1

Intestinal Microbial Flora – Effect of Probiotics in Newborns

Pasqua Betta* and Giovanna Vitaliti
U.O UTIN, Department of Pediatrics,
University of Catania
Italy

1. Introduction

The surface of the human gut has a surplus area of 200-250 m^2 in order to contain, between intraepithelial lymphocytes and lamina propria, Peyer's patches and lymphoid follicles, the lymphoid tissue, while hosts a flora of about 800 different bacteria species with over 7000 strains. The 99% are obligate anaerobes and varies species were then classified using traditional anaerobic culture techniques. More than 50% of the dominant gut microbiota (corresponding to 10^{8}-10^{11} per gram of faeces) cannot be identified using traditional colture ,but molecular approaches, based on the use of 165 ribosomal DNA molecular (Mai & Morris, 2004). Most of these bacteria colonizes the large intestine (in a range of 10^{-12} bacteria/g). The bacterial count of the small intestine (duodedum and jejunum) is considerably lower (approximately 10^{4-7} bacteria/ml) than Streptococcus Lactobacillus, Enterobacteriaceae corresponding to the transient microbiota.

The main bacterial species represented in the human large intestine (colon) are distributed with densities higher than 10^{9-11} per gram of contents, and these high densities can be explained by the slow transit and low redox potential . In this intestinal tract we can mostly find bifidobacteria and bacteroides ,bifidobacterium clostridium. The fecal microbiota contains 10^{9} –10^{11} CFU per gram, and microorganism in about 40% of their weight. The dominant microbiota is represented by strict anaerobes , while the sub-dominant microbiota by facultative anaerobes. In addition to the resident microbiota (dominant and sub dominant), the faeces contain the transient microbiota, that is extremely variable, including Enterobacteriacee (Citrobacter, Klebsiella, Proteus) and Enterobacter (Pseudomonas) and yeast (Candida) CFU per gram (Table 1) (Zoetendal et al, 2004).

2. Intestinal microbiota in newborn

The normal human microflora is a complex ecosystem that somehow depends on enteric nutrients for establishing colonization. At birth ,the digestive tract is sterile. This balance of the intestinal microflora is similar to that of adult from about two years of age (Hammerman et al, 2004).

* Corresponding Author

Mouth	200 species	
Stomach,duodenum	pH 2,5-3,5 destructive to most of bacteria 10^1–10^3 unit /ml Lactobacillus,Streptococcus,	
Jejunum,ileum	10^4–10^6 unit /ml bifidobacteria and bacteroides ,bifidobacterium clostridium	Aerobes
Colon	300-400 several species 10^{10}–10^{11} unit /ml Enterobacteriacee (Citrobacter, Klebsiella,Proteus)o(Pseudomonas) Candida.	Anaerobes

Table 1. Composition and topographical features of intestinal microbiota

Diet and environmental conditions can influence this ecosystem. At birth intestinal colonization derives from microorganism of the vaginal mucoses of the mother and faecal microflora . The microbial imprinting depends on the mode and location of delivery. Literature data shows that infants born in a hospital environment, by caesarean section, have a high component of anaerobic microbial flora (Clostridia) and high post of Gram-negative enterobacteria. Those born prematurely by vaginal delivery and breast-feed have a rather rich in Lactobacilli and Bifidobacteria microflora. (Grönlund et al, 1999; Hall et al, 1990)

Diet can influence the microbiota, while breast-feeding promotes an intestine microbiota in which Bifidobacteria predominate, while coliform, enterococci and bacteroides predominate in formula bottle-fed baby.

Escherichia coli and Streptococcus are included among the first bacteria to colonize the digestive tract. After them, strict anaerobes (Bacteroides, Bifidobacteri ,Clostridium) establish during the first week of life, when the diet plays a fundamental role. (Mackie et al, 1999). The pattern of bacterial colonization in the premature neonatal gut is different from the one of healthy, full term infant gut. Aberrant pre-term infants admitted to NICU, born by caesarean section, are more often separated from their mother and kept in an aseptic intensive care setting, treated with broad-spectrum antibiotics. This is the reason why they show a highly modified bacterial flora, consisting of less than 20 species of bacteria, with a predominance of Staphylococcus (aureus and coagulase negative) among aerobic micro-organisms, and Enterobacteriaceae (Klebsiella), among enterococci and anaerobic Clostridia (Dai et al, 1999; Gothefor, 1989).

It is believed that microbial diversity is an important factor in determining the stability of the ecosystem and that the fecal loss of diversity predisposes the preterm gastrointestinal colonization of antibiotic-resistant bacteria and fungi colonization with a consequent potential risk of infection, thus contributing to the development of necrotizing enterocolitis (NEC) (Fanaro et al, 2003; Sakata et al, 1985)

2.1 Structure and function of intestinal microbial flora

The intestinal microbial flora has numerous functions, even if the most of them has not yet been identified. Among these functions, we can report its anatomical –functional role, its

protective function, in particular the "barrier effect", referring to the physiological capacity of the endogenous bacterial microflora to inhibit colonization of the intestine by pathogenic microorganism. It is already known that the intestinal microbial flora influences food digestion ,absorption and fermentation, the immune system response, peristalsis, production of vitamins such as B-vitamins, influencing moreover the turnover of intestinal epithelial cells. In addition the metabolism of gut microflora influences hormonal secretion.

Bacterial colonization of human gut by environmental microbes begins immediately after birth; the composition of intestinal microbiota, relatively simple in infants, becomes more complex with increasing in age, with a high degree of variability among human individuals. It is believed that microbial diversity is an important factor in determining the stability of the ecosystem and that fecal loss of diversity predisposes the preterm gastrointestinal colonization of antibiotic-resistant bacteria and fungi with the consequent potential risk of infection (Cummings & Macfarlane, 1991; Montalto et al, 2009; Neish, 2002).

2.2 Gut microflora and immunity

The mucosal membrane of the intestines, with an area of approximately 200 m^2, is constantly challenged by the enormous amount of antigens from food, from the intestinal microbial flora and from inhaled particles that also reach the intestines. It is not surprising therefore that approximately the eighty per cent of the immune system is found in the area of the intestinal tract and it is particularly prevalent in the small intestine. The intestinal immune system is referred as GALT (gut-associated-lymphoid tissue). It consists of Peyer's patches, which are units of lymphoid cells, single lymphocytes scattered in the lamina propria and intraepithelial lymphocytes spread in the intestinal epithelia.

The immune system of infants is not fully developed. The structures of the mucosal immune system are fully developed in utero by 28 weeks gestation, but in the absence of intrauterine infections, activation does not occur until after birth. Maturation of the mucosal immune system and establishment of protective immunity is usually fully developed in the first years of life. In addition the exposure to pathogenic and commensal bacteria, the major modifier of the development patterns in the neonatal period, depends on infant feeding practices. (Brandtzaeg, 2001; Gleeson et al, 2004)

Bacterial colonisation of the intestine is important for the development of the immune system. The intestine has an important function in working as a barrier.This barrier is maintained by tight-junctions between the epithelial cells, by production of IgA antibodies and by influencing the normal microbial flora. It is extremely important that only harmless substances are absorbed while the harmful substances are secreted via the faeces.

Studies show that individuals allergic to cow´s milk have defective IgA production and an increased permeability of the intestinal mucosa. This results in an increased absorption of macromolecules by the intestinal mucosa. The increased permeability is most probably caused by local inflammations due to immunological reactions against the allergen. This damages the intestinal mucosa

2.3 Modification of the intestinal flora micro-ecosystem

During the past century our lifestyle has dramatically changed regarding hygienic measures, diet, standards of living and usage of medical drugs. Today our diet largely

includes industrially produced sterilized food and the use of different kinds of preservatives. This has led to a decreased intake of bacteria, particularly lactic acid producing bacteria .

The widespread use of antibiotics in healthcare and agriculture, antibacterial substance is also something new for human kind. We have in so many ways sterilized our environment, which is detrimental to the microbial (Cummings & Macfarlane G.T., 1997; Vanderhoof & Young, 1998).

3. What are probiotics?

The term 'probiotic' was proposed in 1965 to denote an organism or substance that contributes to the intestinal microbial balance. The definition of probiotics has subsequently evolved to emphasise a beneficial effect to health over effects on microbiota composition, underscoring the requirement of rigorously proven clinical efficacy. Most probiotic bacterial strains were originally isolated from the intestinal microbiota of healthy humans and the probiotics most thoroughly investigated thus far belong to the genera lactobacilli and bifidobacteria (Caramia G., 2004).

Probiotics have several effects, including modulating the gut microbiota, promoting mucosal barrier functions, inhibiting mucosal pathogen adherence and interacting with the innate and adaptive immune systems of the host, which may promote resistance against pathogens. The intestinal microbiota constitutes an important aspect of the mucosal barrier the function of which is to restrict mucosal colonisation by pathogens, to prevent pathogens from penetrating the mucosa and to initiate and regulate immune responses

3.1 Proved beneficial effects on the host

Prerequisites for probiotics' efficacy are human origin, resistance transit gastric capacity to colonize survival in and adhesion, competitive exclusion of pathogens or harmful antigens to specific areas of the gastrointestinal tract, vitality, verifiable and stability conservation, production substances with antimicrobial action, exclusion of resistance transferable antibiotic. No pathogenicity and / or toxicity has ever been demonstrated on the host.

3.2 Effect of probiotics

Among their effects, the most important are: competition to the more valid nutrients and enteric epithelial anchorage sites; reduction of intestinal pH values for high production of lactic acid from lactose and acetic acid from carbohydrates, which selects the growth of lactobacilli; production of bacteriocins, peptides with bactericidal activity towards related bacteria species; metabolism of certain nutrients in the volatile fatty acids; activation of mucosal immunity, with increased synthesis of secretory IgA, and phagocytosis; stimulation of production of various cytokines

3.3 Mechanism of action of probiotics

The functional interactions between bacteria, gut epithelium, gut mucosal immune system and systemic immune system are the basis of the mechanisms of direct and indirect effects of probiotics. The direct effect of probiotics in the lumen are: competition with pathogens for

nutrients, production of antimicrobial substances and in particular organic acids competitive inhibition on the receptor sites, change in the composition of mucins hydrolysis of toxins, receptorial hydrolisis, and nitric oxide (NO), while the indirect effect largely depends on the site of interaction between the probiotic and the effectors of the immune response, topographically located in the intestinal tract.

There is evidence, in vitro and in vivo, on effects of different probiotics on specific mechanisms of the immune response. The starting point is the interaction between probiotic and the host intestinal mucosa, but it seems clear that not all probiotics have the same initial contact (immune cells, enterocytes, etc.).

There are several literature data that have demonstrated the interaction between probiotics and the immune system, in particular it has been demonstrated their capacity to stimulate the production of intestinal mucines, their trophic effect on intestinal epithelium, the re-establishment of the intestinal mucosa integrity, the stimulation of the IgA-mediated immune response against viral pathogens. All these effects have been demonstrated in experimental studies and in some clinical studies, even if it is not still clear the main mechanism of action and it is conceivable that different mechanisms of action contribute to the efficacy of probiotics, with a different role in different clinical situations (Vanderhoof & Young, 1998).

3.4 Safety

The oral consumption of viable bacteria in infancy naturally raises safety concerns. Products containing probiotics are widely available in many countries and, despite the growing use of such products in recent years, no increase in Lactobacillus bacteraemia has been detected. Nevertheless, the average yearly incidence of Lactobacillus bacteraemia in Finland between the years 1995 and 2000 was 0.3 cases/100,000 inhabitants. Importantly, 11 out of the 48 isolated strains were identical to Lactobacillus GG, the most commonly used probiotic strain. Lactobacillus bacteraemia is considered to be of clinical significance; immune-suppression, prior prolonged hospitalisation and surgical interventions have been identified as predisposing factors. Nonetheless, clinical trials with products containing both lactobacilli and bifidobacteria have demonstrated the safety of these probiotics in infants and children, and in a recent study, the use of L. casei was found to be safe also in critically ill children

In a trial assessing the safety of long-term consumption of infant formula containing B. lactis and S. thermophilus, the supplemented formulas were demonstrated to be safe and well tolerated. No serious adverse effects have been reported in the trials involving premature neonates, but it should be noted that the studies were not primarily designed to assess their safety (Hammerman et al, 2006)

4. Probiotics and gastrointestinal disorders

The presence of Bifidobacteria in artificial milk can contribute to the induction of a significant increase of Bifidobacteria in the intestinal tract, promotes the development of a protective microflora, similar to that one of the breast- fed newborn, contributes to the modulation of immune-defenses, giving them a major efficiency (Langhendries et al, 1995; Fukushima et al, 1998).

In early 2002, the United States Food and Drug administration accepted a "generally regarded as safe (GRAS) the use of *Bifidobacterium lactis* and *Streptococcus thermophilus* in formula milk for healthy infants aged 4 months or more" (Hammerman et al, 2006).

The clinical efficacy of probiotics in the prevention and treatment of infectious disease in infancy has most comprehensively been documented in diarrhoeal disease. *Lactobacillus* GG or *Lactobacillus reuteri* (ATCC 55730) supplementation has been demonstrated to be effective in the prevention of acute infantile diarrhoea in different settings. *Lactobacillus* GG has also been reported to significantly reduce the duration of acute diarrhoea and the duration of rotavirus shedding after rotavirus infection. Bifidobacteria have also shown promising potential in preventing both nosocomial spread of gastroenteritis and diarrhoea in infants in residential care settings. Meta-analyses of double-blind, placebo-controlled clinical trials have concluded that probiotics, particularly *Lactobacillus* GG, are effective in treatment of acute infectious diarrhoea in infants and children. Probiotics appear also to have some protective effect against antibiotic-associated diarrhoea and acute diarrhoea in children, but the heterogeneity of the available studies precludes drawing firm conclusions (Vanderhoof, 2000).

5. Probitics and atopic disease

Probiotics acts on atopic diseases modulating initial colonisation, intralumenal degradation of allergens, promoting intestinal barrier function, enhancing immune maturation with induction of IgA production, induction of regulatory T cells. In infancy, food allergy and atopic eczema are the most common atopic disorders. Even though atopic disease often becomes manifest during the course of the first year of life, it is well established that the immune pathology leading to clinical disease has its origins in early life, possibly already in the immune environment prevailing *in utero*. Indeed, infantile food allergy could be considered a manifestation of a primary failure to establish tolerance to dietary antigens rather than loss of tolerance characteristic of allergies in later life. Therefore, measures aimed at reducing the risk of atopic diseases should be started in the perinatal period. Thus far, the rationale of most studies assessing means of primary prevention of atopic diseases has been to reduce exposure to the allergens known to most often be associated with sensitisation and provocation of symptoms in allergic individuals, but the success of such measures has been relatively poor. Consequently, probiotics have been investigated as a novel approach with a number of potential effects which might beneficially affect the host immune physiology to a non-atopic mode.

The immune pathology of atopic diseases is characterised by T helper (Th)2-driven inflammatory responsiveness against ubiquitous environmental or dietary allergens. The factors leading to inappropriate Th2 responsiveness, and thus atopic disease, in early immune development remain poorly understood. Th2-type responsiveness is counter-regulated both by Th1 responses, which are usually directed against infectious agents and immunosuppressive, and by tolerogenic regulatory T cell responses. Prescott and colleagues demonstrated that infants with high hereditary risk who subsequently developed atopic disease are characterised by an impaired capacity (compared with healthy infants) to produce both Th1 and Th2 cytokines in the neonatal period. During the first year of life, an increase in Th2 responsiveness is seen in infants developing atopic disease, whereas a reverse development takes place in healthy infants.

5.1 Use of probiotics for prevention of atopic diseases

As previously mentioned, the sequence of bacterial intestinal colonization of neonates and young infants is important in the development of the immune response. Recognition by the immune system of self and nonself, as well as the type of inflammatory responses generated later in life, are likely affected by the infant's diet and acquisition of the commensal intestinal bacterial population superimposed on genetic predisposition.

During pregnancy, the cytokine inflammatory-response profile of the fetus is diverted away from cell-mediated immunity (T-helper 1 [Th1] type) toward humoral immunity (Th2 type). Hence, the Th2 type typically is the general immune response in early infancy. The risk of allergic disease could well be the result of a lack or delay in the eventual shift of the predominant Th2 type of response to more of a balance between Th1- and Th2-type responses (Neaville, 2003).

Administration of probiotic bacteria during a time period in which a natural population of lacticacid– producing indigenous intestinal bacteria is developing could theoretically influence immune development toward more balance of Th1 and Th2 inflammatory responses (Majamaa & Isolauri, 1997). The intestinal bacterial flora of atopic children has been demonstrated to differ from that of nonatopic children. Specifically, atopic children have more Clostridium organisms and fewer Bifidobacterium organisms than do nonatopic study subjects (Björkstén et al, 1999; Klliomaki et al, 2001), which has served as the rationale for the administration of probiotics to infants at risk of atopic diseases, particularly for those who are formula fed.

In a double-blinded RCT, LGG or a placebo was given initially to 159 women during the final 4 weeks of pregnancy. If the infant was at high risk of atopic disease (atopic eczema, allergic rhinitis, or asthma), the treatment was continued for 6 months after birth in both the lactating woman and her infant (Kalliomäki et al, 2003). A total of 132 mother-infant pairs were randomly assigned to receive either placebo or LGG and treated for 6 months while breastfeeding. The primary study end point was chronic recurrent atopic eczema in the infant. Atopic eczema was diagnosed in 46 of 132 (35%) of these study children by 2 years of age. The frequency of atopic eczema in the LGG-treated group was 15 of 64 (23%) versus 31 of 68 (46%) in the placebo group (RR: 0.51 [95% CI: 0.32– 0.84]; P = .01). The number of mother-infant pairs required to be treated with LGG to prevent 1 case of chronic recurrent atopic eczema was 4.5. By 4 years of age, eczema occurred in 26% of the infants in the group treated with LGG, compared with 46% in the placebo group (RR: 0.57 [95% CI: 0.33– 0.97]; P =.01). However, only 67% of the original study group was analyzed at the 4-year follow-up. These results support a preventive effect for giving a probiotic to mothers late in pregnancy and to both mothers and infants during the first 6 months of lactation for the prevention of atopic eczema in infants who are at risk of atopic disease.

Conversely, Taylor et al (2007) found that probiotic supplementation did not reduce the risk of atopic dermatitis in children at high risk with the report of some increased risk of subsequent allergen sensitization. As concluded in a review by Prescott and Björkstén (2007) and in a 2007 Cochrane review (Osborn & Sinn, 2007) despite the encouraging results of some studies, there is insufficient evidence to warrant the routine supplementation of probiotics to either pregnant women or infants to prevent allergic diseases in childhood. Explanations for varied study results include host factors such as genetic susceptibility,

environmental factors such as geographic region and diet, and study variables including probiotic strains and doses used (Prescott & Björkstén, 2007; Penders, 2007).

5.2 Use of probiotics in the treatment of atopic diseases

In an RCT, 53 Australian infants with moderate-to-severe atopic dermatitis were given either Lactobacillus fermentum or placebo for 8 weeks. At final assessment at 16 weeks, significantly more children who received the probiotic had improved extent and severity of atopic dermatitis as measured by the Severity of Scoring of Atopic Dermatitis (SCORAD) index over time compared with those who received placebo (P = .01) (Weston et al, 2005; Viljanen et al, 2005). These results are encouraging, but as summarized in a 2008 Cochrane review (Boyle et al, 2008), probiotics have not yet been proven to be effective in the treatment of eczema.

6. Probiotics and premature infants

Prematurity compromises the anatomical and functional development of all organs, in inverse proportion to the gestational age. Some peculiarities of the preterm are the high incidence of respiratory diseases, the multi-systemic immaturity, even if nutrition constitutes one of the major actual problem to afford.

The preterm infant lacks of the sucking reflex, has a restricted gastric and intestinal capacity, insufficient absorption of the main food, that contribute to both quantitative and qualitative nutritional deficiencies.

The lack of an adequate nutrition decreases the synthesis of surfactant and anti-oxidant molecules, thus causing a delayed lung maturation and both cellular and humoral immune response, responsible for an increase of the catabolism, promoting the use of endogenous proteins. Therefore, the goal of the nutrition of the ELBW infant is the manteinance of his post-natal growth, similarly of what happens in utero, preventing the protein catabolism (through the use of endogenous proteins: lean body mass), avoid the weight loss during the first 2 weeks after birth, assuring a high energetic rate since his first day of life, thus reducing the percentage of preterms with a weight less than 10° percentile at discharge.

Nowadays the first approach to ELBW preterms is the parenteral nutrition since their first day of life (with the prompt introduction of glucose as it is the main source of energy and it reduces the catabolism of endogenous proteins since the first 2 hours after birth, and the introduction of lipids since the first 24 hours after birth). It is also important the introduction of low quantities of milk (minimal enteral feeding) via oral or nasal-gastric way in order to promote the feeding tolerance and the increase of enteral production of cholecystokinin that stimulates the bile function, protecting the liver from hepatic steatosis due to parenteral nutrition.

It is important that these procedures are managed in a gradual way in order to avoid the tiredness of the infant and the aspiration of milk with regurgites. For this reason it is conceivable using a fortified maternal formula for premature infants, with a daily increase of the feeding, paying attention to abdominal distension, vomit, gastric stagnation, apneas, and diarrhea.

It is conceivable to stop the parenteral nutrition when the energetic rate reach a quote of 80cal/Kg/die and the daily increase of milk must not be more than 10ml/Kg/die, and

sometimes it is necessary the continuous or discontinuous enteral feeding, via nasal-gastric tube, in order to suspend the parenteral nutrition.

The passage from enteral nutrition to nursing depends on the acquisition of sucking, deglutition, epiglottis and larynx closure ability and on the nasal passage, as well as the esophageal motility and a synchronized process is usually absent before 34 weeks of gestation. The sucking ability is usually reached when the infant has a weight over 1500 gr even if sometimes it is necessary to proceed with the tactile stimulation of the infant tongue (Tsang et al, 2005).

Enzymatic digestive functions in preterm more than 28 weeks of gestation are mature enough to allow the adequate digestion and absorption of proteins and carbohydrates. Lipids are well adsorbed and unsaturated fatty acids and lipids in maternal milk are better adsorbed than the components of the formula milk.

The weight gain in infants with a birth weight less than 2000 gr should be adequate when the mother shows a protein intake of 2.25-2.75/Kg/die, because they should provide a good intake of essential aminoacids, in particular tryptophan and threonine, that are important for the cerebral development.

The maternal milk, through specific immunologic factors, can potentiate the defensive mechanisms of preterms, contributing to ameliorate the immune defense against infectious agents. Recent studies highlighted that the maternal milk not only promote a passive protection, but can directly modify the immunologic development of the infant.

The maternal milk contains immunologic and non-immunologic factors, and immune-modulant factors, such as the bifidogenic factor, that promotes the development of the *Lactobacillus Bifidus,* that by competition promotes the decrease of the intestinal pH and inhibits the growth of *Escherichia Coli.* The maternal milk must be fortified, while the formula for preterm infants do not contain the bifidogenic factor (Heiman & Schanler, 2007).

It is also well established that the composition of the intestinal microbiota is aberrant and its establishment delays in neonates who require intensive care, with an increased risk of developing NEC. As discussed above, probiotics have been shown to enhance the intestinal barrier, inhibit the growth and adherence of pathogenic bacteria and to improve altered gut micro-ecology In preterm infants, administration of the probiotic *Lactobacillus* GG has been shown to affect colonisation patterns. Data from experimental animal models suggest that bifidobacteria reduce the risk of NEC in rats. Consequently, it could be hypothesised that probiotics might have potential in reducing the risk of NEC in premature infants.

The supplementation of probiotics since the first day of life represents a valid help in influencing the growth of a favourable intestinal ecosystem, decreasing the quote of Clostridium, Bacillus and Bacteroides Fragilis and increasing the rate of bifidobacteria, also improving the intestinal barrier with a way of action similar to that of the maternal milk, protecting the gut from bacteria and fungal colonization, avoiding the development of NEC.

7. Probiotics and necrotizing enterocolitis

Necrotizing enterocolitis (NEC) is a serious anoxic and ischemic disease particularly affecting premature newborns, affecting almost the ileo-colic area, with bacteria

proliferation, production of gas inside gastric walls (cystic pneumatosis), associated with edema and inflammation. Its incidence rate is 1-3 cases for 1000 newborns, with a mortality rate ranging between 10-50%. The prematurity is the most important risk factor, as well as the low birth weight (< 1500 gr). This risk increases after the colonization or the infection of pathogens such as Clostridium, Escherichia, Klebsiella, Salmonella, Shigella, Campylobacter, Pseudomonas, Streptococcus, Enterococcus, Staphylococcus aureus and coagulase negative Staphylococcus. Other factors that can increase its incidence are the intestinal immaturity, the decrease of the intestinal motility, the increase of permeability to macromolecules and the excessive volume of milk. Certainly breast feeding represents a protective factor, as it is shown by the decreased incidence of NEC in breast-fed infants. Moreover literature data supporting the benefits of probiotics are increasing in the last decades.

The role of intestinal micro-organisms has been largely described, even if it is still not clear. Advances in molecular biology and intestinal microbiology allow a better characterization of the intestinal microbiota in children affected by NEC. Nowadays, literature data describe different methods of characterization of the microbic genotype and of identification of its genes, expression of the specific proteins and production of metabolites. The application of these techniques on bioptic samples of infected and non-infected subjects could better the comprehension of the persistence of NEC in premature newborns. Deshpande et al. (2007) published a meta analysis that confirms the benefit of probiotic supplements in reducing death and disease in preterm newborns.

The mechanism of action of probiotics in the protection of NEC seem to be the increased production of anti-inflammatory cytokines, blockage of the passage of bacteria and their products through the mucose, competitive action with some pathogen groups, modification of the response of the host towards microbial products, improving the enteral nutrition, decreasing the duration of the parenteral nutrition, responsible for late sepsis.

Different studies highlight that the supplementation of probiotics reduces the risk of NEC. In the most recent literature, the study of Bin-Nun et al. (2005) showed a lower frequency of serious diseases in newborns with a low birth weight when in their feeding was added a probiotic mixture. Desphande's meta analysis, published in Lancet in 2007, showed the same results. As a matter of fact the first studies on probiotics in premature children were leaded in order to reduce the incidence of NEC in this group of children.

8. Probiotics and infections

The most valid indication of the probiotic remains the decrease of intestinal infections. In fact, the literature shows that the probiotic can reduce the severity and number of episodes of diarrhea.

Weizman & Alsheikh made a double-blind placebo-controlled study using a formula supplemented with L. reuteri or B. bifidium for 12 weeks. In the group of infants in therapy with probiotics, less gastrointestinal infectious episodes have been detected, fewer episodes of fever compared to placebo, with consequent reduce of antibiotic therapy. The fetus and the newborn are particularly vulnerable to the injuries caused by infectious agents or immunological mechanisms related to the immaturity of the immune system. The improvement of perinatal care has led to increased survival of high-risk infant (ELBW,

respiratory distress, surgery), neonatal research priorities on the prevention and treatment of sepsis in NEC and bronchopulmonary dysplasia (CLD) (Weizman & Alsheikh, 2006).

In view of the role of mediators of inflammation in CLD and in sepsis is therefore important to modulate the immune response in these young patients. Some studies have shown that probiotics can alter the intestinal microflora and reduce the growth of pathogenic microorganisms in the intestines of preterm infants, decreasing the incidence of necrotizing enterocolitis and sepsis. Moreover, a study performed in rats with immune deficiency has shown that the administration of LGG reduced the risk of colonization and sepsis by Candida.

One of our retrospective study, performed in 2002 at the University of Catania TIN, showed that supplementation from birth for at least 4-6 weeks of a symbiotic (lactogermine plus 3.5 x109 ucf / day) decreased the incidence and intensity of gastrointestinal colonization of Candida, and subsequently its related infections in a group of preterm infants. Another randomized study on 80 preterm infants has confirmed that the administration of LGG (at a dose of 6 billion cfu / day) from the first day of life for a period of six weeks reduced the fungal enteric colonization with no side effects (Romeo et al, 2011).

Newborns submitted to greater surgical interventions (esophageal atresia, hernia diaframmatica, intestinal malformations) have an increased risk of bacterial and/or mycotic infections due to the use of drains, central venous catheter, NPT, persistent nose-gastric probe that can be the cause of serious sepsis and pneumonias.

In a recent study that we presented at ESPHGAN, we demonstrated that surgical infants admitted to our NICU and supplemented with probiotics have a reduced risk of bacterial and *Candida* infections and an improved clinical outcome (Figure 1) (Betta et al, 2007)

In another recently published study on preterm infants, the use of probiotics appeared to be effective in the prevention of both bacterial and mycotic infections, in the attenuation of gastrointestinal symptoms and in a more rapid weaning from total parenteral nutrition with a reduction in the central venous catheter time and the number of days in hospital. These results were evident both in a group of preterm newborns and in a group of surgical newborn treated with a supplementation of probiotics (Figure 2).

9. Probiotics and respiratory tract infections (RTI)

Two studies have examined the effect in adults of a combined multi-strain probiotic and multivitamin/mineral supplement containing L. gasseri, B. longum and B. bifidum on the incidence, duration and severity of common cold infections and aspects of immune function (de Vrese et al, 2006; Winkler et al, 2005). Both studies found a reduction in severity and duration, as well as enhanced expression of immune cells, while only Winkler et al. (2005) found a reduction in incidence. The major difference between studies is dose—the same probiotic strains were used for both, as well as the same assessment methods for the illness—suggesting that although the dose used by de Vrese et al. (2006) (5 9 107 CFU) was enough to attenuate symptoms and duration, a higher dose such as that used by Winkler et al. (2005) (5 9 108 CFU per day) was needed for prevention of infections. The lower dose may promote a systemic immune response sufficient to reduce severity and duration but not incidence, while the higher dose may stimulate systemic immunity via the mechanism of

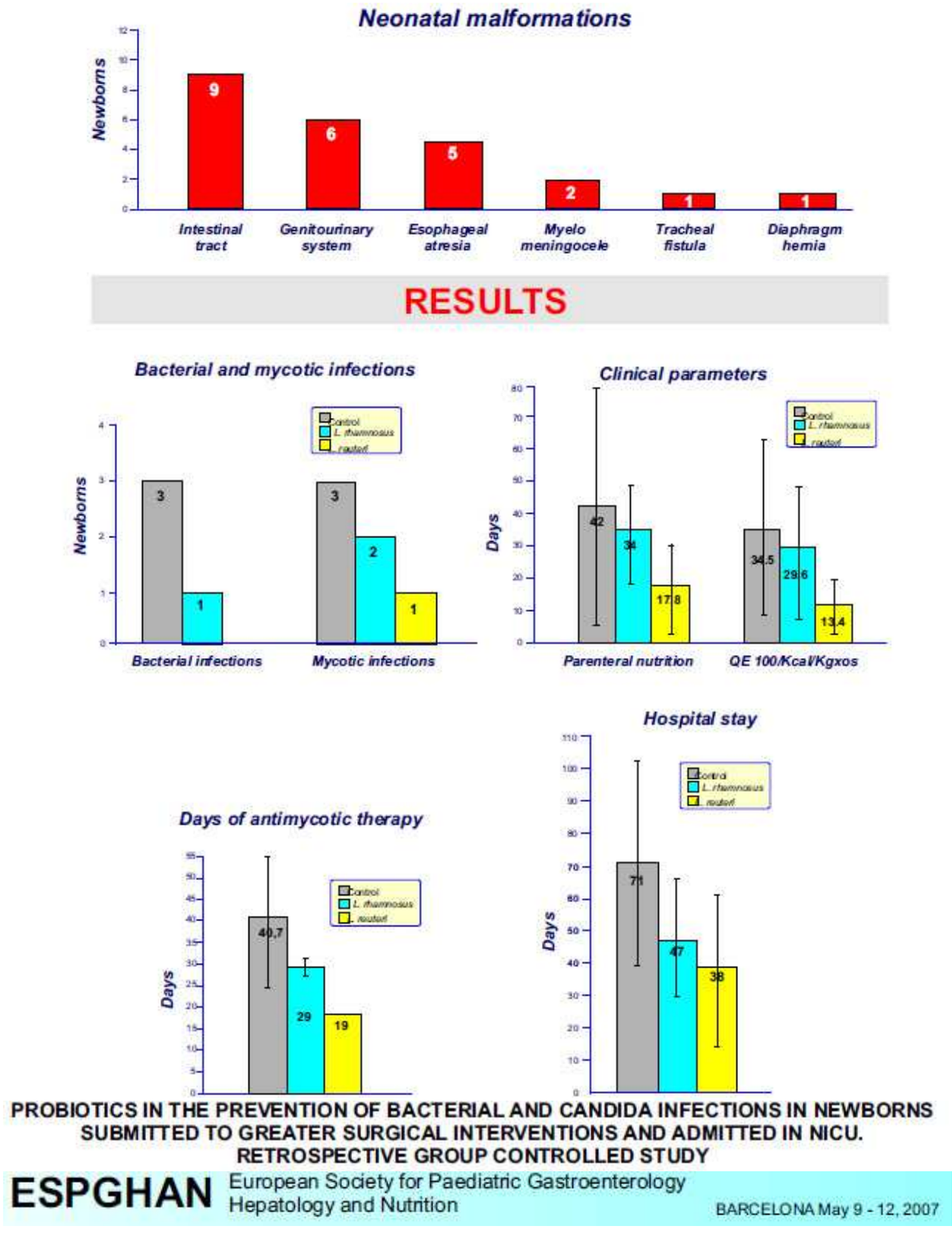


Fig. 1. The direct introduction of probiotics, that positively influences the intestinal microbial population, determining a reduction of more pathogenic species in the bowel reservoir, can improve enteral nutrition reducing time of dependence on intravenous nutrition and might contribute to a better outcome in high risk newborns.

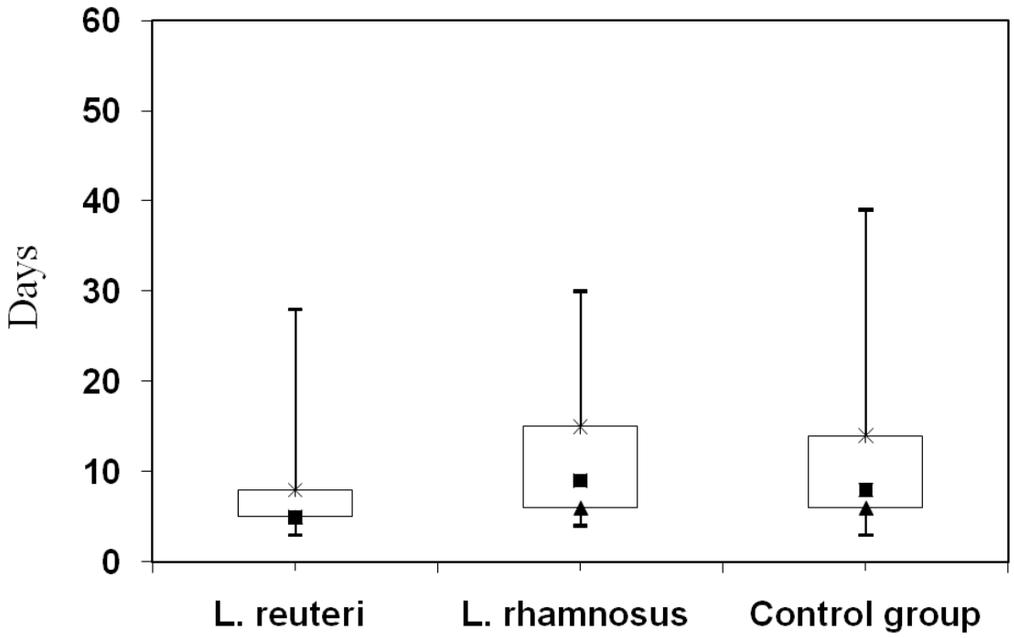


Fig. 2.

distribution of T and B lymphocytes, primed in the gut, which proliferate to the mucosal-associated lymphoid tissue (MALT), where the B cells differentiate into immunoglobulin-producing cells after specific antigenic exposure, leading to an inhibition of colonisation by pathogenic strains.

Olivares et al. (2006) also found an immunostimulatory effect in subjects given a multistrain probiotic containing L. gasseri and L. corniformis, compared with a standard yoghurt containing S. thermophilus and L. bulgaricus, although this study provides no evidence for the efficacy of a greater number of strains, since two non-comparable treatments were used.

Gluck and Gebbers (2003) investigated colonisation by nasal pathogens and showed a 19% reduction in the group given probiotics (L. rhamnosus GG, Bifidobacterium lactis, L. acidophilus, S. thermophilus) compared to no reduction with placebo. Despite this reduction in colonisation, no data are given as to whether subjects became unwell during the study period, making conclusions as to actual health benefits difficult to draw. In a similar study, Hatakka et al. (2007) found no effect of a probiotic mixture on incidence and duration of otitis and upper respiratory infections on children aged 6 months to 10 years; a lower dose than that used by Gluck and Gebbers (2003) may explain the disparity between results. It may also be that ingested probiotics have less effect on the aural mucosa compared to that on the nasal mucosa, or that the effects are strain-specific.

In a 7-month study with over 1,000 subjects, Lin et al. (2009) examined the protective effect of two single probiotics (L. casei and L. rhamnosus, given individually) and one multi-strain mixture containing the 2 lactobacilli and 10 other organisms. Reduced physician visits, as well as decreased incidence of bacterial, and viral respiratory disease were seen in all groups compared with placebo, but there was no significant difference in effectiveness between the preparations even though the multi-strain probiotic was given at a tenfold higher dose than the individual strains. However, in the case of prevention of gastro-intestinal tract

infections, the probiotic mixture was significantly more effective than the single strains. This may be due to the exceptionally high dose given in the multi-strain treatment, resulting in larger numbers of probiotic bacteria competing with pathogens for binding sites and or nutrients in the gut.

Another point of interest in this study is that despite large differences in dose, the two single strains did not have statistically different effects, suggesting strain-specificity in dose and effect for individual species. These data support the theory that supplementation with certain multi-strain probiotics can reduce severity, duration, and possibly incidence of RTIs, and in the case of Lin et al. (2009) that a multi-strain probiotic may be more effective than a single-strain. There is some evidence for immunostimulation, even in cases where illness still occurs. Further consistency could be added to this evidence with the establishment, by testing varied concentrations of probiotic bacteria, of an optimum dose that prevents pathogenic colonisation of the mucosa as well as the incidence and severity of illness. Testing this dose with and without vitamin and mineral supplementation may reveal a synergy between both types of supplement.

Further work should be done to determine the relative efficacy of single- and multi-strain probiotics in this area.

10. Conclusion

The direct introduction of probiotics, can positively influence the intestinal microbial population, include a reduction in the bowel reservoir of more pathogenic species, improve enteral nutrition, and reduce dependence on intravenous nutrition, favour an increased gut mucosal barrier to bacteria and bacterial products, and up regulation in protective immunity.

It is important to establish what probiotic it should be used, the right dosage, the right time of use, and furthermore controlled studies should answer to these questions, in order to describe specific indications on the type of probiotic that must be used in a specific situation, thus better clarifying the structure of the probiotic and its characteristics, selecting the right probiotic for each kind of disease.

It is important to underline that the use of probiotics is safe even at high dosages, without any side effect in preterm infants. After birth the rapid development of the intestinal microflora regulates all the different gastro-intestinal and immunologic functions that are included in the so called mutualism bacteria- host organism. This kind of relationship starts from birth and regulate different aspects of the immune system of the newborn.

Recent epidemiologic data support the hypothesis that in the last 20 years some immunologic modification can find a cause in the modification of the intestinal microflora.

Different therapeutic actions could be potentially able to alter the normal relationship between the intestinal microbiota and the host organism. The international medical community has to be aware of the increasing importance that initial colonising intestinal microflora could have on the health and well-being of the host later in life. It is of great importance to know that the initial bacterial colonisation of the neonate appears to play a crucial role in inducing immunity in the immature human being, and that a suboptimal process could have definite consequences. The optimal early interface between the microbes

and the intestinal mucosa of the host may have been somewhat disturbed by modern perinatal care. It is fundamental to try to decrease these possible negative influences and to discover in the near future the possible means to help manipulate positively the gut microbiotia of infants (Rautava, 2007).

11. References

Betta, P.; Sciacca, P.; Trovato, L. et al. (2007) Probiotics in the prevention of Bacterial and Candida infections in newborns submitted to greater surgical interventions and admitted in NICU. Retrospective Group Controlled Study. ESPGHAN, Barcelona, May 9-12, 2007

Bin-Nun, A.; Bromiker, R.; Wilschanski, M. et al. (2005) Oral probiotics prevent necrotizing enterocolitis in very low birth weight neonates. J Pediatr, 147(2):192-6.

Björkstén, B.; Naaber, P.; Sepp, E. & Mikelsaar, M. (1999) The intestinal microflora in allergic Estonian and Swedish 2-year-old children. Clin Exp Allergy, 29(3):342–346

Boyle, R.J.; Bath-Hextall, F.J.; Leonardi-Bee, J. et al (2008). Probiotics for treating eczema. Cochrane Database Syst Rev, (4):CD006135

Brandtzaeg, P. (2001) Nature and function of gastrointestinal antigen-presenting cells. Allergy,56 Suppl 67:16-20.

Cummings, J.H. & Macfarlane, G.T. (1991) The control and consequences of bacterial fermentation in the human colon. J Appl Bacteriol, 70:443-59.

Cummings, J.H. & Macfarlane, G.T. (1997) Colonic microflora: nutrition and health. Nutrition, 13:476-8.

Dai, D. & Walker, W.A. (1999). Protective nutrients and bacterial colonization in the immature human gut. Adv Pediatr,46:353-82

Deshpande, G.; Rao, S. & Patole, S. (2007) Probiotics for prevention of necrotising entercolitis in preterm neonates with very low birthweight: a systematic reiew of randomised controlled trials, Lancet, 369:1614-20

de Vrese, M.; Winkler, P.; Rautenberg, P. et al, (2006) Probiotic bacteria reduced duration and severity but not the incidence of common cold episodes in a double blind, randomized,controlled trial. Vaccine, 24(44–46):6670–6674

Fanaro, S.; Chierici, R.; Guerrini P. & Vigi, V. (2003) Intestinal microflora in early infancy :composition and development Acta Paediatr Suppl, 91:48-55.

Fukushima, Y.; Kawata, Y.; Hara, H.; Terada, A. & Mitsuoka, T. (1998) . Effect of a probiotic formula on intestinal immunoglobulin A production in healthy children, 42:39-44.

Caramia, C. (2004) Probiotics: from Metchnikoff to the current preventive and therapeutic possibilities. Pediatr Med Chir, 26:19-33.

Gleeson, M.; Pyne, D.B. & Callister R. (2004) The missing links in exercise effects on mucosal immunity. Exerc Immunol Rev, 10:107-28.

Gluck, U. & Gebbers, J.O. (2003) Ingested probiotics reduce nasal colonization with pathogenic bacteria (Staphylococcus aureus, Streptococcus pneumoniae, and beta-hemolytic streptococci). Am J Clin Nutr, 77(2):517–520

Gothefors, L. (1989) Effects of diet on intestinal flora. Acta Paediatr Scand Suppl, 351:118-21.

Grönlund, M.M.; Lehtonen, O.P.; Eerola, E. & Kero, P. (1999) Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after cesarean delivery. J Pediatr Gastroenterol Nutr, 28:19-25

Hall, M.A.; Cole, C.B., Smith, S.L.; Fuller, R. & Rolles, C.J. (1990) Factors influencingthe presence of faecal Lactobacilli in early infancy. Arch Dis Child, 65:185-8.

Hammerman, C.; Bin-Nun, A. & Kaplan, M. (2004) Germ warfare: probiotics in defence of premature gut. Clin Perinat,31: 489-500.

Hammerman, C.; Bin-Nun, A. & Kaplan, M. (2006). Safety of probiotics: comparison of two popular strains. , 11;333:1006-8.

Heiman, H. & Schanler, R.J. (2007) Enteral nutrition for premature infants: the role of human milk. Semin Fetal Neonatal, 12:26-34.

Hatakka, K.; Blomgren, K.; Pohjavuori, S. et al. (2007). Treatment of acute otitis media with probiotics in otitis-prone children-a doubleblind, placebo-controlled randomised study. Clin Nutr, 26(3):314–321

Kalliomäki, M.; Kirjavainen, P.; Eerola, E. et al. (2001) Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. J Allergy Clin Immunol, 107(1):129 –134

Kalliomäki, M. ; Salminen, S. ; Poussa, T. et al (2003) Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. Lancet, 361(9372):1869 –1871

Karvonen, A.; Casas, I. & Vesikari, T (2001). Safety and possible antidiarrhoeal effect of the probiotic Lactobacillus reuteri after oral administration to neonates, Clin Nutr ,20;63:abstract 216.

Langhendries, J.P.; Detry, J.; Van Hees, J. et al (1995) Effect of a fermented infant formula containing viable bifidobacteria on the fecal flora composition and pH of healthy full-term infants. J Pediatr Gastroenterol Nutr, 21:177-81.

Lin, J.S.; Chiu, Y.H.; Lin, N.T. et al. (2009) Different effects of probiotic species/strains on infections in preschool children: A double-blind, randomized, controlled study. Vaccine, 27(7):1073–1079

Mackie, R.I.; Sghir, A. & Gaskins, H.R. (1999) Developmental microbial ecology of the neonatal gastrointestinal tract. Am J Clin Nutr, 69:1035S-1045S.

Mai ,V.; & Morris, J.G. (2004). Colonic bacterial flora: changing understandings in the molecular age, J Nutr, 134:459-64

Majamaa, H. & Isolauri, E. (1997) Probiotics: a novel approach in the management of food allergy. J Allergy Clin Immunol, 99(2): 179 –185

Manzoni, P.; Pedicino, R.; Stolfi, I.; Decembrino, L.; Castagnola, E.; Pugni, L.; Corbo, M. & the Neonatal Fungal Infections Task Force of the Italian Neonatology Society. (2004). Criteri per una corretta Diagnosi delle Infezioni Fungine Sistemiche Neonatali in TIN: i suggerimenti della Task Force per le Infezioni Fungine Neonatali del G.S.I.N, Pediatr Med Chir, 26:89-95.

Montalto, M.; D'Onofrio, F.; Gallo, A.; Cazzato, A. & Gasbarrini G. (2009) Intestinal microbiota and its functions. Digestive and Liver Disease Supplements 3: 30–34

Neaville, W.A.; Tisler, C.; Bhattacharya, A. et al. (2003) Developmental cytokine response profiles and the clinical and immunologic expression of atopy during the first year of life. J Allergy Clin Immunol, 112(4): 740 –746

Neish, A.S. (2002) The gut microflora and intestinal epithelial cells: a continuing dialogue. Microbes Infect, 4: 309-17

Olivares, M.; Diaz-Ropero, M.P.; Gomez, N. et al (2006) The consumption of two new probiotic strains, Lactobacillus gasseri CECT 5714 and Lactobacillus coryniformis CECT 5711, boosts the immune system of healthy humans. Int Microbiol 9(1):47–52

Oliveri, S.; Trovato, L.; Betta, P.; Romeo, M.G. & Nicoletti, G. (2008). Experience with the Platelia Candida ELISA for the diagnosis of invasive candidosis in neonatal patients, Clinical Microbiology and Infection,14:377-397.

Osborn, D.A. & Sinn, J.K. (2007) Probiotics in infants for prevention of allergic disease and food hypersensitivity. Cochrane Database Syst Rev, (4):CD006475

Pappas, P.G.; Rex, J.H.; Sobel, J.D.; Filler, S.D.; Dismukes, W.E.; Walsh, T.J. & Edwards, J.E. (2004). Guidelines for treatment of Candidiasis, Clin Infectious Disease,38:161-89

Penders, J.; Stobberingh, E.E.; van den Brandt, P.A. et al. (2007) The role of the intestinal microbiota in the development of atopic disorders. Allergy, 62(11):1223–1236

Prescott, S.L. & Björkstén, B. (2007) Probiotics for the prevention or treatment of allergic diseases. J Allergy Clin Immunol, 120(2): 255–262

Ramesh, A.; Nidhi, S.; Rama, C.; Ashok, D.; Vinod, K.P.; Ira, G.H. & Pinaki, P. (2003). Effect of oral Lactobacillus GG on Entyeric Microflora in Low Birth-Weight in Neonates. JPGN, 36:397-402.

Rautava, S. (2007) Potential uses of probiotics in the neonate. Semin Fetal Neonatal Med, 12(1):45-53.

Romeo, M.G.; Romeo, D.M.; Trovato, L. et al. (2011) Role of probiotics in the prevention of the enteric colonization by Candida in preterm newborns: incidence of late-onset sepsis and neurological outcome. J Perinatol, 31(1):63-9.

Sakata, H.; Yoshioka, H. & Fujita, K. (1985) Development of the intestinal flora in very low birth weight infants compared to normal full-term newborns. Eur J Pediatr,144:186-90.

Taylor, A.L.; Dunstan, J.A. & Prescott SL. (2007) 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, 119(1): 184 –191

Tsang, R.C.; Uauy, R.; Koletzko, B. & Zlotkin, S.H. Nutrition of the Preterm infant. Scientific Basis and Pratical Guidelines. Cincinnati, Digital Educational Publishing 2005.

Vanderhoof, J.A. & Young, R.J. (1998) Use of probiotics in childhood gastrointestinal disorders Journal of pediatrics, 27:323-332

Vanderhoof, J.A. (2000) Probiotics and intestinal inflammatory disorders in infant and children. Journal of Pediatric Gastroenterology and Nutrition, 30:534-38

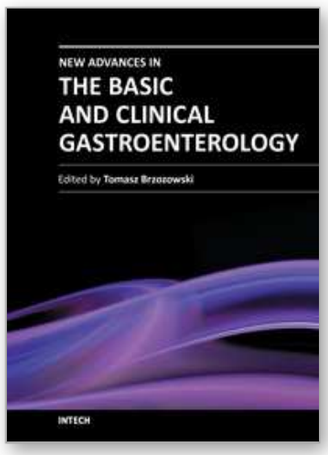
Viljanen, M.; Savilahti, E.; Haahtela, T. et al. (2005) Probiotics in the treatment of atopic eczema/dermatitis syndrome in infants: a double-blind placebo-controlled trial. Allergy, 60(4):494 –500

Weizman, Z. & Alsheikh, A. (2006) Safety and tolerance of a probiotic formula in early infancy comparing two probiotic agents: a pilot study. J Am Coll Nutr, 25(5):415-9.

Weston, S.; Halbert, A.; Richmond, P. et al (2005) Effects of probiotics on atopic dermatitis: a randomised controlled trial. Arch Dis Child. 90(9):892– 897

Winkler, P.; de Vrese, M.; Laue, C. et al. (2005) Effect of a dietary supplement containing probiotic bacteria plus vitamins and minerals on common cold infections and cellular immune parameters. Int J Clin Pharmacol Ther, 43(7):318–326

Zoetendal, E.G.; Collier, C.T.; Koike, S.; Mackie, R.I. & Gaskins, H.R. (2004) Molecular ecological analysis of the gastrointestinal microbiota: a review. J Nutr, 134:465-72.



New Advances in the Basic and Clinical Gastroenterology

Edited by Prof. Tomasz Brzozowski

ISBN 978-953-51-0521-3
Hard cover, 546 pages
Publisher InTech
Published online 18, April, 2012
Published in print edition April, 2012

The purpose of this book was to present the integrative, basic and clinical approaches based on recent developments in the field of gastroenterology. The most important advances in the pathophysiology and treatment of gastrointestinal disorders are discussed including; gastroesophageal reflux disease (GERD), peptic ulcer disease, irritable bowel disease (IBD), NSAIDs-induced gastroenteropathy and pancreatitis. Special focus was addressed to microbial aspects in the gut including recent achievements in the understanding of function of probiotic bacteria, their interaction with gastrointestinal epithelium and usefulness in the treatment of human disorders. We hope that this book will provide relevant new information useful to clinicians and basic scientists as well as to medical students, all looking for new advancements in the field of gastroenterology.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen