleukin (IL)-10 and/or tumor growth factor (TGF)- β both Th1 and Th2 responses and hence the development of both atopic and autoimmune diseases^{38,39}. Indeed, the importance of a delicate balance between allergen-specific Treg cells and allergen-specific Th2 cells in healthy and allergic immune responses to common environmental allergens was demonstrated in a study conducted by Akdis et al⁴⁰. Moreover, duodenal biopsies of healthy infants and infants with multiple food allergy showed that the dominant mucosal abnormality was not Th2 deviation but impaired generation of TGF- β -producing Treg cells⁴¹. The epi-

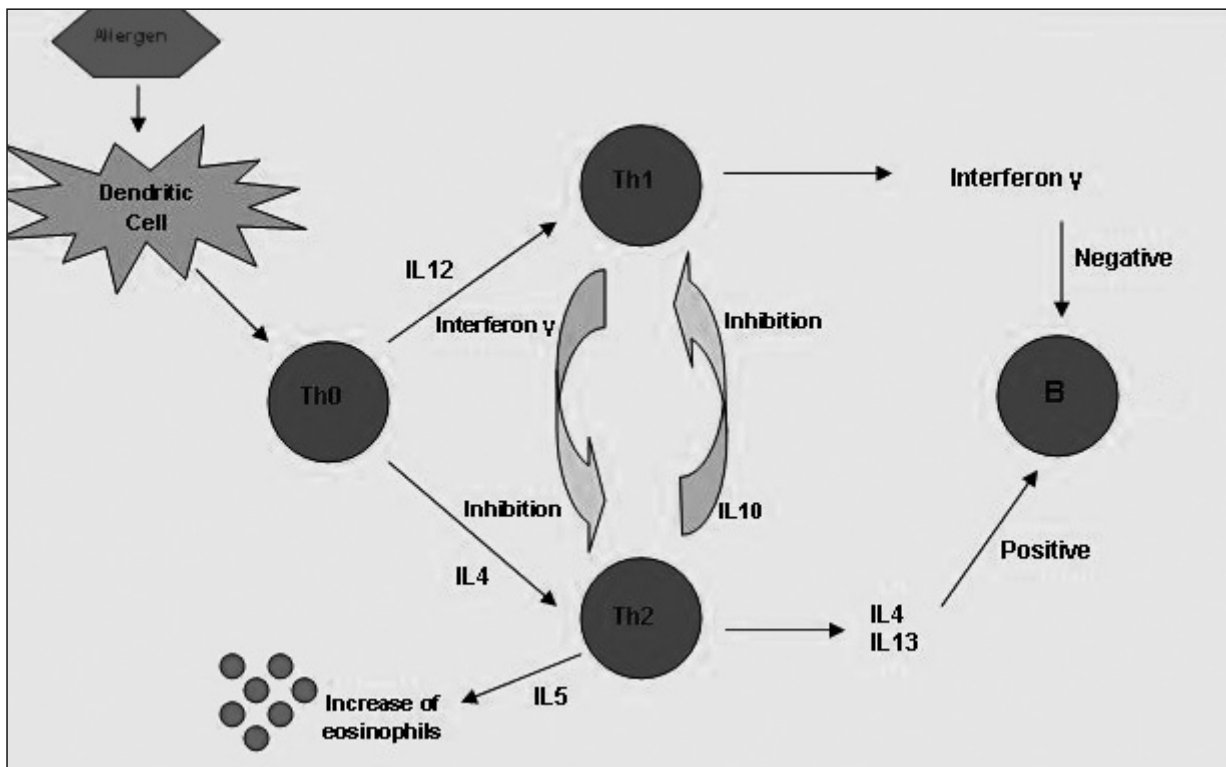


Figure 2. Interactions between microbiota and immune cells in allergic diseases development.

demographical findings and the experimental evidence available so far suggest that both the reduced immune suppression by T-reg cells and the lack of immune deviation from a Th2 to a Th1 profile are involved⁴². Moreover, the impact of the gut microbiota on the development of IgA antibody responses, which contribute to pathogen and allergen exclusion in the gut lumen, may also be involved⁴³. In fact, the effects of the gut microbiota on the immune system may not only be related to food antigens but also to aero-allergens and to the manifestation of allergic airway symptoms. Noverr et al⁴⁴ developed a mouse model to demonstrate experimentally that antibiotic therapy leading to bacterial and fungal microbiota changes could predispose a host to allergic airway diseases. In addition, oral treatment with *Lactobacillus reuterii* has recently been shown to inhibit the allergic airway response in mice⁴⁵. These results support the possibility that afferent events in allergic sensitization may occur outside of lungs and involve host-microbiota communication. The gut can affect the systemic immune system and local inflammation in remote tissues, such as the respiratory tract, remains to be determined. However, it has been shown that inhaled

particles, fluids and microbes are also swallowed. Thus, the GI tract is exposed to any antigens as the respiratory tract. Since ingestion of antigens can induce tolerance to that antigen (oral tolerance), the GI tract may act as a “sensor” for the development of tolerance to inhaled antigens^{46,47}. In addition, induced regulatory T cells may move to other tissues throughout the body as the respiratory tract⁴⁸.

Gut Microbiota and Inflammatory Bowel Diseases

The term inflammatory bowel disease (IBD) comprises two types of chronic intestinal disorders: Crohn’s disease (CD) and ulcerative colitis (UC). CD is characterized by deep inflammation with granulomas, and may affect any part of the GI tract, although it most commonly affects the distal ileum and caecum. UC is largely limited to the colon, particularly the distal colon and the rectum⁴⁹, and causes more superficial ulceration. The initial trigger responsible for the onset of IBD is not known yet but it seems that it involves a complex interplay between the immune system, environmental factors, such as stress, di-

et and enteric infections caused by some pathogens (i.e. *Mycobacterium avium* subspecies *paratuberculosis*, *Clostridium difficile*, enterotoxigenic *Bacteroides fragilis*, adherent/invasive *E. coli*, *Campylobacter* spp, *Salmonella* spp.), and host genotype. Accumulating evidence suggests that IBD results from an inappropriate inflammatory response to intestinal microbes in a genetically susceptible host. The role of the microbiota in IBD has been inferred by the following experiments: (1) suppressing micro-organisms (using antibiotics or gnotoxenic animals); (2) adding micro-organisms or microbial components (e.g. probiotics, CpG-DNA, culture supernatants); (3) altering the composition of the microbiota using prebiotic substrates; (4) studies in knockout animals lacking receptors to specific microbial signals⁵⁰. These experiments have shown microbial and host specificity⁵⁰. Studies in knockout animals have revealed cross talk between the intestinal microbes and the host. Microbial molecules such as lipopolysaccharide, peptidoglycans, flagellins and bacterial DNA, are recognised by specific receptors on intestinal and/or immune cells. These receptors comprise the TLRs and nucleotide oligomerisation binding domains (NOD)-like receptors. Noticeably, polymorphisms of some of these receptors [NOD2, TLR4 and others, such as caspase recruitment domain family, member 15 (CARD15)] are associated with an increased risk of IBD in animal models and in humans⁵¹. On the other hand, the normal intestine secretes various peptides with anti-microbial properties, including defensins, lysozyme, cathelicidins and secretory immunoglobulins. Defensins are synthesised in Paneth cells and released in the intestinal crypts and (other ones) at the epithelial surface and trapped in the mucus layer. Noticeably again, several studies have shown a reduced expression of α -defensins in ileal CD and an attenuated induction of β -defensins in the colon of patients with colonic IBD. Moreover, reports on faecal samples using modern molecular techniques have shown that, as compared with control subjects, patients with CD and those UC have depletion of and reduced diversity in both faeces and mucosa-associated phyla *Firmicutes* and *Bacteroidetes*⁵²⁻⁵⁵, which promote GI health in multiple ways⁵³. Researchers have led studies which shown that *in vitro* peripheral blood mononuclear cell stimulation by *F. prausnitzii* (a major member of the family *Firmicutes*) decreased IL-12 and IFN- γ production and stimu-

lated secretion of IL-10. Oral administration of either live *F. prausnitzii* or its supernatant reduced the severity of TNBS (2,4,6-trinitrobenzenesulfonic acid) colitis and corrected the associated dysbiosis. In parallel, the abundance of *E. coli* is increased in IBD. *E. coli* isolated from CD patients express uropathic-like virulence factors that are postulated to facilitate mucosal invasion⁵⁶. Compositional changes of the microbiota (dysbiosis) in IBD subsets may contribute to disease severity, since abnormal microbiotas correlated with the occurrence of abscesses in CD patients, and IBD patients with dysbiosis underwent surgery at a younger age than those with normal microbiotas⁵⁴.

Treatment of Atopic Diseases: Probiotics

Probiotics such as *Lactobacilli* spp., certain types of *Streptococcus*, and *Bifidobacteria* spp are alive microorganisms with a vast array of therapeutic potential. Probiotics have a beneficial effect on intestinal mucosa via several proposed mechanisms that include suppression of the growth and binding of pathogenic bacteria, improvement of the barrier function of the epithelium, and alteration of the immune activity of the host^{57,58}. Probiotics secrete short chain fatty acids with decrease of luminal pH and production of bactericidal proteins⁵⁸. Butyric acid, a byproduct of bacterial fermentation of fiber, has been shown to nourish colonic enterocytes enhancing mucosal integrity^{59,60}. The DNA of probiotic organisms may also inhibit apoptosis of ECs^{61,62}. In addition, probiotics may improve bowel motility⁵⁹.

The “*hygiene hypothesis*” provides a rationale for using probiotics in order to modify gut microbiota and thereby shaping the immune response of the host especially during infancy. Several studies demonstrated that the composition of the gut microbiota differs between healthy and allergic infants, as in countries with a high and low prevalence of allergies⁶³⁻⁶⁷. In particular, main microflora composition changes associated with allergic trait are represented by less frequent colonization with *Lactobacilli* and lower counts of *Bifidobacteria*⁶³⁻⁶⁶. According to these evidences, probiotics are potentially able to modulate and reconstitute the microbiota of allergic patients. Although this area of research is relatively new (the first probiotic intervention trial dates back to 1997), several potential targets for the probiotics can be identified, such as degradation of enteral antigens, normalization of the properties of aber-

rant indigenous microbiota and of gut barrier functions, regulation of the secretion of inflammatory mediators, and promotion of the development of the immune system. The probiotics' effects are strain-specific: different bacteria or components have defined adherence sites, immunologic effects, and varied effects in the healthy versus inflamed mucosal milieu.

Over the time, different studies have been led with the aim of demonstrating probiotics' beneficial effect in several allergic conditions. In the Majamaa study⁶⁸, the clinical and immunologic effects of cow's milk elimination without and with the addition of the *Lactobacillus GG* (LGG) in an extensively hydrolyzed whey formula in children with atopic eczema and cow's milk allergy were evaluated. The second part of the study involved 10 breast-fed infants who had atopic eczema and cow's milk allergy; in this group, LGG was given to nursing mothers. These studies showed a significant change in the SCORAD (a scoring system which combines an assessment of disease extent using the rule of nines with six clinical features of disease intensity: erythema/darkening, oedema/papulation, oozing/crust, excoriation, lichenification/prurigo and dryness) after intervention in infants receiving LGG and no significant change in the other group. In the breast-fed infants the SCORAD score improved statistically after 1 month of treatment. To test the effect of probiotics in the primary prevention of atopic disease, Kalliomaki et al⁶⁹ assigned 159 mothers to receive placebo or LGG daily for 4 weeks before expected delivery. After delivery, LGG or placebo were taken postnatally for 6 months. In children aged 2 years, the frequency of atopic eczema in the probiotic group was half that of the placebo group. The 7-year follow-up visit confirmed that the cumulative risk of developing eczema during the first 7 years of life was significantly lower in the LGG group than in the placebo group. However, atopic sensitization was similar between groups, suggesting that the preventive effect on eczema was not IgE-mediated⁷⁰.

The clinical studies do not completely agree about the efficacy of probiotics in allergic diseases. In the Abrahamsson et al study⁷¹, the cumulative prevalence of eczema was similar in babies receiving *Lactobacillus reuteri* before delivery and up to 12 months old and in the control group. The *L. reuteri* group had a lower impact of IgE-associated eczema during the second year and, therefore, possibly a reduced risk of devel-

oping allergic diseases later. No differences in the frequency of allergic diseases and sensitization were shown between the probiotic mixture (LGG, *Lactobacillus rhamnosus*, *Bifidobacterium breve*, and *propionibacteria*) and placebo groups after 5 years of follow-up in Kuitunen study⁷². In this latter, 1223 mothers with infants at high risk for allergy were randomized to receive the probiotic mixture or placebo during the last month of pregnancy and their infants to additionally receive prebiotic galactoligosaccharide, from birth until the age of 6 months. However, less IgE-associated allergic diseases occurred in cesarean-delivered children receiving a probiotics mixture. The study by Taylor et al⁷³ showed an opposite effect. In 178 newborns of allergic women, *Lactobacillus acidophilus* supplementation for the first 6 months of life failed to reduce the risk of atopic dermatitis and increased the risk of allergen sensitisation.

Thus, these results suggest that further studies are needed to better understand the mechanisms of allergic disorders and the role of probiotics in allergies.

Treatment of IBD: Probiotics

Probiotics can be used as "ecological treatments" even in IBD. The rationale of using them in IBD has been validated by different studies, which focused on their mechanism of action. In particular, it has been shown that:

1. certain probiotic strains can induce the secretion of antimicrobial peptides by intestinal cells. Antimicrobial peptides can be secreted either by bacteria (bacteriocins), or specialized epithelial Paneth cells (defensins) and can regulate the bacterial load to the mucosa⁷⁴. It has been shown that CD is associated with a defect in defensins⁷⁵, and probiotics, which could enhance these peptides, thus appear as attractive candidates;
2. some probiotics can reinforce the integrity of the intestinal barrier. It is well-known that an increase in intestinal permeability is suspected as a major factor in the pathogenesis of IBD⁵⁹. Thus, probiotics seem to be able to normalize intestinal permeability and some *Lactobacilli* can inhibit pathogen adhesion inducing expression of mucins;
3. some probiotics seem to have an immunomodulatory action, since they can stimulate innate immunity and educate adaptive immunity towards Th1, Th2 or Th3 (tolerance) responses.

Probiotics have been assessed in animal models and some clinical trials. Oral administration of the VSL#3 consortium of probiotics⁷⁶ was shown to normalize barrier function in IL-10 $-/-$ mouse model of IBD⁷⁷. VSL#3 is a probiotic cocktail consisting of eight different gram-positive organisms: *Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium breve*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus delbrueckii* spp. *bulgaricus*, *Lactobacillus plantarum*, and *Streptococcus salivarius* spp. *Thermophilus*⁷⁷. The characteristics of probiotic bacteria suggest that some bacterial species may be effective in the treatment of IBD by strengthening the epithelial barrier. For example, VSL#3 treatment of IL-10 $-/-$ mice with IBD resulted in a normalization of colonic physiological function and barrier integrity along with a reduction in mucosal levels of pro-inflammatory cytokines and a significant improvement in histological disease by secretion of soluble factors enhancing the barrier integrity⁷⁷. Other studies have confirmed that probiotic bacteria may enhance the integrity of the tight junctions between the intestinal epithelial cells during infections or inflammatory conditions⁷⁸. Thus, colonization with probiotic bacteria may lead to decreased exposure of immune cells to the bacterial antigens that are believed to drive IBD. Rachmiliwitz et al⁷⁹ showed that protective effects of probiotic microorganisms (VSL#3) in a dextran sulfate sodium model of experimental colitis are mediated by DNA that was recognized by the mucosal TLR9 receptor. This interaction subsequently led to an increased endogenous production of β -defensins, antibacterial peptides that regulate bacterial survival. Additionally, it was reported that the treatment of cultured intestinal epithelial cells with VSL#3 led to an increase of the transepithelial electrical resistance, a change that correlates with decreased permeability⁸⁰. In that study, incubation of intestinal epithelial cells with this probiotic consortium also induced the expression of several mucins, leading to decreased adhesion of microorganisms and their components to the epithelial surface.

All the above mentioned evidences suggest that probiotics may play an important role in the management of IBD in the future, although current clinical trials lack statistical power, probably due to their limited number. In addition, treatments need to be individualized based on compositional alterations in patient subsets. A better understanding of bacterial and host mutualistic in-

teractions and the availability of novel techniques that are able to finely identify microbiota modifications in different clinical subsets seem to be the key to the success of effective probiotics treatment in IBD patients.

References

- 1) LIU Z, LI N, NEU J. Tight junctions, leaky intestines, and pediatric diseases. *Acta Paediatr* 2005; 94: 386-393.
- 2) SRIKANTH CV, MC CORMICK BA. Interactions of the intestinal epithelial with the pathogen and the indigenous microbiota: a three-way crosstalk. *Interdiscip Perspect Infect Dis* 2008; 2008: 626827. Epub 2008 Oct 29.
- 3) NEU J. Perinatal and neonatal manipulation of the intestinal microbiome: a note of caution. *Nutr Rev* 2007; 65(6 Pt 1): 282-285.
- 4) STAPPENBECK TS, HOOPER LV, GORDON JI. Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. *Proc Natl Acad Sci USA* 2002; 99: 15451-15455.
- 5) CONROY ME, WALKER WA. Intestinal immune health. *Nestle Nutr Workshop Ser Paediatr Program* 2008; 62: 111-121; discussion 121-5.
- 6) RAKOFF-NAHOUM S, PAGLINO J, ESLAMI-VARZANEH F, EDBERG S, MEDZHITOV R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* 2004; 118: 229-241.
- 7) MACPHERSON AJ, HARRIS NL. Interactions between commensal intestinal bacteria and the immune system. *Nat Rev Immunol* 2004; 4: 478-485.
- 8) FALK PG, HOOPER LV, MIDTVEDT T, GORDON JI. Creating and maintaining the gastrointestinal ecosystem: what we know and need to know from gnotobiology. *Microbiol Mol Biol Rev* 1998; 62: 1157-1170.
- 9) ULEVITCH RJ. Endotoxin opens the Tollgates to innate immunity. *Nat Med* 1999; 5: 144-145.
- 10) MEDZHITOV R, JANEWAY C JR. Innate immune recognition: mechanisms and pathways. *Immunol Rev* 2000; 173: 89-97.
- 11) AKIRA S, HEMMI H. Recognition of pathogen-associated molecular patterns by TLR family. *Immunol Lett* 2003; 85: 85-95.
- 12) STROBER W, FUSS IJ, BLUMBERG RS. The immunology of mucosal models of inflammation. *Annu Rev Immunol* 2002; 20: 495-549.
- 13) BLAND PW. Mucosal T cell-epithelial cell interactions. *Chem Immunol* 1998; 71: 40-63.
- 14) FUNDA DP, TUĚKOVÁ L, FARRE MA, IWASE T, MORO I, TLASKALOVÁ-HOGENOVÁ H. CD14 is expressed and released as soluble CD14 by human intestinal epithelial cells in vitro: lipopolysaccharide activation of epithelial cells revisited. *Infect Immun* 2001; 69: 3772-3781.

- 15) LINDEN SK, FLORIN TH, MCGUCKIN MA. Mucin dynamics in intestinal bacterial infection. *PLoS One* 2008; 3: e3952.
- 16) LINDEN SK, SHENG YH, EVERY AL, MILES KM, SKOOG EC, FLORIN TH, SUTTON P, MCGUCKIN MA. MUC1 limits *Helicobacter pylori* infection both by sterich in-drance and by acting as a releasable decoy. *PLoS Pathog* 2009; 5: e1000617.
- 17) CAMPBELL DJ, BUTCHER EC. Intestinal attraction: CCL25 functions in effector lymphocyte recruitment to the small intestine. *J Clin Invest* 2002; 110: 1079-1081.
- 18) GARSIDE P, MILLINGTON O, SMITH KM. The anatomy of mucosal immune responses. *Ann N Y Acad Sci* 2004; 1029: 9-15.
- 19) KUNKEL EJ, CAMPBELL DJ, BUTCHER EC. Chemokines in lymphocyte trafficking and intestinal immunity. *Microcirculation* 2003; 10: 313-323.
- 20) MACDONALD TT, MONTELEONE G. Immunity, inflammation, and allergy in the gut. *Science* 2005; 307(5717): 1920-1925.
- 21) SUZUKI R, NAKAO A, KANAMARU Y, OKUMURA K, OGAWA H, RA C. Localization of intestinal intraepithelial T lymphocytes involves regulation of alphaEbeta7 expression by transforming growth factor-beta. *Int Immunol* 2002; 14: 339-345.
- 22) NEUTRA MR, MANTIS NJ, KRAEHNBUHL JP. Collaboration of epithelial cells with organized mucosal lymphoid tissues. *Nat Immunol* 2001; 2: 1004-1009.
- 23) BEASLEY R, KEIL U, VON MUTIUS E, ET AL. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. *Lancet* 1998; 351(9111): 1225-1232.
- 24) ROMAGNANI S. Coming back to a missing immune deviation as the main explanatory mechanism for the hygiene hypothesis. *J Allergy Immunol* 2007; 119: 1511-1513.
- 25) SEPP E, JULGE K, VASAR M, NAABER P, BJÖRKSTEN B, MIKELSAAR M. Intestinal microflora of Estonian and Swedish infants. *Acta Paediatr* 1997; 86: 956-961.
- 26) BENNET R, ERIKSSON M, TAFARI N, NORD CE. Intestinal bacteria of newborn Ethiopian infants in relation to antibiotic treatment and colonisation by potentially pathogenic gram-negative bacteria. *Scand J Infect Dis* 1991; 23: 63-69.
- 27) ADLERBERTH I, CARLSSON B, DE MAN P, JALIL F, KHAN SR, LARSSON P, MELLANDER L, SVANBORG C, WOLD AE, HANSSON LA. Intestinal colonization with Enterobacteriaceae in Pakistani and Swedish hospital-delivered infants. *Acta Paediatr Scand* 1991; 80: 602-610.
- 28) ADLERBERTH I, STRACHAN DP, MATRICARDI PM, AHRNÉ S, ORFEI L, ABERG N, PERKIN MR, TRIPODI S, HESSELMAR B, SAALMAN R, COATES AR, BONANNO CL, PANETTA V, WOLD AE. Gut microbiota and development of atopic eczema in 3 European birth cohorts. *J Allergy Clin Immunol* 2007; 120: 343-350.
- 29) PENDERS J, THIJIS C, VINK C, STELMA FF, SNIJDERS B, KUMMELING I, VAN DEN BRANDT PA, STOBBERINGH EE. Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics* 2006; 118: 511-521.
- 30) PALMER C, BIK EM, DIGIULIO DB, RELMAN DA, BROWN PO. Development of the human infant intestinal microbiota. *PLoS Biol* 2007; 5: e177.
- 31) STRACHAN DP. Hay fever, hygiene, and household size. *Br Med J* 1989; 299(6710): 1259-1260.
- 32) STRACHAN DP. Family size, infection and atopy: the first decade of the "hygiene hypothesis". *Thorax* 2000; 55(Suppl 1): S2-S10.
- 33) ROMAGNANI S. Regulatory T cells: which role in the pathogenesis and treatment of allergic disorders? *Allergy*. 2006; 61: 3-14.
- 34) ROMAGNANI S. Immunologic influences on allergy and the TH1/TH2 balance. *J Allergy Clin Immunol* 2004; 113: 395-400.
- 35) MATRICARDI PM, BONINI S. High microbial turnover rate preventing atopy: a solution to inconsistencies impinging on the hygiene hypothesis? *Clin Exp Allergy* 2000; 30: 1506-1510.
- 36) BAKER BS. The role of microorganisms in atopic dermatitis. *Clin Exp Immunol* 2006; 144: 1-9.
- 37) YAZDANBAKHSH M, KREMSNER PG, VAN REE R. Allergy, parasites, and the hygiene hypothesis. *Science* 2002; 296(5567): 490-494.
- 38) RAUTAVA S, KALLIOMAKI M, ISOLAURI E. New therapeutic strategy for combating the increasing burden of allergic disease: probiotics – A Nutrition, Allergy, Mucosal Immunology and Intestinal Microbiota (NAMI) Research Group report. *J Allergy Clin Immunol* 2005; 116: 31-37.
- 39) ROOK GA, BRUNET LR. Old friends for breakfast. *Clin Exp Allergy* 2005; 35: 841-842.
- 40) AKDIS M, VERHAGEN J, TAYLOR A, KARAMLOO F, KARAGIANNIDIS C, CRAMERI R, THUNBERG S, DENIZ G, VALENTA R, FIEBIG H, KEGEL C, DISCH R, SCHMIDT-WEBER CB, BLASER K, AKDIS CA. Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med* 2004; 199: 1567-1575.
- 41) PEREZ-MACHADO MA, ASHWOOD P, THOMSON MA, LATCHAM F, SIM R, WALKER-SMITH JA, MURCH SH. Reduced transforming growth factor-beta1-producing T cells in the duodenal mucosa of children with food allergy. *Eur J Immunol* 2003; 33: 2307-2315.
- 42) ROMAGNANI S. The increased prevalence of allergy and the hygiene hypothesis: missing immune deviation, reduced immune suppression, or both? *Immunology* 2004; 112: 352-363.
- 43) RAUTAVA S, RUUSKANEN O, OUWEHAND A, SALMINEN S, ISOLAURI E. The hygiene hypothesis of atopic disease—an extended version. *J Pediatric Gastroenterol Nutr* 2004; 38: 378-388.
- 44) NOVERR MC, NOGGLE RM, TOEWS GB, HUFFNAGLE GB. Role of antibiotics and fungal microbiota in driving pulmonary allergic responses. *Infect Immun* 2004; 72: 4996-5003.

- 45) FORSYTHE P, INMAN MD, BIENENSTOCK J. Oral treatment with live *Lactobacillus reuteri* inhibits the allergic airway response in mice. *Am J Respir Crit Care Med* 2007; 175: 561-569.
- 46) NOVERR MC, HUFFNAGLE GB. The microflora hypothesis of allergic diseases. *Clin Exp Allergy* 2005; 35: 1511-1520.
- 47) NOVERR MC, FALKOWSKI NR, McDONALD RA, MCKENZIE AN, HUFFNAGLE GB. Development of allergic airway disease in mice following antibiotic therapy and fungal microbiota increase: role of host genetics, antigen, and interleukin-13. *Infect Immun* 2005; 73: 30-38.
- 48) PRESCOTT SL, DUNSTAN JA, HALE J, BRECKLER L, LEHMANN H, WESTON S, RICHMOND P. Clinical effects of probiotics are associated with increased interferon-gamma responses in very young children with atopic dermatitis. *Clin Exp Allergy* 2005; 35: 1557-1564.
- 49) PODOLSKY DK. Inflammatory bowel disease. *N Engl J Med* 2002; 347: 417-429.
- 50) PACKEY CD, SARTOR RB. Interplay of commensal and pathogenic bacteria, genetic mutations, and immunoregulatory defects in the pathogenesis of inflammatory bowel diseases. *J Intern Med* 2008; 263: 597-606.
- 51) NOOMEN CG, HOMMES DW, FIDDER HH. Update on genetics in inflammatory disease. *Best Pract Res Clin Gastroenterol* 2009; 23: 233-243.
- 52) TURNER JR. Molecular basis of epithelial barrier regulation: from basic mechanisms to clinical application. *Am J Pathol* 2006; 169: 1901-1909.
- 53) BACKHED F, LEY RE, SONNENBURG JL, PETERSON DA, GORDON JI. Host-bacterial mutualism in the human intestine. *Science* 2005; 307(5717): 1915-1920.
- 54) FRANK DN, St AMAND AL, FELDMAN RA, BOEDEKER EC, HARPAZ N, PACE NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci U S A* 2007; 104: 13780-13785.
- 55) SWIDSINSKI A, LOENING-BAUCKE V, VANECHOUTTE M, DOERFFEL Y. Active Crohn's disease and ulcerative colitis can be specifically diagnosed and monitored based on the biostructure of the fecal flora. *Inflamm Bowel Dis* 2008; 14: 147-161.
- 56) BAUMGART M, DOGAN B, RISHNIW M, WEITZMAN G, BOSWORTH B, YANTISS R, ORSI RH, WIEDMANN M, McDONOUGH P, KIM SG, BERG D, SCHUKKEN Y, SCHERL E, SIMPSON KW. Culture independent analysis of ileal mucosa reveals a selective increase in invasive *Escherichia coli* of novel phylogeny relative to depletion of Clostridiales in Crohn's disease involving the ileum. *ISME J* 2007; 1: 403-418.
- 57) COLLINS SM. A case for an immunological basis for irritable bowel syndrome. *Gastroenterology* 2002; 122: 2078-2080.
- 58) O'MAHONY L, MCCARTHY J, KELLY P, HURLEY G, LUO F, CHEN K, O'SULLIVAN GC, KIELY B, COLLINS JK, SHANAHAN F, QUIGLEY EM. *Lactobacillus* and *Bifidobacterium* in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. *Gastroenterology* 2005; 128: 541-551.
- 59) GUYONNET D, CHASSANY O, DUCROTTE P, PICARD C, MOURET M, MERCIER CH, MATUCHANSKY C. Effect of a fermented milk containing *Bifidobacterium animalis* DN-173 010 on the health-related quality of life and symptoms in irritable bowel syndrome in adults in primary care: a multicentre, randomized, double-blind, controlled trial. *Aliment Pharmacol Ther* 2007; 26: 475-486.
- 60) MOAYYEDI P, FORD AC, TALLEY NJ, VAISMAN N. The efficacy of probiotics in the therapy of irritable bowel syndrome: a systematic review. *Gut* 2008; 59: 325-332.
- 61) NIV E, NAFTALI T, HALLAK R, VAISMAN N. The efficacy of *Lactobacillus reuteri* ATCC 55730 in the treatment of patients with irritable bowel syndrome—a double blind, placebo-controlled, randomized study. *Clin Nutr* 2005; 24: 925-931.
- 62) NOBAEK S, JOHANSSON ML, MOLIN G, AHRNÉ S, JEPSSON B. Alteration of intestinal microflora is associated with reduction in abdominal bloating and pain in patients with irritable bowel syndrome. *Am J Gastroenterol* 2000; 95: 1231-1238.
- 63) BJORKSTEN B, NAABER P, SEPP E, MIKELSAAR M. The intestinal microflora in allergic Estonian and Swedish 2-year-old children. *Clin Exp Allergy* 1999; 29: 342-346.
- 64) WATANABE S, NARISAWA Y, ARASE S, OKAMATSU H, IKENAGA T, TAJIRI Y, KUMEMURA M. Differences in fecal microflora between patients with atopic dermatitis and healthy control subjects. *J Allergy Clin Immunol* 2003; 111: 587-591.
- 65) KALLIOMAKI M, KIRJAVAINEN P, EEROLA E, KERO P, SALMINEN S, ISOLAURI E. Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *J Allergy Clin Immunol* 2001; 107: 129-134.
- 66) BJORKSTEN B, SEPP E, JULGE K, VOOR T, MIKELSAAR M. Allergy development and the intestinal microflora during the first year of life. *J Allergy Clin Immunol* 2001; 108: 516-520.
- 67) PENDERS J, THUIS C, VAN DEN BRANDT PA, KUMMELING I, SNIJDERS B, STELMA F, ADAMS H, VAN REE R, STOBBERINGH EE. Gut microbiota composition and development of atopic manifestations in infancy: the KOALA Birth Cohort Study. *Gut* 2007; 56: 661-667.
- 68) MAJAMAA H, ISOLAURI E. Probiotics: a novel approach in the management of food allergy. *J Allergy Clin Immunol* 1997; 99: 179-185.
- 69) KALLIOMÄKI M, SALMINEN S, ARVILOMMI H, KERO P, KOSKINEN P, ISOLAURI E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 2001; 357: 1076-1079.
- 70) KALLIOMÄKI M, SALMINEN S, POUSSA T, ISOLAURI E. Probiotics during the first 7 years of life: a cumulative risk reduction of eczema in a randomized, placebo-controlled trial. *J Allergy Clin Immunol* 2007; 119: 1019-1021.

- 71) ABRAHAMSSON TR, JAKOBSSON T, BÖTTCHER MF, FREDRIKSON M, JENMALM MC, BJÖRKSTÉN B, OLDAEUS G. Probiotics in prevention of IgE-associated eczema: a double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol* 2007; 119: 1174-1180.
- 72) KUITUNEN M, KUKKONEN K, JUNTUNEN-BACKMAN K, KORPELA R, POUSSA T, TUURE T, HAAHTELA T, SAVILAHTI E. Probiotics prevent IgE-associated allergy until age 5 years in cesarean-delivered children but not in the total cohort. *J Allergy Clin Immunol* 2009; 123: 335-341.
- 73) TAYLOR AL, DUNSTAN JA, PRESCOTT SL. Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: a randomized controlled trial. *J Allergy Clin Immunol* 2007; 119: 184-191.
- 74) FLYNN S, VAN SINDEREN D, THORNTON GM, HOLO H, NES IF, COLLINS JK. Characterization of the genetic locus responsible for the production of ABP-118, a novel bacteriocin produced by the probiotic bacterium *Lactobacillus salivarius* subsp. *Salivarius* UCC118. *Microbiology* 2002; 148(Pt 4): 973-984.
- 75) WEHKAMP J, SALZMAN NH, PORTER E, NUDING S, WEICHENTHAL M, PETRAS RE, SHEN B, SCHAEFFELER E, SCHWAB M, LINZMEIER R, FEATHERS RW, CHU H, LIMA H JR, FELLERMANN K, GANZ T, STANGE EF, BEVINS CL. Reduced Paneth cell alpha-defensins in ileal Crohn's disease. *Proc Natl Acad Sci U S A* 2005; 102: 18129-18134.
- 76) CHICHLOWSKI M, CROOM J, McBRIDE BW, MCKAIGNEY C, JUON H, YACHIMEC C, DOYLE J, JEWELL L, DE SIMONE C. Metabolic and physiological impact of probiotics or direct-fed-microbials on poultry: a brief review of current knowledge. *Int J Poult Sci* 2007; 6: 694-704.
- 77) MADSEN K, CORNISH A, SOPER P, MCKAIGNEY C, JUON H, YACHIMEC C, DOYLE J, JEWELL L, DE SIMONE C. Probiotic bacteria enhance murine and human intestinal epithelial barrier function. *Gastroenterology* 2001; 121: 580-591.
- 78) CHICHLOWSKI M, CROOM WJ, EDENS FW, McBRIDE BW, QIU R, CHIANG CC, DANIEL LR, HAVENSTEIN GB, KOCI MD. Microarchitecture spatial relationship between bacteria and ileal, cecal, and colonic epithelium in chicks fed a direct-fed microbial, PrimaLac, and salinomycin. *Poult Sci* 2007; 86: 1121-1132.
- 79) RACHMILEWITZ D, KATAKURA K, KARMELI F, HAYASHI T, REINUS C, RUDENSKY B, AKIRA S, TAKEDA K, LEE J, TAKABAYASHI K, RAZ E. Toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis. *Gastroenterology* 2004; 126: 520-528.
- 80) OTTE JM, PODOLSKY DK. Functional modulation of enterocytes by gram-positive and gram-negative microorganisms. *Am J Physiol Gastrointest Liver Physiol* 2004; 286: G613-G626.