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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 vitamine production. It also provides colonocyte substrates such as (9) butyric acid and butyrate. This process 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 its 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 are available and 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

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) it is essential to understanding the molecular basis of the 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. This knowledge now helps to understand old observations, still relevant. 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 and atopy. Presently known as the hygiene hypothesis (25), although it is still not

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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 behave as major histocompatibility complex (MHC) II and activate T cells via recognition by alpha/beta T cell receptor proteins (27-29). 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 of 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 the understanding of such complex diseases. Activation of specific genes that affect antigen presentation and the handling of cells by innate immunity may lead to autoimmunity with the subsequent 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 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). The presence of these mutations may depend on the microbiological ecology of a population and therefore their frequency varies in different populations. 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 may contribute 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). HBD-2 in ulcerative colitis can be influenced by the use of synbiotics (49). *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 (NOVEL) FOODS

Probiotics beneficially affect the host by improving the properties of the indigenous microflora. Probiotics also are important in the intestinal colonization and 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, in particular nosocomial rotavirus gastroenteritis (59). A systematic Cochrane review in 2004 concluded that probiotics are a useful adjunct to rehydration therapy in treating acute, infectious diarrhea in adults and children. More research is needed to explore the particular probiotic regimens in specific patient groups (60). *Saccharomyces boulardii* has been shown recently its benefit in antibiotic-associated diarrhea (61).

Prebiotics, such as inulin-type fructans have been improved the metabolic functions of the commensal flora. Clinical and experimental data suggest an improvement of the affected gut mucosal barrier, as well as the trophic functions of the flora. Prebiotics may 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 "functional" foods. In Europe the term "new" or "novel" foods is used. This concept suggests that foods with proven natural or added beneficial components are important to improve or maintain health. Examples are certain peptides found in Japan that have found to be helpful 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 therapeutic and prophylactic effects, probiotics are not 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, any part of 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 may 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 using real time PCR

in patients with pouchitis in remission 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 has been 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). In inflammation 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).

BENEFICIAL EFFECTS OF SYNBIOTICS

Few studies have focused on the effects of synbiotics on the intestinal mucosal immune system.

Recent observations on the use of synbiotics in ulcerative colitis are encouraging (49,76). A randomized placebo-controlled trial, 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. Increases in faecal butyrate, propionate and short-chain fatty acid concentrations were significant in the bifidobacteria-fermented milk, but not in 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 case of suspition, isolates should be sent to reference centers for molecular characterization and confirmation.

THE CHALLENGE FOR THE FUTURE

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

We all can agree with the statement made by Bohm and Kruis (78) –"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 control of intestinal inflammation allows the prediction that probiotics will have a place in the standard management of gastrointestinal disease.

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