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Intestinal flora, probiotics, prebiotics, synbiotics and novel foods

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INTRODUCTION

Observations in experimental animal models and in humans suggest that the intestinal flora represents a great number of commensal micro-organisms that have evolved in harmony with their host and resulted in improvement of the health of their hosts (1). These bacteria are involved in the normal development of the immune system, in the regulation of the response to pathogens and are essential for the establishment and maintenance of mucosal immune tolerance (2-6). The intestinal flora participates in several physiological processes, such as digestion and motility (7,8) as well as metabolic functions of the organism, such as such as vitamine production. It also provides colonocyte substrates such as butyrate (9). Butyric acid and butyrate which is present in the colonic lumen after digestion of e.g. fiber-rich foods, regulate the differentiation of mucosal cells in the large bowel and induces apoptosis which is important to control inflammation and in avoiding the development of cancer (10,11).

THE IMPORTANCE OF THE UBIQUITOUS INTESTINAL FLORA IN DISEASE PREVENTION

The evidence that the ubiquitous intestinal flora protects man from diseases such asthma, allergy and chronic inflammatory bowel diseases as well as the known molecular mechanisms will be reviewed. Scientific studies on this topic are now possible since new technology permits the study of human fecal samples by using culture-independent PCR protocols. Also the detection and identification of predominant bacteria using the 16S rRNA-gene-targeted group-specific primers and terminal restriction fragment length polymorphisms is now available. The technology allows the study of the composition and the dynamics of the intestinal microflora without the previous time-consuming culture techniques (12-14).

These new advances in technology are paralleled by advances in the knowledge of the basis of host-microbial symbiosis and the interaction of the intestinal microflora with the innate and acquired or adapted immunity.

THE MOLECULAR BASIS OF HOST-BACTERIAL SYMBIOSIS

One of the key advances that are bound to stimulate further research and contribute to resolve the dilemma of using isolated bacteria strain or mixtures of different strains in the regulation of intestinal inflammation (15), synbiotics (16-20), cationic antimicrobial peptides (21) or zwitterionic capsular polysaccharides (ZPS) are the advances in understanding the molecular basis for host-bacterial symbiosis (22).

Recent insight into one of the archetypal molecule of commensal bacteria that mediates development of the host immune system (23) suggest that ZPS is able to modulate the complex ecosystem of the gastrointestinal tract. In this perspective this knowledge now helps to understand old observations, however still relevant today. Strachan from the London School of Hygiene and Tropical Medicine proposed in 1989 (24) that changes in the bacterial flora were responsible for the increase incidence of immune disorders such as allergy ans atopy. At present

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we know this as the hygiene hypothesis (25), although it is still not known whether the absence of commensal bacteria such as *Lactobacilli* and/or *bifidobacteria* predisposes an individual to suffer from inflammatory bowel disease. For example, there is evidence that *Lactobacillus plantarum* induces the synthesis and secretion in macrophages and T-cells derived from the inflamed colon of the anti-inflammatory cytokine IL-10 (26). The ubiquitous flora is able to synthesize ZPS. These bacterial molecules utilize major histocompatibility complex (MHC) II presentation to activate T cells via recognition by alpha/beta T cell receptor proteins (27-29). The seminal observations providing evidence for the biological importance of T cell activation by these zwitterionic polymers was provided not long ago. Human CD4+ T cells stimulated with these molecules *in vitro* and adoptively transferred to rats *in vivo* conferred protection against intra-abdominal abscesses induced by viable bacterial challenge (30,31).

INNATE IMMUNITY

The advances on the innate immunity and its relationship with the acquired immunity are providing a good insight in the mechanisms that control the inflammation in the gut. The identification and functional characterization, including the discovery of mutants which completely abolish NF-κB signal transduction, like the extracellular toll-like receptors (TLRs) and the intracellular NOD/CARDs receptors are providing new facts to understand the relationships between intestinal bacteria & host and intestinal bacteria & clinical disease.

TOLL-RELATED PROTEINS

The toll-related proteins, of which until to date about 12 have been identified, are highly conserved through evolution. The TLRs are expressed on both enterocyte and immune cells. They recognize specific microbial components through leucine rich region domains (LRRs), such as surface determinants, lipopolysaccharide (LPS) of Gram-negative bacteria (TLR2 and TLR4) and unmethylated CpG DNA sequences (TLR9). Their activation induce the production of T-helper 1 (Th1) cytokines through a process dependent on NF-κB activation (32,33). LPS and the Lipid A fraction of LPS is recognized by TLR4. In humans the D299G mutation, affecting the LRR domain of TLR4, is associated with a blunted response to inhalated LPS. An increased expression of this receptor in the epithelial cells of patients with inflammatory bowel diseases has been related to changes in the intestinal flora (34).

NOD-LRR FAMILY OF PROTEINS

Other products of intestinal bacterial flora such as peptidoglycan of Gram-positive bacteria are able to stimulate specific receptors, the family of NOD proteins. These are cytoplasmic receptors which are also characterized by the presence LRRs. NOD1 and NOD2 receptors are able to stimulate the NF-κB. The presence of caspase activating regions in NOD proteins suggests their importance in apoptosis (35).

ACQUIRED IMMUNITY, THE T-CELL COMPONENT

In normal circumstances, the intestinal immune response to the resident bacteria will be limited by a suppressive immune response (a so called TH2 response) with predominance of IgA and IL-10. Recent findings have revealed that specific T regulatory cells, such as Th3, which produce transforming growth factor-β (TGF-β) and Tr1 cells which produce IL-10 downregulate the mucosal inflammatory response. Deficiency of either cytokine or cell type leads to mucosal inflammation as a consequence of an abnormal response to the ubiquitous enteric flora. It has been shown that *Lactobacillus* prevents the development of spontaneous colitis in interleukin 10-deficient mice (36) and continuous feeding of *Lactobacillus plantarum* attenuates the inflammation in this model (37).

BARRIER DISEASES

Crohn's disease and ulcerative colitis are chronic inflammatory autoimmune conditions of the gastrointestinal tract. Other organs, such as the eyes, skin and joints are often affected. IBD may be accompanied by other diseases of autoimmune origin. Recent advances in genetics and in the molecular mechanisms of the proteins coded by these genes have given rise to a new vision in understanding these complex diseases. Activation of specific genes that affect antigen presentation and the handling of cells by innate immunity may lead to autoimmunity with the consequent activation of the MHC and multiple cytokines which are involved in the regulation of acquired immunity.

This constellation of diseases may best be classified as barrier diseases and probably are due to the lack of adaptation of the innate immune system to the environment and the "westernization" of civilization (38). These diseases affect 1-5 of 1,000 individuals and represent a major burden on the national health systems of many countries on different continents. On a world scale, a major challenge would be to generate interventions to prevent the development of these diseases in Asia, Latin America and Africa (39).

INNATE IMMUNITY AND CROHN'S DISEASE

NOD proteins

Studies have demonstrated that a gene coding for NOD2, the *CARD15* gene, is the first gene involved in the susceptibility of Crohn's disease. NOD2 is also expressed in intestinal epithelial and paneth cells. Mutations in the *CARD15* gene may serve as a key component of abnormal innate mucosal responses to luminal bacteria. Therefore, failure in this interaction may contribute to the development of Crohn's disease (40). Although these mutations depend on the microbiological ecology of a population and therefore their frequency varies in different populations. This probably explains that in Asiatic (41-44) and African (45) populations no significant differences in carriers of *CARD15* mutations between patients with Crohn's disease and controls have been found.

Alpha and beta defensins

NOD1 and NOD2 appear to exert bactericidal activity by modulating the epithelial production of defensins and explain the reduced expression of alpha defensins in the ileum of patients with Crohn's disease (46,47).

Human beta-defensins (HBD-2) are increased exclusively in ulcerative colitis but not in Crohn colitis (48). The increased HBD-2 in ulcerative colitis can be diminished by the use of synbiotics (49) and *E.coli Nissle* which has been show to maintain remission in ulcerative colitis (50) induces HBD2 expression (51).

The DNA copy number of the beta-defensin gene cluster on chromosome 8p23.1 is highly polymorphic within the healthy population and there is recent evidence that a lower HBD-2 gene copy number in the beta-defensin locus predisposes to colonic Crohn's disease, most likely through diminished beta-defensin expression (52). Therefore, the regional localizations of CD, either ileal or colonic disease, can be linked to different defects in defensin expression (53).

PROBIOTICS, PREBIOTICS, SYNBIOTICS, NEW OR NOVEL FOODS

Probiotics beneficially affect the host by improving the properties of the indigenous microflora. Probiotics also are important in the intestinal colonization in the maturation of humoral immunity in early infancy (54,55). They are useful in the treatment and prevention of acute infectious diarrhea in infants and children (56); this has been shown in randomized, double-blind, and placebo-controlled trials (57,58). Prophylactic use of orally administered *Lactobacillus GG* significantly reduced the risk of nosocomial diarrhea in infants, particularly nosocomial rotavirus gastroenteritis (59). A systematic Cochrane review in 2004 concluded that probiotics appear to be a useful adjunct to rehydration therapy in treating acute, infectious diarrhea in adults and children. More research is needed to inform the use of particular probiotic regimens in specific patient groups (60). *Saccharomyces boulardii* has been shown recently to be of benefit in antibiotic-associated diarrhea (61).

Prebiotics, such as inulin-type fructans have been shown to improve the metabolic functions of the commensal flora. Clinical and experimental data suggest that they improve the gut mucosal barrier, modulate the trophic functions of the flora. Prebiotics could help in the prevention of inflammatory bowel diseases (62).

It appears that the rationale to use synbiotics, i.e. products resulting from the combination of probiotics and prebiotics, is based on observations showing the improvement of survival of the probiotic bacteria during the passage through the upper intestinal tract. A more efficient implantation in the colon as well as a stimulating effect of the growth of probiotics and ubiquitous bacteria contribute to maintain the intestinal homeostasis and a healthy body. The Japanese introduced the term of functional foods and in Europe the term of new or novel foods is being used. It suggest that certain components may have influence in reducing the blood pressure or reducing cholesterol levels by the use of plant sterols. However, no data exists concerning the use of these nutrients in the management of IBD.

RATIONALE OF USING PROBIOTICS IN INFLAMMATORY BOWEL DISEASE

Despite the therapeutic and prophylactic effects of probiotics they are still not a part of the standard management of inflammatory bowel diseases or of motility disorders of the gastrointestinal tract. In ulcerative colitis the inflammation is confined to the mucosa and submucosa of the colon; the most common symptom of presentation is bloody diarrhea. In Crohn's disease, the inflammation can extend through the intestinal wall and the entire gastrointestinal tract can be involved. Characteristically, areas of inflammation are in continuity with normal mucosa.

Advances in the pathogenesis of both acute and chronic intestinal inflammation suggest that probiotics, prebiotics and/or synbiotics will be helpful in the management of these disorders. The use of *Lactobacillus casei* strain *Shirota* has shown improvement in murine chronic inflammatory bowel disease and is associated with the down-regulation IL-6 and IFN-gamma production in the lamina propria of colonic specimens (63).

A mixture of several probiotics strains have shown beneficial effects in the treatment of mild ulcerative colitis (64-66), in the treatment of pouchitis and maintenance of remission (67,68). Studies of patients with pouchitis in remission using real time PCR have shown that VSL#3 increased the total number of bacterial cells

($p = 0.002$) and modified the spectrum of bacteria in favor of anaerobic species. Taxa specific clone libraries for *Lactobacilli* and *Bifidobacteria* showed that the richness and spectrum of these bacteria were altered under probiotic therapy. Restoration of the integrity of a "protective" intestinal mucosa could therefore be a potential mechanism of the beneficial effects of probiotic bacteria in inflammatory barrier diseases of the lower gastrointestinal tract (69).

BENEFICIAL EFFECTS IN OTHER INDICATIONS

The safety and efficacy of VSL#3 in patients with quiescent IBD who suffered from arthralgia for more than two weeks was recently studied in an open-label trial (70). Based on these preliminary clinical observations, we put forward the hypothesis that probiotics may be helpful in the management of common extraintestinal manifestations such as arthralgia in patients with ulcerative colitis and Crohn's disease (71). However, randomized controlled studies are indicated.

Promising results of probiotics in the treatment of the irritable bowel syndrome are now available (72,73).

BENEFICIAL EFFECTS OF SYNBIOTICS

Not yet many studies have focused on the effects of synbiotics on the intestinal mucosal immune system. Recent studies suggest that probiotics could inhibit NF-κB activation in lymphocytes isolated from the lamina propria of the intestinal biopsy specimens and downregulate inflammatory cytokine secretion from inflamed tissues of patients with active ulcerative colitis (74,75).

Recent observations on the use of synbiotics in ulcerative colitis are encouraging (49,76). A randomized placebo-controlled trial in Japan, in 20 patients with ulcerative colitis, using 100 ml/day bifidobacteria-fermented milk supplementation or placebo during 12 weeks showed that the clinical, the endoscopic and histological activity index was significantly lower in the bifidobacteria-fermented milk group than in the placebo group after treatment. Increases in faecal butyrate, propionate and short-chain fatty acid concentrations were significant in the bifidobacteria-fermented milk, but not the placebo group (77).

An additional advantage of the use of synbiotics is the lack of pathogenicity even in immunocompromised patients and it is safe for both children and adults. Although the strains used for probiotics are chosen from the commensal flora of humans and carry no intrinsic resistance to antibiotics, vigilance regarding the detection of possible rare cases of infection due to probiotics should be maintained. In this case, isolates should be sent to reference centers for molecular characterization and confirmation.

THE CHALLENGE FOR THE FUTURE

The challenge for the experts working on the medical use of functional foods and those working in the field of probiotics, prebiotics, synbiotics and novel foods is to apply the new knowledge which is generated by basic scientists in the field of intestinal flora and the development of synbiotics to improve the treatment and possibly prevent allergy, atopy and inflammatory bowel diseases.

We all can agree with the statement made by Bohm and Kruis (78) that –"probiotic research at the intersection of gastroenterology, immunology and microbiology is highly dynamic in both the basic and the clinical field. Further understanding of the complex molecular mechanisms leading to the effectiveness of probiotics will also spur the development of more successful probiotic formulations".

For the practical gastroenterologists few indications exist that are evidence-based. The development of new technology to assess the effect that different strains of probiotics, alone or in combination, have in the modification of the intestinal flora and the role of these changes in the control of intestinal inflammation allow predicting that probiotics will have one day a definite role in the management of gastrointestinal disease.

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REFERENCES

1. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell 2006; 124: 837-48.
2. Underhill DM, Ozinsky A, Hajjar AM, Stevens A, Wilson CB, Bassetti M, et al. The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. Nature 1999; 401: 811-5.
3. Aderem A, Ulevitch RJ. Toll-like receptors in the induction of the innate immune response. Nature 2000; 406: 782-7.
4. Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, et al. A Toll-like receptor recognizes bacterial DNA. Nature 2000; 408: 740-5.
5. Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, et al. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature 2001; 410: 1099-103.
6. Yamamoto M, Sato S, Hemmi H, Sanjo H, Uematsu S, Kaisho T, et al. Essential role for TIRAP in activation of the signalling cascade shared by TLR2 and TLR4. Nature 2002; 420: 324-9.

7. Massi M, Ioan P, Budriesi R, Chiarini A, Vitali B, Lammers KM, et al. Effects of probiotic bacteria on gastrointestinal motility in guinea-pig isolated tissue. World J Gastroenterol 2006; 12: 5987-94.
8. Bazzocchi G, Gionchetti P, Almerigi PF, Amadini C, Campieri M. Intestinal microflora and oral bacteriotherapy in irritable bowel syndrome. Dig Liver Dis 2002; 34 (Supl. 2): S48-53.
9. Gassull MA. Review article: The intestinal lumen as a therapeutic target in inflammatory bowel disease. Aliment Pharmacol Ther 2006; 24 (Supl. 3): 90-5.
10. Collard TJ, Guy M, Butt AJ, Perks CM, Holly JM, Paraskeva C, et al. Transcriptional upregulation of the insulin-like growth factor binding protein IGFBP-3 by sodium butyrate increases IGF-independent apoptosis in human colonic adenoma-derived epithelial cells. Carcinogenesis 2003; 24: 393-401.
11. Giermasz A, Grzela T, Nowis D, Makowski M, Czajka A, Stoklosa T, et al. Butyric acid enhances in vivo expression of hTNF-alpha in transduced melanoma cell line. Anticancer Res 2001; 21: 4001-4.
12. Brigidi P, Swennen E, Vitali B, Rossi M, Matteuzzi D. PCR detection of Bifidobacterium strains and Streptococcus thermophilus in feces of human subjects after oral bacteriotherapy and yogurt consumption. Int J Food Microbiol 2003; 81: 203-9.
13. Matsuki T, Watanabe K, Fujimoto J, Miyamoto Y, Takada T, Matsumoto K, et al. Development of 16S rRNA-gene-targeted group-specific primers for the detection and identification of predominant bacteria in human feces. Appl Environ Microbiol 2002; 68: 5445-51.
14. Nagashima K, Hisada T, Sato M, Mochizuki J. Application of new primer-enzyme combinations to terminal restriction fragment length polymorphism profiling of bacterial populations in human feces. Appl Environ Microbiol 2003; 69: 1251-62.
15. Karimi O, Pena AS. Probiotics: Isolated bacteria strain or mixtures of different strains? Two different approaches in the use of probiotics as therapeutics. Drugs Today (Barc) 2003; 39: 565-97.
16. Rioux KP, Fedorak RN. Probiotics in the treatment of inflammatory bowel disease. J Clin Gastroenterol 2006; 40: 260-3.
17. Passeron T, Lacour JP, Fontas E, Ortonne JP. Prebiotics and synbiotics: Two promising approaches for the treatment of atopic dermatitis in children above 2 years. Allergy 2006; 61: 431-7.
18. Bengmark S. Synbiotics and the mucosal barrier in critically ill patients. Curr Opin Gastroenterol 2005; 21: 712-6.
19. Sartor RB. Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: Antibiotics, probiotics, and prebiotics. Gastroenterology 2004; 126: 1620-33.
20. Kanamori Y, Sugiyama M, Hashizume K, Yuki N, Morotomi M, Tanaka R. Experience of long-term synbiotic therapy in seven short bowel patients with refractory enterocolitis. J Pediatr Surg 2004; 39: 1686-92.
21. Peyrin-Biroulet L, Vignal C, Dessein R, Simonet M, Desreumaux P, Chamaillard M. NODs in defence: From vulnerable antimicrobial peptides to chronic inflammation. Trends Microbiol 2006; 14: 432-8.
22. Mazmanian SK, Kasper DL. The love-hate relationship between bacterial polysaccharides and the host immune system. Nat Rev Immunol 2006; 6: 849-58.
23. Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell 2005; 122: 107-18.
24. Strachan DP. Hay fever, hygiene, and household size. BMJ 1989; 299: 1259-60.
25. Anderson WJ, Watson L. Asthma and the hygiene hypothesis. N Engl J Med 2001; 344: 1643-4.
26. Pathmakanthan S, Li CK, Cowie J, Hawkey CJ. Lactobacillus plantarum 299: Beneficial in vitro immunomodulation in cells extracted from inflamed human colon. J Gastroenterol Hepatol 2004; 19: 166-73.
27. Cobb BA, Kasper DL. Zwitterionic capsular polysaccharides: The new MHCII-dependent antigens. Cell Microbiol 2005; 7: 1398-403.
28. Stingele F, Corthesy B, Kusy N, Porcelli SA, Kasper DL, Tzianabos AO. Zwitterionic polysaccharides stimulate T cells with no preferential V beta usage and promote anergy, resulting in protection against experimental abscess formation. J Immunol 2004; 172: 1483-90.
29. Cobb BA, Wang Q, Tzianabos AO, Kasper DL. Polysaccharide processing and presentation by the MHCII pathway. Cell 2004; 117: 677-87.
30. Tzianabos AO, Finberg RW, Wang Y, Chan M, Onderdonk AB, Jennings HJ, et al. T cells activated by zwitterionic molecules prevent abscesses induced by pathogenic bacteria. J Biol Chem 2000; 275: 6733-40.
31. Tzianabos AO, Chandraker A, Kalka-Moll W, Stingele F, Dong VM, Finberg RW, et al. Bacterial pathogens induce abscess formation by CD4(+) T-cell activation via the CD28-B7-2 costimulatory pathway. Infect Immun 2000; 68: 6650-5.
32. Korhonen R, Korpela R, Saxelin M, Maki M, Kankaanranta H, Moilanen E. Induction of nitric oxide synthesis by probiotic Lactobacillus rhamnosus GG in J774 macrophages and human T84 intestinal epithelial cells. Inflammation 2001; 25: 223-32.
33. Miettinen M, Lehtonen A, Julkunen I, Matikainen S. Lactobacilli and Streptococci activate NF-kappa B and STAT signaling pathways in human macrophages. J Immunol 2000; 164: 3733-40.
34. Cario E, Podolsky DK. Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. Infect Immun 2000; 68: 7010-7.
35. Inohara N, Ogura Y, Fontalba A, Gutiérrez O, Pons F, Crespo J, et al. Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. J Biol Chem 2003; 278: 5509-12.
36. Madsen KL, Doyle JS, Jewell LD, Tavernini MM, Fedorak RN. Lactobacillus species prevents colitis in interleukin 10 gene-deficient mice. Gastroenterology 1999; 116: 1107-14.
37. Schultz M, Veltkamp C, Dieleman LA, Grenther WB, Wyrick PB, Tonkonogy SL, et al. Lactobacillus plantarum 299V in the treatment and prevention of spontaneous colitis in interleukin-10-deficient mice. Inflamm Bowel Dis 2002; 8: 71-80.
38. Schreiber S, Rosenstiel P, Albrecht M, Hampe J, Krawczak M. Genetics of Crohn disease, an archetypal inflammatory barrier disease. Nat Rev Genet 2005; 6: 376-88.
39. Pena AS. Contribution of genetics to a new vision in the understanding of inflammatory bowel disease. World J Gastroenterol 2006; 12: 4784-7.
40. Hisamatsu T, Suzuki M, Reinecker HC, Nadeau WJ, McCormick BA, Podolsky DK. CARD15/NOD2 functions as an antibacterial factor in human intestinal epithelial cells. Gastroenterology 2003; 124: 993-1000.
41. Croucher PJ, Mascheretti S, Foelsch UR, Hampe J, Schreiber S. Lack of association between the C3435T MDR1 gene polymorphism and inflammatory bowel disease in two independent Northern European populations. Gastroenterology 2003; 125: 1919-20; author reply 1920-1.
42. Inoue N, Tamura K, Kinouchi Y, Fukuda Y, Takahashi S, Ogura Y, et al. Lack of common NOD2 variants in Japanese patients with Crohn's disease. Gastroenterology 2002; 123: 86-91.
43. Leong RW, Armuzzi A, Ahmad T, Wong ML, Tse P, Jewell DP, et al. NOD2/CARD15 gene polymorphisms and Crohn's disease in the Chinese population. Aliment Pharmacol Ther 2003; 17: 1465-70.
44. Sugimura M, Kinouchi Y, Takahashi S, Aihara H, Takagi S, Negoro K, et al. CARD15/NOD2 mutational analysis in Japanese patients with Crohn's disease. Clin Genet 2003; 63: 160-2.
45. Zouiten-Mekki L, Zaouali H, Boubaker J, Karoui S, Fekih M, Matri S, et al. CARD15/NOD2 in a Tunisian population with Crohn's disease. Dig Dis Sci 2005; 50: 130-5.
46. Wehkamp J, Salzman NH, Porter E, Nuding S, Weichenthal M, Petras RE, et al. Reduced Paneth cell alpha-defensins in ileal Crohn's disease. Proc Natl Acad Sci USA 2005; 102: 18129-34.
47. Wehkamp J, Schmid M, Fellermann K, Stange EF. Defensin deficiency, intestinal microbes, and the clinical phenotypes of Crohn's disease. J Leukoc Biol 2005; 77: 460-5.
48. Wehkamp J, Harder J, Weichenthal M, Mueller O, Herrlinger KR, Fellermann K, et al. Inducible and constitutive beta-defensins are differentially expressed in Crohn's disease and ulcerative colitis. Inflamm Bowel Dis 2003; 9: 215-23.
49. Furrie E, Macfarlane S, Kennedy A, Cummings JH, Walsh SV, O'Neil DA, et al. Synbiotic therapy (Bifidobacterium longum/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: A randomised controlled pilot trial. Gut 2005; 54: 242-9.
50. Kruis W, Schutz E, Fric P, Fixa B, Judmaier G, Stolte M. Double-blind comparison of an oral Escherichia coli preparation and mesalazine in maintaining remission of ulcerative colitis. Aliment Pharmacol Ther 1997; 11: 853-8.

51. Wehkamp J, Harder J, Wehkamp K, Wehkamp-von Meissner B, Schlee M, Enders C, et al. NF-kappaB- and AP-1-mediated induction of human beta defensin-2 in intestinal epithelial cells by Escherichia coli Nissle 1917: A novel effect of a probiotic bacterium. Infect Immun 2004; 72: 5750-8.
52. Fellermann K, Stange DE, Schaeffeler E, Schmalzl H, Wehkamp J, Bevins CL, et al. A chromosome 8 gene-cluster polymorphism with low human beta-defensin 2 gene copy number predisposes to Crohn disease of the colon. Am J Hum Genet 2006; 79: 439-48.
53. Wehkamp J, Stange EF. A new look at Crohn's disease: Breakdown of the mucosal antibacterial defense. Ann N Y Acad Sci 2006; 1072: 321-31.
54. Gronlund MM, Arvilommi H, Kero P, Lehtonen OP, Isolauri E. Importance of intestinal colonisation in the maturation of humoral immunity in early infancy: A prospective follow up study of healthy infants aged 0-6 months. Arch Dis Child Fetal Neonatal Ed 2000; 83: F186-92.
55. Gronlund MM, Salminen S, Mykkanen H, Kero P, Lehtonen OP. Development of intestinal bacterial enzymes in infants-relationship to mode of delivery and type of feeding. Apmis 1999; 107: 655-60.
56. Van Niel CW, Feudtner C, Garrison MM, Christakis DA. Lactobacillus therapy for acute infectious diarrhea in children: A meta-analysis. Pediatrics 2002; 109: 678-84.
57. Szajewska H, Mrukowicz JZ. Probiotics in the treatment and prevention of acute infectious diarrhea in infants and children: A systematic review of published randomized, double-blind, placebo-controlled trials. J Pediatr Gastroenterol Nutr 2001; 33 (Supl. 2): S17-25.
58. Szajewska H, Mrukowicz JZ. Probiotics in prevention of antibiotic-associated diarrhea: Meta-analysis. J Pediatr 2003; 142: 85.
59. Szajewska H, Kotowska M, Mrukowicz JZ, Armanska M, Mikolajczyk W. Efficacy of Lactobacillus GG in prevention of nosocomial diarrhea in infants. J Pediatr 2001; 138: 361-5.
60. Allen SJ, Okoko B, Martinez E, Gregorio G, Dans LF. Probiotics for treating infectious diarrhoea. Cochrane Database Syst Rev 2004: CD003048.
61. Can M, Besirbellioglu BA, Avci IY, Beker CM, Pahsa A. Prophylactic Saccharomyces boulardii in the prevention of antibiotic-associated diarrhea: A prospective study. Med Sci Monit 2006; 12: PI19-22.
62. Guarner F. Inulin and oligofructose: Impact on intestinal diseases and disorders. Br J Nutr 2005; 93 (Supl. 1): S61-5.
63. Matsumoto S, Hara T, Hori T, Mitsuyama K, Nagaoka M, Tomiyasu N, et al. Probiotic Lactobacillus-induced improvement in murine chronic inflammatory bowel disease is associated with the down-regulation of pro-inflammatory cytokines in lamina propria mononuclear cells. Clin Exp Immunol 2005; 140: 417-26.
64. Guslandi M, Giollo P, Testoni PA. A pilot trial of Saccharomyces boulardii in ulcerative colitis. Eur J Gastroenterol Hepatol 2003; 15: 697-8.
65. Zocco MA, dal Verme LZ, Cremonini F, Piscaglia AC, Nista EC, Candelli M, et al. Efficacy of Lactobacillus GG in maintaining remission of ulcerative colitis. Aliment Pharmacol Ther 2006; 23: 1567-74.
66. Gionchetti P, Rizzello F, Lammers KM, Morselli C, Sollazzi L, Davies S, et al. Antibiotics and probiotics in treatment of inflammatory bowel disease. World J Gastroenterol 2006; 12: 3306-13.
67. Gionchetti P, Amadini C, Rizzello F, Venturi A, Campieri M. Treatment of mild to moderate ulcerative colitis and pouchitis. Aliment Pharmacol Ther 2002; 16 (Supl. 4): 13-9.
68. Gionchetti P, Rizzello F, Poggioli G, Morselli C, Lammers KM, Campieri M. Probiotic therapy to prevent pouchitis onset. Dis Colon Rectum 2005; 48: 1493; author reply 1493-4.
69. Kuhbacher T, Ott SJ, Helwig U, Mimura T, Rizzello F, Kleessen B, et al. Bacterial and fungal microbiota in relation to probiotic therapy (VSL#3) in pouchitis. Gut 2006; 55: 833-41.
70. Karimi O, Peña AS, van Bodegraven AA. Probiotics (VSL#3) in arthralgia in patients with ulcerative colitis and Crohn's disease: A pilot study. Drugs Today (Barc) 2005; 41: 453-9.
71. Karimi O, Peña AS. Probiotics in arthralgia and spondyloarthropathies in patients with inflammatory bowel disease. Prospective randomized trials are necessary. Rev Esp Enferm Dig 2005; 97: 570-4.
72. Camilleri M. Probiotics and irritable bowel syndrome: Rationale, putative mechanisms, and evidence of clinical efficacy. J Clin Gastroenterol 2006; 40: 264-9.
73. Harris LA, Chang L. Irritable bowel syndrome: New and emerging therapies. Curr Opin Gastroenterol 2006; 22: 128-35.
74. Duncker SC, Lorentz A, Schroeder B, Breves G, Bischoff SC. Effect of orally administered probiotic E. coli strain Nissle 1917 on intestinal mucosal immune cells of healthy young pigs. Vet Immunol Immunopathol 2006; 111: 239-50.
75. Bai AP, Ouyang Q, Xiao XR, Li SF. Probiotics modulate inflammatory cytokine secretion from inflamed mucosa in active ulcerative colitis. Int J Clin Pract 2006; 60: 284-8.
76. Haskey N, Dahl WJ. Synbiotic therapy: a promising new adjunctive therapy for ulcerative colitis. Nutr Rev 2006; 64: 132-8.
77. Kato K, Mizuno S, Umesaki Y, Ishii Y, Sugitani M, Imaoka A, et al. Randomized placebo-controlled trial assessing the effect of bifidobacteria-fermented milk on active ulcerative colitis. Aliment Pharmacol Ther 2004; 20: 1133-41.
78. Bohm SK, Kruis W. Probiotics: Do they help to control intestinal inflammation? Ann N Y Acad Sci 2006; 1072: 339-50.