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REVIEW

Bioecological control of inflammatory bowel disease

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Summary
It is today generally accepted, that the intestinal bacterial flora is deeply involved in the pathogenesis of human inflammatory bowel diseases (IBDs), although the exact presence of unwanted or lack of specific crucial bacteria are not yet known. Westerners lack to large extent important immunomodulatory and fibre-fermenting lactic acid bacteria (LAB), bacteria which are present in all with a more primitive rural lifestyle. Acute reduction of flora is observed in disease, including IBD, as well as in mental and physical stress. Some observations suggest the mucosa has lost its ability of holding back the pathogenic flora and prevent close contacts between resident microflora and the epithelial surface. Among the manifestations of IBD are increased inflammation and coagulability, impaired cellular membrane function, exaggerated nitric oxide production and impaired short-chain fatty acid production. Animal studies suggest, in addition to reduced flora, an intimate association with immunostimulatory DNA, malfunctioning trifoil factors, increased splanchnic metabolism and reduced availability of natural antioxidants. Treatment with plant fibres, antioxidants and sometimes probiotics have had limited success. The most dramatic effects are seen in the few cases where total faecal replacement (TFR) has been tried. The general experience this far is that the best effects are obtained with compositions of probiotics rather than with single LAB treatments.

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The role of flora

The lower intestine of mammals and vertebrates contains normally an extremely dense and diverse microflora of non-pathogenic microbes, which salvage the energy of otherwise indigestible dietary carbohydrates (plant fibres). These microbes are also important for control of various pathogenic microorganisms, which compete for the same available energy. The intestinal mucosa is normally highly adapted to the presence of these so-called commensals. The IgA secreted in the presence of the commensals accounts for $>70\%$ of total-body immunoglobulin production.[1] Inflammation is, despite the presence of billions and billions of bacteria, relatively rare in the intestine, which might be explained by the fact that the commensal flora has the capacity to induce B and T cells, and this without triggering the inflammation, typical to a pathogenic infection.

Immune system and dendritic cells (DCs)

Antigen-presenting cells (APCs) such as monocytes, DCs and macrophages are responsible for detecting microbes and presenting their antigenic structure to the T cells. Both pathogens and non-pathogens express specific molecular pattern, which are identified by the Toll-like receptors (TLRs) of the innate immune system. APCs are found in most tissues. Especially DCs seem to have a disproportionate large effect on innate immune responses. They have a pivotal position in the intersection of innate and adaptive immunity and are suggested to be gatekeepers to/conductors of the immune response. Intestinal DCs have a unique ability to, without disrupting the barrier function, continuously sample bacterial and other antigens in the gut by sending processes into the gut lumen. This function is important as IgA-coated bacteria such as commensals will otherwise not penetrate mucosal surfaces. It has been observed that DCs obtained from mucosal tissues preferentially induce a Th2 phenotype whereas splenic DCs induce a Th1 phenotype.[2] DCs possess a special ability to activate naïve T cells, to determine if active or non-responsive (tolerance) response, and type of T cell response: Th1, Th2 or un-polarized response. Neonatal immune response is initially more leaning towards a Th2 profile, but will during the process of gut colonization be driven towards a more balanced Th1/Th2 immune response.

Gut DCs are localized at interfaces to the environment: lamina propria, Peyer's patches, cryptopatches, lymphocyte-filled villi and adjacent mesenteric lymph nodes (MLNs). One DC is said to influence the function between 300 and 1000 T cells.[3] DCs recognize and respond to microbial molecular structure through a family of pattern recognition receptors designated as TLRs, which are suggested to be key to regulation of the immune response but also to constitute a link between innate and adaptive immune functions. DCs are constantly trafficking in and out of the gut compartment; in steady state with a turnover time said to be of a few days.[4] In response to the signals received from the environment the DCs undergo maturation and migrate to adjacent draining lymph nodes. It is in this process that they develop their expression of MHC class II and co-stimulatory molecules, and acquire the ability to stimulate naïve T cells. The monocytic response to commensal bacteria, and cytokines produced, changes dramatically when monocytes differentiate into DCs, and become largely unresponsive to commensal (probiotic) Gram-positive bacteria.[4] It was recently shown that DCs can retain small numbers of commensals inside the cell for several days,[1] in sharp contrast to macrophages, which kills them within 4 h.[5] As a matter of fact, all live commensal bacteria isolated from MLNs are residing inside DCs.[1]

The epithelial structures, which form the intestinal epithelial barrier, consist in apical plasma membranes and intracellular tight junctions (TJs) of the enterocytes. The bilipid composition of the enterocyte membrane provides a diffusion barrier against transcellular permeation of large, water-soluble molecules. The TJs are normally sealed against paracellular penetration of noxious substances from the lumen including bacteria, bacterial toxins, bacterial by-products, digestive enzymes, degraded food products and various luminal antigens.[6] Patients with inflammatory bowel disease (IBD) are known to have a defective intestinal TJ barrier and an increased intestinal permeability, resulting in increased translocation of both commensals and pathogens, but also of larger molecules such as lactulose and cellobiose.

Flora and immune response

It is generally agreed that TNF-α plays a central role in the inflammation associated with IBD, and it was recently shown, that the TNF-α-induced increase in permeability

occurs over NF-κB activation with subsequent cytoplasmic-to-nuclear translocation of NF-κB, increased NF-κB binding to DNA binding sites, down-regulation and alteration in junctional localization of the zonula occludens-1 proteins.[3] DCs have themselves the capacity to express TJ proteins in order to preserve the integrity of the intestinal barrier and prevent translocation. The pattern of cytokine production by the DCs is aimed to stimulate IgA production and induce non-responsiveness by T cells to orally fed antigens and to commensal flora. DCs have the pronounced ability to produce a whole range of pro- and anti-inflammatory cytokines, including IL-10, IL-12 and TGF-β, depending on the nature of stimulus. Also cell wall components of lactic acid bacteria (LAB) stimulate the immune response: for example components from *Bifidobacterium longum* predominantly stimulate the Th2 cytokine IL-10 while lipopolysaccharides (LPS) predominantly induce Th1 cytokine IL-12, but also some IL-10.[3] Observations like these support an assumption that the modulation of the inflammatory response by the DCs is, at least partly, influenced by flora and supplied probiotic bacteria. A recent study suggests that non-pathogenic intestinal Gram-negatives (*Escherichia coli*, *Bacteroides vulgatus*, *Veillonella parvula*, *Pseudomonas aeruginosa*) but not Gram-positives (*Bifidobacterium adolecentis*, *Enterococcus faecalis*, *Lb plantarum* and *Staphylococcus aureus*) prime DCs for either Th1 or Th2 development.[7] It is demonstrated that Gram-negative bacteria- (GnB) matured monocyte-derived DCs (moDCs) express elevated levels of p19 and p28mRNA, e.g. critical subunits of IL-23 and IL-27. IL-23 functions primarily on effector T cells[8] while IL-27 has profound effects on naïve Th cells and is of great importance for initial and early IFN-γ production,[9] alone or in synergy with IL-12. A study comparing six different lethally irradiated LAB (*Lb reuteri* DSM 12246, *Lb plantarum* Lb1, *Lb fermentum* Lb20, *Lb casei* subsp *alactus* CHCC3137, *Lb plantarum* 299 V and *Lb johnsonii* Lal) demonstrated great differences in their ability to activate murine DC. The strains with the greatest capacity to induce IL-12 were also the most effective to upregulate MHC class II and B7-2 (CD86), indicative of CD maturation.[10] Striking differences were observed both for IL-12 and TNF-α: ranging from strong (*Lb. casei*) to no (*Lb. reuteri*) influence in falling order: *Lb casei* $\gg$ *Lb plantarum* Lb1 $>$ *Lb fermentum* Lb20$\sim$*Lb johnsonii* La1$\sim$*Lb plantarum* 299v $\gg$ *Lb reuteri*. These observations suggest that there exists a possibility that the Th1/Th2/Th3-driving capacity of gut DCs varies with the composition of the gut flora. A recent study looked at the capacity of a probiotic cocktail, VSL#3 (*Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium breve*, *Lb acidophilus*, *Lb casei*, *Lb delbruecki* subsp *bulgaricus* and *Streptococcus salivarius* subsp *thermophilus*) (Sigma-Tau, Pomezia, Italy, and VSL Pharmaceuticals, Fort Lauderdale, USA) to influence the cell surface antigen expression and cytokine production by murine CDs.[11] Supply of the probiotic composition did significantly upregulate the expression of CD80;CD86; CD40 and major histocompatibility complex class II I-A as well as the ability to induce allogeneic T-cell proliferation and enhance of IL-10 release. Furthermore, it was observed that the response to the composition of LAB was significantly stronger than to individual LABs (Drake M, pers. comm..).

Chronic intestinal inflammation

It is today generally accepted, that the intestinal bacterial flora is deeply involved in the pathogenesis of human IBDs, although the exact presence of unwanted or lack of specific crucial bacteria are not yet known. Among the observations to support such a view are the fact that luminal contents in IBD trigger inflammation,[12] and that diversion of faecal stream is effective in ameliorating especially Crohn's disease (CD).[13] The observation that the incidence of CD, but not UC, increases with reduced sero-prevalence of *Helicobacter pylori* (odds ratio 0.18) is interesting.[14] Similar differences in seropositivity are, however, also reported with regard to *Yersinia*, *Escherichia coli*, *Salmonella*, *Listeria*, MAC other microbial antibodies, most likely an indicating a generally broken mucus barrier in CD.

Idiopathic IBDs, especially CD, are rare in rural areas of developing countries, and are globally significantly more common in the North than South, particularly distinct in Europe, where the incidence is several-fold higher in Scandinavia than in Southern Europe.[16] Increased domestic hygiene seems to dramatically increase the incidence, which observation has lead to the suggestion that the innate immune system has never had a chance to fully develop and mature in those predisposed to IBD, explaining the exaggerated immune response also to small immunological challenges such as minor infections.[14] In support of the role of exorbitant hygiene as a link to IBD are observations such as that the incidence of CD is increased 5-fold with the availability of hot water in the house, and 3-fold when separate toilets are available.[15] However, studies of flora on populations with higher hygiene standards has this far failed to show any noticeable reduction of bacterial burden in terms of absolute numbers and diversity of intestinal bacteria. However, a significantly richer commensal flora have in observed in those consuming larger amounts of fresh plant foods. Fresh fruits and vegetables seem to provide a daily ''booster dose'' of a variety of bacteria, which normally reside on the surface of the plants.

Human commensal flora

Only 10% of the total cells within the human body are eukaryotic. The large majority of cells, 90% in a healthy individual, are prokaryotic, e.g. represent a population living in or on the host, collectively referred to as microbionta. The large intestine of the healthy individual contains an especially rich flora, consisting in $>$400 microbial species with a density of 10,[14] totally weighing 1–2 kg. However, about 99% of the human GI microbionta is constituted by 30–40 microbial species.[17] Among the bacterial genera, which are commonly detected as components of the intestinal microflora in humans are: *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Enterococcus*, *Eubacterium*, *Fusobacteria*, *Peptostreptococcus*, *Ruminococcus*, *Lactobacillus* and *Escherichia*. Each human being has his/her own unique collection especially of *Bifidobacterium* and *Lactobacillus* strains, and can well be identified on the basis of the personal intestinal microflora.[17] The flora is established early in life and new species cannot be added to the flora later in life. Probiotic bacteria supplied later in life

will stay at the best for a few days but will as we know it today never permanently colonize the individual.

The different microorganisms live collectively in symbiosis with each other but also in a more or less intensive competition with the host. Single microorganisms, which each have a narrow metabolic function, work in synergy with each other and provide nutrients for each other and for the host. It is likely that millions of different nutrients, vitamins and antioxidants are released and absorbed as a result of microbial enzymes in the intestine. In addition, microbiota provide colonization resistance—a first line of defence against invasion of exogenous pathogenic organisms or indigenous opportunists.[18] Fibre-fermenting bacteria such as some *Lactobacilli* are significantly more dominating among those, who consume larger amounts of plant fibres and fermented foods. Every rural Asian and African person possesses a more or less richer and diversified flora, not only of LABs, but also of other non-pathogenic Gram-negatives. *Lb plantarum* is one of the dominating species in fermented plant foods: sour dough, sauerkraut, green olives, natural wines, beers and in most Third world staple foods: African *ogi, kenkey and wara*[19] and Asian (Indonesian) *tempeh*. A study reported in 1983 demonstrated that *Lb plantarum* is found in only app 25% of omnivorous Americans and in about 2/3 of vegetarian Americans.[20] Nothing supports the assumption that this has changed to the better. A more recent Scandinavian study suggest the largest LAB taxa on the rectal mucosa in healthy humans are *Lb plantarum*, *Lb rhamnosus* and *Lb paracasei* ssp *paracasei,* isolated in 52%, 26% and 17%, respectively.[21] The colonization rates for commonly used milk-born probiotic bacteria such *Lb casei*, *Lb Reuteri* and *Lb acidophilus* were according to the same study only 2%, 2% and 0% respectively. Great differences in volume and diversity of flora have also been observed between different human cultures. It is reported that Scandinavian children have compared to Parkistani children a much reduced flora.[22] Interestingly, great differences are observed also between people living in close geographic proximity, but with obvious differences in lifestyle. Such a difference was observed between Swedish and Estonian children during the Sovjet occupation of Estonia,[23] a difference which most likely has disappeared with westernization of lifestyle in the Baltic countries.

Flora and modern lifestyle

It is clear that many species in human flora do not tolerate Western lifestyle, easting habits and stress. An extreme example is the flora of cosmonauts, who on return from space flights have lost most of their commensal flora including *Lactobacillus* species such as *Lb plantarum* (lost to almost 100%), *Lb casei* (lost to almost 100%), *Lb fermentum* (reduced by 43%), *Lb acidophilus* (reduced by 27%), *Lb salivarius* (reduced by 22%) and *Lb brevis* (reduced by 12%).[24] These changes could be attributed to poor eating (dried food, no fresh fruits and vegetables) and consequently much reduced supply of plant fibres and antioxidants, but also to mental and physical stress and eventually lack of physical exercise. Clearly many Westerners live today a type of ''astronaut-like lifestyle'' with unsatisfactory consumption of fresh fruits, vegetables, too much stress and no or little outdoor/sport activities. Nor does the flora tolerate chemicals including pharmaceuticals. Flora is generally reduced in disease, and severely sick—critically ill patients have most often lost their entire *Lactobacillus* flora.[25] The dramatic change in physical environment at mucosal surfaces in the critically ill, induced by disease and lack of nutrients; pH, redox state, osmolarity and counter-regulatory hormones seems to significantly change the virulence of the PPMs. There are more nerve endings in the colon than in any other organ in the body. Increased luminal release of norepinephrine in mental or catabolic stress is a strong inducer of increased virulence of the luminal bacteria.[26] Much suggest that PPMs, normally indolent colonizers, under stress changes their phenotype and become sometimes life-threatening pathogens.[27] Alverdy et al.[27] also suggests that these bacteria will adhere to the host cell walls for nutritional purposes, and mentions as an example *Escherichia coli*, which induces contact-depending activation of the signal transduction pathways within the mucosal cells, resulting in disruption of epithelial TJ permeability, cytokine release, activation of neutrophils and cellular apoptosis.

Flora in IBD

It was suggested by Gilliland and Speck[28] already in the 1970s that patients with UC have a deranged flora, which could partly be corrected by supply of *Lactobacillus* species. We demonstrated about 15 years later a significantly reduced flora in patients with UC. In contrast to previous studies, our studies were based on mucosal biopsies and not on stool samples. The material consisted in 30 patients with ulcerative colitis (UC; 12 with active and 18 with inactive disease) and 30 control patients.[29] All patients with active disease showed, in sharp contrast to patients with inactive disease, a significant reductions not only in *Lactobacillus* flora but also in total numbers Gram-negatives.[29] We also observed that 10/20 patients with UC had a significant overgrowth of *Proteus mirabilis* in contrast to 0/20 control patients. A more recent study confirms significant reductions in numbers of LAB subspecies in UC patients (average 18 subspecies) compared to controls (average 32 subspecies).[30] In this study, a more frequent growth of *Bacteroides thetaiotaomicron* was observed in UC patients (8/10 patients) compared to controls (4/10 patients). A reduction in density of endogenous *Lactobacillus* and *Bifidobacteria* has also been reported in CD.[31,32]

Only some 10–40% of the very complex colonic microbionta has this far been identified as the majority of colonic bacteria are fastidious in culture. One can still not exclude that the flora harbours one, several or many unidentified pathogens, which are responsible for some not fully understood conditions such as autism, IBD and rheumatoid arthritis. It is a great step forward when new DNA-based methodologies has been made available. A study of 57 patients with active IBD and 46 controls, using 16S rDNA based single-strand conformation polymorphism (SSCP) fingerprint, cloning experiments, and real-time polymerase chain reaction (PCR) was recently published.[33] A total of 1019 clones (most of them were identical) were fully sequenced to investigate the overall diversity of the

intestinal flora. The main bacterial groups identified were *Streptococcus* species (34%), *Ruminococcus* species (22%). *Escherichia* (13%) and *Clostridium* (6.5%). About 1% or less was found to be *Enterobacter*, *Fusobacterium*, *Peptostreptococcus* and *Eubacterium*. A recent observation that the diversity of microflora is reduced in IBD is of great interest: in CD with app 50% and in UC with app 30%. Most of the loss of diversity is seen among normal anaerobic bacteria such as *Bacteroides* species, *Eubacterium* species and *Lactobacillus* species. However, it still remains to be investigated whether the observed changes are pathogenic, secondary to disease or methodical, since no correlation was done to cultures or to FISH.

The mucosa in IBD seems, in addition to the observed derangement of flora, loose its ability to holding back the pathogenic flora and prevent a close contact.[34] The microbial density at intestinal mucosal surface increases significantly in parallel to increased severity of disease. Patients with $>10{,}000$ cfu/ml have a thick bacterial "band" attached to the mucosa, and patients with $>50{,}000$ cfu/ml show in addition inclusions of polymorphic bacteria also inside enterocytes close to the lamina propria. A recent study reports high prevalence of adherent-invasive *E. coli* AIEC) in the intestinal mucosa of patients with CD but not with UC.[35] AIEC strains were found in 22% of CD chronic lesions vs. 6% in controls. The prevalence of AIEC was even higher (36%) in so-called neoterminal ileum e.g. the last 10 cm of ileum before an ileo–colic anastomosis. Ability of *Escherichia coli* strains to survive and replicate within macrophages has been clearly demonstrated, but its role in initiation and perpetuation of inflammation is not yet defined. It is, however, reasonable to assume that bacterial strains, which can invade and survive within macrophages, can also translocate across the intestinal barrier, move into deeper tissues, induce fibroplasia and form granulomas. CD patients have recently been reported to suffer mutation in the *NOD2/CARD15* gene.[36,37] CARD15 is an integrate part of the innate immune system and is mainly expressed at the basal level of phagocytes including monocytes, macrophages, DCs and polymorphoneclear cells. Recent studies suggest that the expression of CARD15 is not confined to only these cells, as also epithelial cells and especially Paneth cells are involved. Paneth cells, which have a high density in terminal ileum, secrete various antimicrobial substances such is lysozyme, phospholipase A2 and various defensins, and a deranged Paneth cell function might well be responsible for some depressed antimicrobial and anti-inflammatory functions in CD patients.

The intestinal epithelium not only provides a physical barrier to invasion of luminal bacteria and other potential invaders, it also provides a series of antimicrobial peptides to limit the access of various potentially pathogenic microorganisms. One such class of peptides, which recently got attention, is the family of defensins, consisting in cationic arginine-rich peptides with a molecular weight of 3–5 kd. It is of special interest that recent studies have identified a decrease in human β-defensin 1(HBD-1) in both UC and CD in addition to a specific lack in induction of human β-defensin 2 and 3 (HBD-2, HBD-3), seen only in CD.[14,38] This is of special interest as it also was shown that probiotics such as *Escherichia coli* Nissle and some other probiotics, both single *Lactobacillus* species and a combination of some *Lactobacillus* species (Synbiotic 2000, see below) in contrast to more than 40 different other *Escherichia coli* strains tested, strongly induce expression of HBD-2 in Caco-2 intestinal epithelial cells.[39]

Mucus function in IBD (Fig. 1A–D)

The mucus layer covering the mucosa in healthy individuals is important for maintenance of intestinal and general health. A recent study using fluorescent in situ hybridization with bacterial 16S RNA probes (Swidsinski et al., this issue) reports that mucus portion adjacent to the mucosa was free of bacteria in approx. 80% of the normal appendices obtained by appendicectomies and in biopsies from healthy controls (Fig. 1A). The thickness of the mucus layer decreases with increasing severity of inflammation. The epithelial surface will in parallel to increased degree of inflammation show increasing bacterial adherence, increasing epithelial tissue defects and increasing deep mucosal infiltration with bacteria and leucocytes. In inflammatory conditions such as UC, so-called self-limiting colitis (SLC), and acute appendicitis will bacteria and leucocytes, in sharp contrast to the mucus of healthy individuals, infiltrate and even penetrate the mucosal layer (Fig. 1B). Swidsinski et al. did also observe an inverse correlation between the concentration of bacteria within mucus and the numbers of leucocytes.

Swidsinski et al. did also with fluorescent in situ hybridization with bacterial 16S RNA probes study the effects of various established treatments. Treatment with azathioprine reduced dramatically the infiltration of leucocytes into the mucus layer, while 5-ASA treatment, demonstrated no influence on migration of leucocytes into the mucus layer. However, 5-ASA treatment did induce a dramatic decrease in mucosal concentrations of bacteria in all studied IBD patients. In sharp contrast to this, the azathioprine treatment induced an in average 28-fold increase concentration of mucosal bacteria compared to 5-ASA group treated IBD patients and a 1000-fold increase in comparison to healthy controls. Furthermore, it was observed that 5-ASA and azathioprine, when used in combination seemed to neutralize each other's effects on the mucosal flora and the barrier function as no statistically significant difference was observed when compared to untreated IBD patients either in mucus leucocyte migration or in concentrations of bacteria in the mucus layer.

Swidsinski et al. did also study the effects combined metronidazole and ciprofloxacin therapy on the mucus layer in IBD. The presence and concentrations of bacteria were significantly reduced both in patients treated for only 2 h, 1 day and in patients treated for 7–14 days (Fig. 1C). However, these suppressing effects of antibiotics on the mucosal flora, was accompanied by a dramatic and massive rebound effect on cessation of supply of antibiotics as the concentrations of mucosal bacteria were already after 1 week dramatically increased (Fig. 1D), and remained, compared to patients with no antibiotic treatment, increased for at least a period of 5 months; the number of counted mucus bacteria being after 1–4 weeks $13.2 \pm 4.3 \times 10^{10}$/ml, after 3–5 months 5.8 ± 2; 6–9 months 1.1 ± 0.8 compared $0.5 \pm 0.4 \times 10^{10}$/ml in untreated IBD patients.

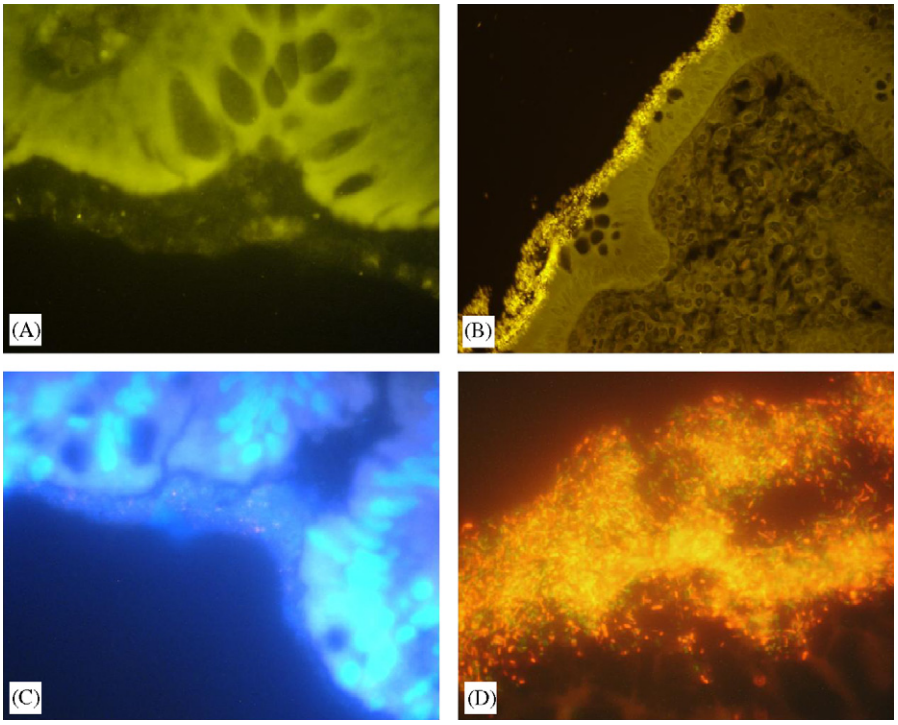

Figure 1 Microscopy of hybridized biopsies of colonic mucosa. (A) Normal mucosa covered with mucus. This section is hybridized with Eub338 Cy 3 probe, regarded as universal for all bacteria. The histologic structures can be well perceived. The mucus contains no bacteria. (B) Mucosa from a patient with UC. The bacteria are visualized with Eub 338 Cy3 probe and have yellow fluorescence. A prolific bacterial biofilm is observed. (C) Mucosa after supply of antibiotics. The numbers of bacteria within the mucus of a patient with UC are markedly reduced already 2 h after induction of antibiotic treatment. The DAPI stain (blue fluorescence showing all DNA structures) is covered by the yellow fluorescence of bacteria hybridized with a Eub 338 Cy3 stained probe. Only single bacteria within the mucus hybridize positively with this probe. The mucus contains also DAPI stained clouds which might correspond to autolytic bacteria, which do not hybridize with bacterial probes. D. Mucosa from a patient with UC 1 week after cessation of antibiotic therapy. The bacterial biofilm has completely recovered and is enhanced. The hybridization is performed with Bac 303 Cy3 probe representing Bacteroides group (yellow fluorescence), Ebac Cy5 probe representing Enterobacteriaceae (red fluorescence) and Eub 338 FITC probe, representative for all other bacteria (green fluorescence). By courtesy of A Swidsinski.

State of exaggerated inflammation

Increased inflammation and coagulability: Chronic diseases from Alzheimer to prostatic hyperplasia are all expressing an exaggerated acute phase response, which probably because of the continuous long-lasting inflammatory pressure should be called "chronic" phase response—see further Bengmark.[40] Signs of increased inflammation are often observed in various chronic conditions during weeks, months, sometimes years, before obvious clinical signs of manifest disease. Not only cytokines such as IL-6 and TNF-α, but also acute phase proteins such as C-reactive protein (CRP), fibrinogen and PAI-1 are significantly elevated, changes, which signal a state of ongoing inflammation and increased coagulation somewhere in the body. Associated manifestations are increased deposits of fibrin on vascular endothelium accompanied by increased incidence of thrombosis. High levels of insulin, blood glucose and non-esterified fatty acids (NEFAs) are commonly observed. Several similarities exist between IBD (and RA) and other metabolic syndrome-associated chronic diseases, and some studies report also increased incidence of insulin resistance also in IBD patients,[41] but a clear association has never been fully verified. When observed, it seems more likely that these manifestations are secondary, as they commonly disappear when the disease enters into remission. Clearly, chronically elevated levels of pro-inflammatory cytokines and of increased coagulability are seen in IBD,[42,43] as are signs of both epithelial and endothelial dysfunction, changes demonstrated to significantly relate to disease activity, especially in UC. Changes in IGF-1 and in its binding protein (IGFBP) have also been observed and shown to relate to the disease activity.[44] mRNA of both IGF-1 and IGFBP, and of TGFβ express high activity in the intestinal wall of patients with active disease of CD, changes which have been suggested responsible for the increased collagen synthesis and increased connective tissue in the intestinal wall, characteristic of CD.[45] Such changes are in CD observed in all layers of the intestinal wall but in UC only in lamina propria and the submucosa.[46] When present, such changes will indicate a malfunctioning innate immune system and an exaggerated inflammatory reaction, manifestations known to be modulated by flora and supplied probiotics.

Impaired cellular membranes: Polyunsaturated fatty acids (PUFAs) are important ingredients in cellular membranes, especially of prokaryotic cells, and are precursors of

eicosanoids. PUFAs are important for microbial hydrophobicity and adherence of microbes, also non-pathogenic, to mucus and epithelial surfaces.[47] Significantly increased plasma levels of both omega-3 and omega-6 PUFAs have been described both in active and inactive CD and UC,[48,49] but its pathogenetic significance is not fully understood. Increased levels of platelet-activating factor (PAF),[50] an endogenous phospholipids, and of the eicosanoids PGE_2 and LBT_4[51] are described both in UC and CD. Omega-6 fatty acids are known to upregulate inflammation and increase epithelial permeability.[52] Free PUFAs seem to inhibit the function of *lactobacillus* species. An important recent observation is that presence of PUFAs, even in lower concentrations than provided in fermented dairy products such as yoghurt, makes *Lactobacillus* to loose their ability to adhere to mucous membranes and to grow, an observation suggesting that dairy products might not be ideal as carriers of probiotics.[53]

Exaggerated nitric oxide production: The role of nitric oxide (NO) in IBD is far from fully understood. Clearly, NO production is exaggerated and NO-synthase (NOS) activities dramatically increased of colonic mucosa in IBD. A correlation between increase in NO and loss of mucosal barrier function has also been documented.[54,55] However, clear anti-inflammatory effects of NO are observed in the stomach, which seems to suggest that the NO effects are either depending on the tissue affected, the type of inducing enzyme, or amount of NO released. A recent study in trinitrobenzene sulphonic acid (TNBS)-induced colitis reports significant reduction in mucosal damage, activity of myeloperoxidase and nitric oxide synthesis, and luminal NO production, when the animals are orally supplied with *Lb farciminis*,[56] a LAB known for its ability to produce NO. These results are similar to those we obtained in acetic acid colitis with treatment with *Lb reuteri*.[57] The fact that substances can both stimulate and inhibit has been called chemical hormesis.[58] It is a well known observation that a broad range of chemicals, and also microbes, are stimulatory/preventive in low doses and inhibitory/damaging in larger doses,[58] a phenomenon often referred to as Arndt–Schultz law.

Impaired SCFA production: SCFAs, especially butyrate, are preferred fuel for the intestinal mucosa and essential for its continuous replacement and healing. A constant fermentative activity and uninterrupted production of SCFAs is desirable not only for the function but also protection of the mucosa. It was recently shown that SCFAs, in addition to the nutritive importance, and particularly butyrate, induce heat shock proteins (HSPs), whose substances are of significant importance for survival of cells under stressful conditions. HSPs are known to stabilize critical cellular components and processes such as cytoskeletal and mitochondrial functions and to inhibit apoptotic pathways.[59] Increased expression of particularly HSP 25 has also been observed with oral supply of prebiotics (substrate for bacterial fermentation) such as pectin[59] and probiotic bacteria such as *Lb plantarum*.[60]

F&D-induced inflammation

Modern foods such as dairy products and gluten-containing grains (wheat, rye and barley) will especially in individuals with predisposing HLA molecules induce mucosal damage and increased translocation, changes especially seen in individuals with autism and autoimmune diseases, but also in a wide range of other conditions, IBD being no exception. A whole range of chemicals from aspirin and indomethacin to various cytostatics and chemo-therapeutics do also induce deleterious effects to mucosal membranes and functions. However, both food-induced[61] and drug-induced mucosal damages[62–64] are successfully prevented by supply of pro- and synbiotics.

Specific lessons from animal experiments

Imunostimulatory DNA: Immunostimulatory DNA (ISS-DNA) is known to have a broad range of activities especially directed towards TLR ligands.[65] ISS-DNA induces secretion of Th-1 like cytokines such interferons (IFNs) and IL-12 and up-regulates cell-surface molecules on DCs such as CD 40, B7-1 and B7-2.[66] The potent immunostimulatory effect of ISS-DNA has been tried in efforts to support Th1 immunity and enhance host defence, especially in UC. Th2 cytokines are found at high levels in UD, in contrast to CD, where Th-1 cytokines (IL-12) are usually elevated. This knowledge stimulated studies in both experimental and spontaneous murine colitis, using a synthetic analogue, called synthetic oligonucleotide analogue, ISS-ODNs.[67] In both the models tried, the ISS-ODNs ameliorate clinical, biochemical and histological signs of colonic inflammation. It also inhibited induction of colonic proinflammatory cytokines and chemokines and suppressed the induction of colonic matrix metalloproteinases.

Trefoil factors (TFFs): TFF belongs to a class of non-mitogenic peptides found to protect and repair intestinal epithelium.[68,69] Protective and healing effects of TFFs have been reported from animal studies in various conditions, including colitis.[70,71] Intragastric administration of *Lactococcus lactis*, engineered to secrete bioactive murine TFF, was in sharp contrast to the purified TFF, recently shown to effectively prevent and heal acute dextran sulphate (DSS)-induce colitis and to effectively improve the condition of chronic colitis in in $IL10^{-/-}$ mice.[72] This novel possibility opens new and attractive ways to use LAB for delivering of drugs to the site where they are most needed, the colonic mucosa, a technology, which in the future most likely will be extensively tried to target the colonic mucosa with various compounds.

Pyruvate: Patients with IBD, both UC and CD, have a significantly increased splanchnic metabolism. It was shown already 20 years ago that uptake of glucose precursors; lactate and glycerol is three times higher in IBD patients compared to controls and of pyruvate five times higher.[73] It is likely that there often occurs a shortage of nutrients at the level of the colonic mucosa, which almost entirely is dependent on nutrients produced by the flora. Nutrient deficiency occurs especially in the distal colon, where the shortage in prebiotic substrates, necessary for fermentation and production of nutrients, is the largest. Pyruvate is a key metabolite from fermentation by some LAB, and is a substrate for biosynthesis of important amino acids such as L-tryptophan, L-tyrosine and alanine. It is also known to be an effective antioxidant and strong inhibitor of

inflammation. Pyruvate supply is also reported to significantly reduce ischaemic injuries in various organs and to ameliorate inflammation, microvascular hypoperfusion, gut mucosal damage and prevent translocation in experimental models of mesenteric ischaemia and reperfusion.[74,75]

Natural antioxidants: It has been suggested that development of IBD is due to an imbalance between pro-oxidant and antioxidant mechanisms.[76,77] Significant decreases in activity of key enzymes involved in synthesis of important intracellular antioxidants, especially of the tripeptide glutathione (GSH) have also been reported.[78] Low levels of glutathione have also been registered in colonic biopsies from patients with CD.[79] Flora releases numerous antioxidants from consumed plants, including important antioxidants such as glutathione and folic acid. Special attention has in the last years been given to curcumin, a substance obtained from the rhizomes of the plant *Curcuma longa Linn*, and an ingredient in the spice turmeric, which contains 1–5% of the active substance. Also this substance is most likely released and absorbed after microbial fermentation in the large intestine. Curcumin has in the recent few years, in experimental studies, shown strong anti-inflammatory effects including capacity to prevent neurodegenerative diseases such as Alzheimer and various cancers. Curcumin is a strong inducer of heat shock response,[80] strong antioxidant and inhibitor of COX-2, LOX, NF-κB[81,82] and of the Th1 profile of CD4T cells.[83] Significant prevention of cellular injury have obtained in animal studies both in livers subjected to carbon tetrachloride[84] and alcohol-induced[85] liver injuries and on intestinal mucosa when colitis is induced by TNBS.[86,87]

Comments: It is important to point out the results obtained in animal studies are almost generally better than what were later obtained in clinical trials. Effects obtained with pre- and probiotics constitute no exception. A whole series of different LAB have, when tried in cytokine-deficient and normal animals been quite successful, but subsequent trials in patients have yielded less successful results.

Role of plant fibres (prebiotics)

Both IBD and IBS are typical Western diseases, and Westerners under-consume dietary fibres, but there is little evidence that lack of dietary fibre plays a role in the pathogenesis of these diseases. The ability of maintaining remission in UC patients by a daily supply of 10 g of Plantago ovata seeds (also called psyllium or Ispaghula husk) was compared with daily treatment with 500 mg of mesalamine and a combination of the two.[88] Twelve months of treatment failed to demonstrate any difference in clinical benefits between the three groups. Germinated barley foodstuff (GBF), a by-product from breweries, rich in hemicellulose and in glutamine, was tried in 39 patients with mild-to-moderate active UC.[89] Daily supply of 30 g reduced significantly the disease activity, increased concentration of SCFAs, and increased in stool the numbers of *Bifidobacterium and Eubacterium*. It can well be that the observed effect were more due to increased supply of glutamine and other antioxidants such as various B vitamins than to the fibre per se as these compounds are known to be rich in by-products from breweries. Glutamine, as well as other antioxidants, are known to attenuate proinflammatory cytokines such as TNF-α and to enhance release of HSP (HSP-72).[90] A controlled study using oat bran as fibre source was recently reported from a study in 22 patients+10 controls with quiescent UC. Daily supply during 3 months of as much as 60 g of oat bran (eqv. to 20 g dietary fibre) resulted in a significant increase in faecal butyrate (average 36%) but also reduction in abdominal pain. All the treated patients tolerated well the large dose of fibre but signs of relapse of diseases were seen in none of the colitis patients.[91] Butyrate has been shown to inhibit NF-κB activation of lamina propria macrophages, and to reduce the number of neutrophils in crypts and surface epithelia, as well as the density of lamina propria lymphocytes/plasma cells in patients with ulcerative colitis[92]—all findings which correlate well with the observed decreased disease activity. Twenty patients with ileal pouch-anal anastomosis received daily for 2 weeks 24 g of inulin. Significant reduction in inflammation was observed with endoscopy and histology. In addition significant increase in faecal concentrations of butyrate and reductions in faecal pH, faecal content of secondary bile acids, and growth of *Bacteroides fragilis* were observed.[93]

Comments: Future studies with use of other fibre sources, especially pectin, known to be a strong substrate for fermentative production of butyrate, but also a strong antioxidant, will certainly be of great interest.

Role of antioxidants

LAB produce themselves and/or release a whole range of important vitamins and antioxidants from consumed plants. One such example is the essential B vitamin, folate, known to have a strong effect in reducing homocysteine/s, and ability to prevent some chronic diseases. Folate is synthesised by LABs such as *Lactococcus lactis* and *Lb plantarum*. Other LABs, however, such as *Lb gasseri,* are net comsumers of folate. A recent publication describes successful transfer of five genes essential for folate biosynthesis from *Lactococcus lactis* to *Lb gasseri,* turning *Lb gasseri* into a net producer of folate.[94] Anaemia, iron deficiency and folate deficiency is common among patients with IBD.[95,96] In a recent paediatric study of 43 patients and 46 controls, plasma total homocysteine (tHcy) concentrations were shown to be significantly higher in children with IBD than in control subjects, ($P<0.05$), and level of plasma tHcy levels correlated negatively with plasma 5-methyltetrahydrofolate ($P<0.0005$).[97] Another recent study in adult 108 IBD patients and 74 healthy controls found that both UC and CD patients had lower levels of folate ($P<0.05$).[98] The serum concentration of tHcy/s was also in this study significantly higher in both UC (15.9+10.3 mmol/l) and CD patients (13.6 ± 6.5) compared to controls (9.6 ± 3.4, $P<0.05$).

Comments: An eventual contribution of increased levels of the pro-oxidant tHcy to recurrence of active disease is not known. If shown to be of significance, supply of fermented foods rich or enriched with folate as well as supplementation of specific pro- and synbiotics could well prove helpful in preventing activation of disease.

Role of pro- and synbiotics

Several attempts to modify the course of IBD by supplementing probiotics are reported in the literature. The success has with a few exceptions been absent or quite limited. Balfour Sartor did recently provide a comprehensive review on therapeutic manipulation of human microbionta.[99] I fully agree with his conclusions that "*current data for therapeutic efficacy do not withstand rigorous scrutiny or fulfil current evidence based rationale for using antibiotics, probiotics and prebiotics in the treatment of IBD*" and that "*clinical trials have consistently been underpowered to show equivalency or superiority, many have design flaws that preclude definite results, or use outcomes such as disease activity index, that do not conform with widely accepted criteria for disease response or remission*" It is also my observation that "*enthusiasm outstrips scientific support for these therapeutic approaches*". Recommendations of standards for clinical trials in IBD have been available for some time,[100,101] but, as far as I can see, few if not none of the studies presented this far have met these standards.

It is clear that the edge-cutting results sometimes obtained in experimental animals with induced colitis have not been repeatable in patients with true chronic disorders, spontaneously developed colitis as in IBD, e.g. in patients, who often have suffered the disease for years and been objects to various medical treatments with no or limited success. The differences in success observed might also be explained by the fact that the doses of LAB supplied to experimental studies are most often significantly larger in relation to body weight or area of mucosal surfaces than commonly used in treatment of patients. It is not unreasonable to assume that there exists a much higher resistance to treatment in spontaneous chronic diseases than in experimentally induced conditions. Furthermore, it cannot be excluded that species differences also play a role. The greatest effects of both single-strain (mono-strain) and multi-strain pro- and synbiotic treatments have clearly been achieved in acute conditions: reduction of acute diarrhoea, reduction of inflammation and prevention of infection in connection with extensive surgical procedures such as liver transplantation or in critically ill patients.[102,103] Dramatic effects, similar to those observed in acutely ill patients, have never been achieved in patients with chronic diseases, including those of the lower digestive tract. The need of both more and better studies is urgent. With this reservation in mind, one is tempted to conclude that the observed effects of treatment in chronic conditions, including IBD, vary from no to significant as one goes from single-strain to full flora replacement: single-strain probiotic < multistrain probiotic < or ~ single-strain/single fibre synbiotics < multi-strain/multi-fibre synbiotics < total faecal flora replacement.[104,105]

There is certainly wisdom in the way nature has designed our flora to consist in approx. 500 and eventually many more of different species. At least 40 different species occur in larger amounts. It is obvious that our flora functions as an organ, the different microbial species interact between themselves and with the mucosa, influencing each other's growth, producing nutrients for each other etc. Flora constitutes a good example of both symbiosis (living together to the benefit of both and synergy (increase of potency through interaction). The hunt in recent years for a magic single-strain probiotic, a probiotic with all the capacities in one, is unrealistic—the magic bug does not exist and will most likely never be found. As our knowledge about our flora grows we will possibly with time be able to move from a LAB soloist (single-strain) via a "chamber orchestra" of LABs (multi-strain) to a "full synbiotic symphony orchestra". Our present knowledge has this far restricted our efforts to construct formulas for manipulation of flora to contain more than a handful of LABs. It is my present experience that after mixing four eventually five strains, it becomes increasingly difficult to prove additional value from the addition of more strains to a probiotic or synbiotic product.

The results from clinical use of TFR are compelling, but the experience in patients with IBD much limited.[104,105] However, successful treatments with TFR in other conditions, particularly in *Clostridium difficile* colitis (84/150 TFR cases reported are in this group of patients), seem to support an extraordinary efficacy of such treatment. Even if successful, TFR will for understandable reasons never be widely used. Instead the goal should be to construct a synbiotic formulation as close as possible to normal human intestinal content. Psychological reasons will most likely also preclude a wide use of probiotics containing *Escherichia coli* and *Enterococcus faecium* as probiotics, even if proven effective. The studies presented with VSL#3 are important from two aspects: they demonstrate the efficacy of multi-strain treatment but also the necessity to supply larger amounts of probiotics per day than generally is the case today.[106–109] No effects of probiotics have been observed when given, especially in acute conditions, in dose lower than 10^7. Due to this reason, most single-strain probiotics are usually administered in a dose of 10^9 e.g. one billion LAB or more. Doses as high as 3600 billions are used in recent VSL#3 studies, which also might contribute to its clinical success. The greatest problem with VSL#3 is that no data are provided about the characteristics in function of each of the LAB nor is any information provided about the eventual synergy from their use when combined. It might well be that several of the stains included in the composition do not produce any additional values. Some of the strains in VSL#3 are similar to strains commonly used by dairy industry for production of yoghurt, cheeses and similar LAB-containing milk products. Such have documented low or no effects when used as medical probiotics, a conclusion further supported by some recent negative experience when such LABs were tried in patients in connection with surgery and in critically ill patients. A standard commercial product, TREVIS™ (Chr Hansen Biosystem, Denmark), containing *Lb acidophilus LA5*, *Bifidobacterium lactis BP12*, *Streptococcus thermophilus*, *Lb bulgaricus* and combined with 7.5 g oligofructose was supplied to patients in two separate controlled studies.[110,111] Although the treatments in both studies favourably influenced the microbial composition of the upper gastrointestinal tract, did it not influence intestinal permeability, nor was it associated by measurable clinical benefits. See further a published commentary of mine.[112]

The most active fermentation occurs in the right colon where high bacterial counts, high bacterial growth rate, low pH (5.4–5.9) and highest levels of SCFAs (approx. 130 mmol/l)

are found. The fermentation activity decreases dramatically as the food stream approaches the rectum, most likely due to shortage in fermentable fibre, especially in the Western diet. The bacterial growth is already in transverse colon significantly reduced, the pH slightly higher (approx. 6.2) and SCFA levels slightly lower. Usually little fibre is left for the left colon to ferment, instead proteins are fermented and increased levels of phenols, indoles and ammonia observed. This phenomenon is suggested as explanation for the relatively higher incidence of cancer in the left compared to the right. It might also explain the higher incidence of diverticulosis/diverticulitis in the left colon, as the mucosa for its growth and function is very dependent on the supply of butyrate, a product provided by fermentation of fibres such as pectin. The continuous decrease in fibre consumption seem to lead to a deficiency of substrate already in the ascending colon, which is suggested to explain the relative rapid rise in incidence of the right-side colonic cancers, which has occurred in recent decades. The information of lack of substrate in the lowest part of the digestive tract provides a rationale for supply of both substrate and probiotics, e.g. synbiotics to IBD patients and might explain the success with synbiotic enemas.[113] Information like this, support the rationale of simultaneous supplementation of fibres with the supply of probiotics. I certainly agree with a recent conclusion of Sartor that "*the interesting approach of combining probiotic and prebiotic agents (synbiotics) has considerable appeal*" and the suggestion "*that the combination of several compounds may be effective, analogous to cocktails of various probiotic bacteria*".[99] Multi-strain/multi-fibre synbiotics are attractive to me, especially as they have yielded such excellent results in critically ill and postoperative patients.[102,103,114,115] However, only three smaller studies with such a synbiotic formula have this far been reported in IBD. This treatment was clearly ineffective in CD when tried in a daily dose of 40 billion LAB (Chermish et al., this issue) and,[116] but studies with 400, eventually up to 1200 billion LAB are seemingly justified. Greater success was achieved with 2 weeks of rectal instillation of Synbiotic 2000 in patients with distal colitis with dramatic improvements in diarrhoea scores, visible blood in stool, nocturnal diarrhoea, urgency and consistency of stool.[117]

Future aspects

Most future attempts to increase the efficacy of bioecological treatment will most likely in the future focus on multi-strain probiotics and synbiotics. Efforts will be made to find and try other hitherto un-identified prebiotic fibres and probiotic bacteria. Special interest will be given to such natural food ingredients as turmeric, rich in "super-antioxidants" which substances most likely will be tried in combination with existing or new multi-strain pro- and synbiotics.[118] The possibility to clone *Lactobacillus* species genes to compensate for insufficient metabolic and hormonal functions in the body will extensively be tried. Such efforts will also include manipulations in order to develop special strains able to release larger amounts of nutrients such as glutamine, pyruvate and antioxidants such as folic acid, glutathione or curcumin at the level of the GI tract, where the demand is high, the colonic mucosa. New ways for delivery will also be tried. The success with topical application by enemas stimulates to further studies. A recent study found significant attenuation inflammation also from subcutaneous administration of live *Lb salivarius* in IL-10 knockout miceC We have observed significant reduction of inflammation, neutrophil tissue infiltration, tissue destruction after subcutaneous injection in experimental animals of the LAB in Synbiotic 2000.[119,120] Also inhalation of LAB offers an attractive alternative. Clearly the use of pre-, pro-, and synbiotics is in its infancy, and much further studies needed.

References

1. Macpherson AJ, Uhr Th. Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science* 2004;**303**:1662–5.
2. Guarner F, Schaafsma GJ. Probiotics. *Int J Food Microbiol* 1998;**39**:237–8.
3. Stagg AJ, Hart AL, Knight SC, Kamm MA. The dendritic cell: its role in intestinal inflammation and relationship with gut bacteria. *Gut* 2003;**52**:1522–9.
4. Karlsson H, Larsson P, Wold AE, Rudin A. Pattern of cytokine responses to Gram-positive and Gram-negative commensal bacteria is profoundly changed when monocytes differentiate into dendritic cells. *Infect Immun* 2004;**72**:2671–8.
5. Nagl M Kacani L, Mullauer B, Lemberger EM, et al. Phagocytosis and killing of bacteria by professional phagocytes and dendritic cells. *Clin Diagn Lab Immunol* 2002;**9**:1165–8.
6. Ma TY, Iwamoto GK, Hoa NT, et al. TNF-α-induced increase in intestinal epithelial tight junction permeability requires NF-κB activation. *Am J Physiol Gastrointest Liver Physiol* 2004;**286**: G367–76.
7. Smits HH, van Beelan A, Hessle Ch, et al. Commensal Gram-negative bacteria prime human dendritic cells for enhanced IL-23 and IL-27 expression and enhanced Th1 development. *Eur J Immunol* 2004;**34**:1371–80.
8. Oppmann B, Lesley R, Blom B, et al. Novel p19 protein engages IL-12p40 to form cytokine IL-23 with biological activities similar as well as distinct from IL-12. *Immunity* 2000;**13**: 715–25.
9. Pflanz S, Timans CJ, Cheung J, et al. IL-27, a heterodimeric cytokine composed of EB13 and p28 protein, induces proliferation of naïve CD4(+) T cells. *Immunity* 2002;**16**:779–90.
10. Christensen HR, Frokiaer H, Pestka JJ. *Lactobacilli* differently modulate expression of cytokines and maturation surface markers in nurine dendritic cells. *J Immunol* 2002;**168**:171–8.
11. Drakes M, Blanchard T, Czinn S. Bacterial probiotic modulation of dendritic cells. *Infect Immun* 2004;**72**:3299–309.
12. D'Haens G, Geboes K, Peeters M, Baert F, Penninckx F, Rutgeerts P. Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum. *Gastroenterology* 1998;**114**:262–7.
13. Rutgeerts P, Geboes K, Peeters M, et al. Effect of faecal stream diversion on recurrence of Crohn's disease in the neonatal ileum. *Lancet* 1991;**338**:771–4.
14. Fellermann K, Wehkamp J, Herrlinger KR, Stange EF. Crohn's disease: a defensin deficiency syndrome? *Eur J Gastroenterol Hepatol* 2003;**15**:627–34.
15. Gent AE, Hellier MD, Grace RH, et al. Inflammatory bowel disease and domestic hygiene in infancy. *Lancet* 1994;**343**: 766–7.
16. Shivanande S, Lennard-Jones J, Logan R, et al. Incidence of inflammatory bowel disease across Europe: is there a difference between north and south. Results of the European

Collaboration Study on inflammatory bowel diseases. *Gut* 1996;**39**:690–7.
17. Tannock GW. Probiotic properties of lactic-acid bacteria: plenty of scope for fundamental R&D. *Trends Biotechnol (TIBTECH)* 1997;**15**:270–4.
18. van der Waaij D. The ecology of the human intestine and its consequencies for overgrowth by pathogens such as *Clostridium difficile*. *Annu Rev Microbiol* 1989;**43**:69–87.
19. Olasupo NA, Olukoya DK, Odunfa SA. Studies on bacteriocinogenic *Lactobacillus* isolates from selected Nigerian fermented foods. *J Basic Microbiol* 1995;**35**:319–24.
20. Finegold SM, Sutter VL, Mathisen GE. Normal indigenous intestinal flora. In: Hentges DJ, editor. *Human intestinal microflora in health and disease*. London: Academic Press; 1983. p. 3–31.
21. Ahrné S, Nobaek S, Jeppsson B, et al. The normal *Lactobacillus* flora in healthy human rectal and oral mucosa. *J Appl Microbiol* 1998;**85**:88–94.
22. Adlerberth I, Carlsson B, deMan P, et al. Intestinal colonization with *Enterobacteriaceae* in Parkistani and Swedish hospital-delivered infants. *Acta Pediatr Scand* 1991;**80**:602–10.
23. Sepp E, Julge K, Vasur M, et al. Intestinal microflora of Estonian and Swedish infants. *Acta Pediatr* 1997;**86**:956–61.
24. Lencner AA, Lencner CP, Mikelsaar ME, et al. Die quantitative Zusammensetzung der Lactoflora des Verdauungstrakts vor und nach kosmischen Flügen unterschiedlicher Dauer. *Die Nahrung* 1984;**28**:607–13.
25. Knight DJW, Ala'Aldeen D, Bengmark S, Girling KJ. The effect of synbiotics on gastrointestinal flora in the critically ill. *Br J Anaesth* 2004;**92**:307–8.
26. Kinney KS, Austin CE, Morton DS, Sonnenfeld G. Norepinephrine as a growth stimulating factor in bacteria: Mechanistic studies. *Life Sci* 2000;**67**:3075–85.
27. Alverdy JC, Laughlin RS, Wu L. Influence of the critically ill state on host pathogen interactions within the intestine: gut derived sepsis redefined. *Crit Care Med* 2003;**31**:598–607.
28. Gilliland SE, Speck ML. Antagonistic action of *Lactobacillus acidophilus* toward intestinal and food born pathogens in associative cultures. *J Food Prot* 1977;**40**:820–3.
29. Fabia R, Ar'Rajab A, Johansson ML, et al. Impairment of bacterial flora in human ulcerative colitis and in experimental colitis in the rat. *Digestion* 1993;**54**:248–55.
30. Pathmakanthan S. Mucosally associated bacterial flora of the human colon: Quantitative and species-specific differences between normal and inflamed colonic biopsies. *Microb Ecolog Health Dis* 1999;**11**:169–74.
31. Favier C, Neut C, Mizon C, et al. Fecal β-D-galactosidase and bifidobacteria are decreased in Crohn's disease. *Dig Dis Sci* 1997;**42**:817–22.
32. Sartor RB. Microbial factors in the pathogenesis of Crohn's disease, ulcerative colitis and experimental intestinal inflammation. In: Kirsner JG, editor. *Inflammatory bowel diseases*. 5th ed. Philadelphia: Saunders; 1999. p. 153–78.
33. Ott SJ, Wenderoth DF, Hampe J, et al. Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. *Gut* 2004;**53**:685–93.
34. Swidsinski A, Ladhoff A, Pernthaler A, et al. Mucosal flora in inflammatory bowel disease. *Gastroenterology* 2002;**122**: 44–54.
35. Darfeuille-Michaud A, Boudeau J, Bulois P, et al. High prevalence of adherent-invasive *E. coli* associated with ileal mucosa in Crohn's disease. *Gastroenterology* 2004;**127**: 412–21.
36. Ogura Y, Bonen DK, Inqhar N, et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001;**411**:603–6.
37. Hugot JP. Genetic origin of IBD. *Inflamm Bowel Dis* 2004;**10**: 11–5.
38. Wehkamp J, Harder J, Weichenthal M, et al. Inducible and constitutive β-defensins are differently expressed in Crohn's disease and ulcerative colitis. *Inflamm Bowel Dis* 2003;**9**: 215–23.
39. Wehkamp J, Harder J, Wehkamp K, et al. NF-κB and AP-1 mediated induction of human beta defensin-2 in intestinal epithelial cells by *E. coli* Nissle 1917: a novel effect of a probiotic bacterium. *Infect Immun* 2004;**72**:5750–8.
40. Bengmark S. Acute and "chronic" phase response—a mother of disease. *Clin Nutr* 2004;**23**:1256–66.
41. Capristo E, Mingrone G, Addolorato G, et al. Glucose metabolism and insulin sensitivity in inactive inflammatory bowel disease. *Aliment Pharmacol Ther* 1999;**13**: 209–17.
42. Larsen TB, Nielsen JN, Fredholm L, et al. Platelets and anticoagulant capacity in patients with inflammatory bowel disease. *Pathophysiol Haemost Thromb* 2002;**32**:92–6.
43. Saibeni S, Bottasso B, Spina L, et al. Assessment of htrombin-activable fibrinolysis inhibitor (TAFI) plasma levels in inflammatory bowel diseases. *Am J Gastroenterol* 2004;**99**:1966–70.
44. Corkins MR, Gohil AD, Fitzgerald JF. The insulin-like growth factor axis in children with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 2003;**36**:228–34.
45. Zimmermann EM, Li L, Hou YT, et al. Insulin-like growth factor I and insulin-like growth factor binding protein 5 in Crohn's disease. *Am J Physiol Gastrointest Liver Physiol* 2001;**280**: G1022–9.
46. Lawrance IC, Maxwell L, Doe W. Inflammation location, but not type, determines the increase in TGF-beta1 and IGF-1 expression and collagen deposition in IBD intestine. *Inflamm Bowel Dis* 2001;**7**:16–26.
47. Wadström T, Andersson K, Sydow M, et al. Surface properties of lactobacilli isolated from the small intestine of pigs. *J Appl Bacteriol* 1987;**62**:513–20.
48. Esteve-Comas M, Ramirez M, Fernandez-Banãres F, et al. Plasma polyunsaturated fatty acid in active inflammatory bowel disease. *Gut* 1992;**33**:1365–9.
49. Esteve-Comas M, Nunez MC, Fernandez.Banãres F, et al. Abnormal plasma polyunsaturated fatty acid pattern in non-active inflammatory bowel disease. *Gut* 1993;**34**: 1370–3.
50. Eliakim R, Karmeli F, Razin E, Rachmilewitz D. Role of platelet-activating factor in ulcerative colitis. *Gastroenterology* 1988;**95**:1167–72.
51. Rask-Madsen J. Eicosanoids and their role in the pathogenesis of diarrhoeal diseases. *Clin Gastroenterol* 1986;**15**:545–66.
52. Ohtsuka Y, Yamashiro Y, Shimizu T, et al. Reducing cell membrane n-6 fatty acids attenuate mucosal damage in food-sensitive enteropathy in mice. *Pediatr Res* 1997;**42**: 835–9.
53. Kankanpää PE, Salminen SJ, Isolauri E, Lee YK. The influence of polyunsaturated fatty acids on probiotic growth and adhesion. *FEMS Microbiol Lett* 2001;**194**:149–53.
54. Rachmilewitz D, Stamler JS, Bachwind D, et al. Enhanced colonic nitric oxide generation and nitric generation and nitric oxide synthase activity in ulcerative colitis and Crohn's disease. *Gut* 1995;**36**:718–23.
55. Da-Zhong X, Lu Q, Deitsch EA. Nitric oxide directly impairs intestinal barrier function. *Shock* 2002;**17**:139–45.
56. Lamine F, Fioramonti J, Buono L, et al. Nitric oxide released by *Lactobacillus farminis* improves TNBS-colitis in rats. *Scand J Gastroenterol* 2004;**39**:37–45.
57. Fabia R, Ar'Rajab A, Johansson ML, et al. The effect of exogenous administration of *Lactobacillus reuteri* R2LC and oat fibre on acetic acid-induced colitis in the rat. *Scand J Gastroenterol* 1993;**28**:155–62.
58. Calabrese EJ, Baldwin LA. Hormesis as a biological hypothesis. *Environ Health Perspect* 1998;**106**(Suppl 1):S357–62.

59. Ren H, Musch MW, Kojima K, et al. Short-chain fatty acids induce heat shock protein expression in rats and IEC 18 cells. *Gastroenterology* 2001;**121**:631–9.
60. De Angelis M, Di Cagno R, Huet C, et al. Heat shock response in *Lactobacillus plantarum*. *Appl. Environ Microbiol* 2004;**70**: 1336–46.
61. Pelto L, Isolauri E, Lilius EM, et al. Probiotic bacteria down-regulate the milk-induced inflammatory response in milk-hypersensitive subjects but have an immunostimulatory effect in healthysubjects. *Clin Exp Allergy* 1998;**28**:1474–9.
62. Mao Y, Yu JL, Ljungh Å, Molin G, Jeppsson B. Intestinal immune response to oral administration of *Lactobacillus reuteri* R2LC, *Lactobacillus plantarum* DSM 9843, pectin and oatbase on methotraxate-induced enterocolitis in rats. *Microb Ecol Health Dis* 1997;**9**:261–70.
63. Gotteland M, Cruchet S, Verbeke S. Effect of *Lactobacillus* ingestion on the gastrointestinal mucosal barrier alterations induced by indometacin in humans. *Aliment Pharmacol Ther* 2001;**15**:11–7.
64. Montalto M, Maggiono N, Ricci R, et al. *Lactobacillus acidophilus* protects tight junctions from aspirin damage in HT-29 cells. *Digestion* 2004;**69**:225–8.
65. Wagner H. Toll meets CpG-DNA. *Immunity* 2001;**14**:499–502.
66. Martin-Orozco E, Kobayashi H, van Uden J, et al. Enhancement of antigen-presenting cell surface molecules involved in cognate interactions by immunostimulatory DNA sequences. *Int Immunol* 1999;**11**:1111–8.
67. Rachmilewitz D, Karmeli F, Takabayashi K, et al. Immunostimulatory DNA ameliorates experimental and spontaneous murine colitis. *Gastroenterology* 2002;**122**:1428–41.
68. Playford RJ, Marchbank T, Chinery R, et al. Human spasmolytic polypeptide is a cytoprotective agent that stimulates cell migration. *Gastroenterology* 1995;**108**:108–16.
69. Playford RJ, Marchbank T, Goodlad RA, et al. Transgenic mice that overexpress the human trefoil peptide pS2 have an increased resistance to intestinal damage. *Proc Natl Acad Sci USA* 1996;**93**:2137–42.
70. Tran CP, Cook GA, Yeomans ND, et al. Trefoil peptide TFF2 (spasmolytic polypeptide) potently accelerates healing and reduces inflammation in a rat model of colitis. *Gut* 1999;**44**:636–42.
71. Soriano-Izquierdo A, Gironella M, Massaguer A, et al. Trefoil peptide TFF2 treatment reduces VCAM-1 expression of leucocyte recruitment in experimental inflammation. *J Leukoc Biol* 2004;**75**:214–23.
72. Vandenbroucke K, Hans W, Van Huysse J, et al. Active delivery of trefoil factors by genetically modified *Lactococcus lactis* prevents and heals acute colitis in mice. *Gastroenterology* 2004;**127**:502–13.
73. Erikssson LS. Splanchnic exchange of glucose, amino acids and free fatty acids in patients with chronic inflammatory bowel disease. *Gut* 1983;**24**:1161–8.
74. Sappington PL, Fink ME, Yang R, et al. Ethyl pyruvate provides durable protection against inflammation-induced intestinal epithelial barrier dysfunction. *Shock* 2003;**6**:521–8.
75. Uchiyama T, Delude RL, Fink MP. Dose-dependent effects of ethyl pyruvate in mice subjected to mesenteric ischemia and reperfusion. *Intens Care Med* 2003;**29**:2050–8.
76. Grisham MD. Oxidants and free radicals in inflammatory bowel disease. *Lancet* 1994;**344**:859–61.
77. Gross V, Arndt H, Andus T, Palitzsch KD, Scholmerich J. Free radicals in inflammatory bowel diseases: pathophysiology and therapeutic implications. *Hepatogastroenterology* 1994;**41**: 320–7.
78. Sido B, Hack V, Hochlehnert A, et al. Impairment of intestinal glutathione synthesis in patients with inflammatory bowel disease. *Gut* 1998;**42**:485–92.
79. Miralles-Barrachina O, Savoye G, Belmonte-Zalar L, et al. Low levels of glutathione in endoscopic biopsies in patients with Crohn's colitis: the role of malnutrition. *Clin Nutr* 1999;**18**: 313–7.
80. Dunsmore KE, Chen PG, Wong HR. Curcumin, a medical herbal compound capable of inducing the heat shock response. *Crit Care Med* 2001;**29**:2199–204.
81. Bremner P, Heinrich M. Natural products as targeted modulators of the nuclear factor-κB pathway. *J Pharm Pharmacol JPP* 2002;**54**:453–72.
82. Foryst-Ludwig A, Neumann M, Schneider-Brachert W, Naumann M. Curcumin blocks NF-κB and motogenic response in *Helicobacter pylori*-infected epithelial cells. *Biochem Biophys Res Commun* 2004;**316**:1065–72.
83. Kang BY, Song YJ, Kim KM, et al. Curcumin inhibits Th1 cytokine profile in CD4+ T cells by suppressing interleukin-12 production in macrophages. *Br J Pharmacol* 1999;**128**:380–4.
84. Park EJ, Jeon CH, Ko G, Kim J, Sohn DH. Protective effects of curcumin in rat liver injury induced by carbon tetrachloride. *J Pharm Pharmacol* 2000;**52**:437–40.
85. Nanji AA, Jokelainen K, Tipoe GL, et al. Curcumin prevents alcohol-induced liver disease in rats by inhibiting the expression of KF-κB-dependent genes. *Am J Physiol Gastroenterol Liver Physiol* 2003;**284**:G321–7.
86. Sahl B, Assi V, Templeman V, et al. Curcumin attenuates DBN-induced colitis. *Am J Physiol Gastroenterol Liver Physiol* 2003;**285**:G235–43.
87. Ukil A, Maity S, Karmakar S, et al. Curcumin, the major component of food flavour turmeric, reduces mucosal injury in trinitrobenzene sulphonic acid-induced colitis. *B J Pharmacol* 2003;**139**:209–18.
88. Fernadez-Banares F, Hinojosa J, Sanchez-Lombrana JL, et al. Randomized clinical trial of *Plantago ovata* seeds (dietary fiber) as compared with mesalamine in maintaining remission in ulcerative colitis. *Am J Gastroenterol* 1999;**94**:427–33.
89. Kanauchi O, Iwanaga T, Mitsuyama K. Germinated barley foodstuff feeding. A novel neutraceutical strategy for ulcerative colitis. *Digestion* 2001;**63**(Suppl 1):S60–7.
90. Wischmeyer PE, Riehm J, Singleton KD, et al. Glutamine attenuates tumor necrosis factor-α release and enhances heat shock protein 72 in human peripheral blood mononuclear cells. *Nutrition* 2003;**19**:1–6.
91. Hallert C, Björck I, Nyman M, et al. Increasing fecal butyrate in ulcerative colitis patients by diet: controlled pilot study. *Inflamm Bowel Dis* 2003;**9**:116–21.
92. Lührs H, Gerke T, Müller JG, et al. Butyrate inhibits NF-κB activation in lamina propria macrophages of patients with ulcerative colitis. *Scand J Gastroenterol* 2002;**37**:458–66.
93. Welters CF, Heineman E, Thunnissens FB, et al. Effect of dietary inulin supplementation on inflammation of pouch mucosa in patients with an ileal pouch–anal anastomosis. *Dis Colon Rectum* 2002;**45**:621–7.
94. Wegkamp A, Starrenburg M, de Vos WM, et al. Transformation of folate-consuming *Lactobacillus gasseri* into a folate producer. *Appl Environ Microbiol* 2004;**70**:3146–8.
95. Pironi L, Cornia GL, Ursitti MA, et al. Evaluation of oral administration of folic and folinic acid to prevent folate deficiency in patients with inflammatory bowel disease treated with salicylazosulfapyridine. *Int J Clin Pharmacol Res* 1988;**8**: 143–8.
96. Gasche C, Lomer MC, Cavill I, Weiss G. Iron, anaemia, and inflammatory bowel diseases. *Gut* 2004;**53**:1190–7.
97. Nakano E, Taylor CJ, Chada L, et al. Hyperhomocystinemia in children with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 2003;**37**:586–90.
98. Koutroubakis IE, Dilaveraki E, Vlachonikolis IG, et al. Hyperhomocysteinemia in Greek patients with inflammatory bowel disease. *Dig Dis Sci* 2000;**45**:2347–51.

99. Sartor RB. Therapeutic manipulations of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics and prebiotics. *Gastroenterology* 2004;**126**:1620–33.
100. Sutherland LR, Hanauer S, Schmolmerich J. Standards for trials of therapy in inflammatory bowel disease. *Inflamm Bowel Dis* 1997;**3**:277–83.
101. Veldhuyzen van Zanten SJO, Talley NJ, Bytzer P, et al. Design of treatment trials for functional gastrointestinal disorders. *Gut* 1999;**45**(Suppl 2):1169–77.
102. Bengmark S. Bioecological control of perioperative and ITU morbidity. *Langenbecks Arch Surg* 2004;**389**:2–154.
103. Bengmark S. Bioecological control of inflammation and infection in transplantation. *Transplant Rev* 2004;**18**:38–53.
104. Borodo TJ, Warren EF, Leis S, et al. Treatment of ulcerative colitis using fecal bacteriotherapy. *J Clin Gastroenterol* 2003;**37**:42–7.
105. Borody TJ, Warren EF, Leis SM, et al. Bacteriotherapy using fecal flora. Toying with human motions. *J Clin Gastroenterol* 2004;**38**:475–83.
106. Venturi A, Gionchetti P, Rizzello F, et al. Impact on the composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. *Aliment Pharmacol Ther* 1999;**13**:1103–8.
107. Gionchetti P, Rizzello F, Venturi A, et al. Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. *Gastroenterology* 2000;**119**:305–9.
108. Mimura T, Helwig U, Paggioli G, et al. One daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. *Gut* 2004;**53**:108–14.
109. Gionchetti P, Rizzello F, Helwig U, et al. Prophylaxis of pouchitis onset with probiotic therapy: a double-bland, placebo-controlled trial. *Gastroenterology* 2003;**124**:1202–9.
110. Jain PK, McNaught CE, Anderson ADG, et al. Influence of synbiotic containing *Lactobacillus acidophilus LA5, Bifidobacterium lactis BP12, Streptococcus thermophilus, Lactobacillus bulgaricus* and oligofructose on gut barrier function and sepsis in critically ill patients: a randomized controlled trial. *Clin Nutr* 2004;**23**:467–75.
111. Woodcock NP, McNaught CE, Morgan DR, et al. An investigation into the effect of a probiotic on gut immune function in surgical patients. *Clin Nutr* 2004;**23**:1069–73.
112. Bengmark S. Synbiotics to strengthen gut barrier function and reduce morbidity in critically ill patients. *Clin Nutr* 2004;**23**: 441–5.
113. Pathmakanthan S, Walsh M, Bengmark S, et al. Efficacy and tolerability treating acute distal ulcerative colitis with synbiotic enema's: a pilot trial. *Abstr. Gut* 2002;**51**(Suppl. III):A307.
114. Rayes N, Seehofer D, Theruvath T, et al. Combined perioperative enteral supply of bioactive pre- and probiotics abolishes postoperative bacterial infections in human liver transplantation—a randomised, double-blind clinical trial. *Am J Transplant* 2005;**5**:125–30.
115. Bengmark S. Prevention of ITU infections; bioecological control and synbiotic treatment. In: Cresci G, editor. *Nutritional support for the critically ill patients: a guide to practise*. Boca Raton, FL: Taylor & Francis, CRC Press; 2005.
116. Rutgeerts P, D'Haens G, Baert F, et al. Randomized placebo controlled trial of pro-and prebiotics (Synbiotics cocktail) for maintenance of infliximab induced remission of luminal Crohn's disease (CD). *Gastroenterology* 2004;**126**: A-467 (T1310)
117. Pathmakanthan S, Walsh M, Bengmark S, et al. Efficacy and tolerability treating acute distal ulcerative colitis with synbiotic enema's: a pilot trial. *Abstr. Gut* 2002;**51**(Suppl. III):A307.
118. Bengmark S. Curcumin: an atoxic antioxidant and natural NF-κB, COX-2, LOX and iNOS inhibitor—a shield against acute and chronic diseases. *J Parenter Enteral Nutr* 2006;**30**: 45–51.
119. Sheil B, McCarthy J, O'Mahony L, et al. Is the mucosal route of administration essential for probiotic function? Subcutaneous administration is associated with attenuation of murine colitis and arthritis. *Gut* 2004;**53**:694–700.
120. Ilkgul O, Aydede H, Erhan Y, et al. Subcutaneous administration of live *Lactobacillus* prevents sepsis-induced lung organ failure in rats. *Br J Int Care* 2005;summer:52–7.