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Pre-, Pro-, and Synbiotics

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Abstract: About three thirds of the immune system is localised in the gastrointestinal tract. The saliva and the gastrointestinal secretions, but also flora, resident and supplied (probiotics) as well as supplied fibres (prebiotics) are important for optimal function. Probiotic bacteria have been shown to influence the immune system through several molecular mechanisms. Pre-, pro-, and synbiotics (products produced by fermentation) offer both protection against and cure of a variety of endemic and acute diseases. This review summarizes the present experience in various forms of diarrhoea, inflammatory bowel disease, Helicobacter infections, in intensive care patients and in connection with extensive surgery.

Gastrointestinal secretions important

Digestion depends on two principally different, but equally powerful, digestive systems. The system best explored is based on enzymes provided at all levels of the GI tract by the very rich gastrointestinal secretions, amounting to up to ten litres per day: saliva (2.5 lit), gastric secretion (2.5 lit), bile (0.5 lit), pancreatic secretion (1.5 lit) and small intestinal and colonic secretions (between 1.0 to 5.0 liters). These secretions are essential for the food digestion, but they are also important as they provide a whole series of important factors, necessary for immune and infection control, such as immunoglobulins, lactoferrin, lysozyme, fibronectin. In addition they provide mucin, which is veryimportant as matrix and protection of the flora but also substrate for fermentation and nutrition of the bacteria. The secretions, especially saliva, are very rich in important growth factors such as epidermal growth factor. Elimination of the salivary glands leads to gastrointestinal ulcers, poor wound healing and reduced regeneration of organs such as the liver. It is thus of the greatest importance that the gastrointestinal secretions are stimulated, and not inhibited, as often is the case in the very sick and critically ill patient.

The microb organ

The second digestive system, so far much less explored, is based on the 1-2 kg of bacteria harboured by the gut and mainly located in the large intestine. These bacteria break down complex food ingredients, indigestible by the enzymes of the GI secretions, which reach the colon untouched. Among the foods that are metabolised by microbial enzymes are mostly non-processed fresh fruits, vegetables, pulses, tubers, but also unprocessed cereals. This food is often referred to as colonic food. It is suggested that at least 10 % of our daily-ingested calories or at least ¼ of the food weight should be of the type colonic food, a recommendation often expressed as “five to eight fresh fruits and vegetables per day”. But also complex proteins constitute important sources for fermentation, the main sources being the rich protein content of pancreatic juice (contains about the same amount of protein as is daily needed by the body) and the almost half a kilogram of gastrointestinal mucosa cells, which are replaced each day. Both the pancreatic juice protein and the apoptotic mucosa cell remnants are subsequently fermented by the commensal flora, and its molecular components absorbed and recycled.

Although the various functions of the flora are still not explored to the extent one would wish, it is clear that this system is large and very complex in its function. The number of microbial

cells in the body is ten to twenty times larger (10^{14}) than the total number of eukaryotic cells of the whole body (10^{13}). Usually some 500 microbial strains are to be found in a human colon, but only some 35 to 40 strains seem to occur in any larger amount. An indication of the complexicity of the flora and its numerous metabolic functions is the observation that the flora of an individual contains about 300 000 genes, to be compared to about 65 000 in the rest of the body. Dominating genera of the human gut are *Bacteroides, Bifidobacterium, Clostridium, Enterococcus, Eubacterium, Fusobacterium, Peptostreptococcus, Ruminococcus, Lactobacillus* and *Escherichia.* Of the lactic acid bacteria (LAB) do usually fibre-fermenting LAB such as the fruit/vegetable born LAB: *Lactobacillus plantarum, Lactobacillus rhamnosus* and *Lactobacillus paracasei* ssp *paracasei* occur more frequently and in larger amounts, compared to mainly milk-born LAB: *Lactobacillus casei, Lactobacillus reuteri* and *Lactobacillus acidophilus*.

Biogenics, Prebiotics, Probiotics, Synbiotics

The term probiotic was originally designed to describe what has been regarded as ”health-promoting” lactic acid bacteria, when supplied orally. A synonymous term sometimes used is biogenics. Similarly was the term prebiotics given to the foods, mainly plant fibres, consumed and used by the gut flora as substrate for fermentation. The combined products of pre- and probiotics are increasingly called synbiotics. It documented that our Palaeolithic forefathers not only consumed millions of times more of lactic acid bacteria but also several times more of fruit and vegetable fibres. Furthermore, the selection of plants eaten was significantly larger. It is suggested that they ate in average from about 500 plant species, which gave the humans of that time a much larger variation in prebiotics supplied and consequently a wider production of various synbiotics. This larger variation in plant consumption facilitated compared to today a better availability of a larger amount of the more than two million chemical compounds that constitute the human body. It may or may not be a co-incidence that increases in inflammatory conditions in general, allergic conditions, obesity, coronary-heart disease, and cancers in the Western world have paralleled the decreased consumption of probiotics and prebiotics but also a reduced variation in consumed prebiotics.

Availability of prebiotics and variation in consumption essential

Dietary fibres are usually classified into three groups: *soluble fibres* such as pectins and various gums, *insoluble fibres* such as cellulose and *mixed type fibres* such as bran. The most important characteristic for them all is that they are resistant to hydrolysis by human alimentary tract enzymes, which make some of them ideal as substrate for microbial fermentation in the lower GI tract. Soluble fibres constitute an important source for bacterial fermentation and microbial production of nutrients, antioxidants, vitamins, and growth and other important factors. This far, the main interest has focused on the production of various short chain fatty acids (SCFAs) and fermentation by-products such as hydrogen, methane and carbon dioxide. But the content of other nutrients; various bioactive amino acids, polyamins, antioxidants and various growth factors, just to mention a few, are equally or more important – for review see Bengmark(1-5).

The following three criteria should be met for a substrate to be classified as prebiotic: 1. the substrate must not be hydrolysed or absorbed in the stomach or small intestine. 2. it must be selective for beneficial commensal bacteria in the colon and stimulate growth of the organisms, and 3. alter the microflora to a healthy composition and induce beneficial luminal/systemic effects on the host.

Variation in supply of prebiotics (fibres from plant cell walls) is important as availability and content of various fibres limits the production of synbiotic compounds produced. The important source of fibres is daily fruit and vegetable consumption, but supplementation of extra fibre, especially to the sick, seems also to be of importance. Among the many fibres of available and possible to be use in clinical nutrition as prebiotic supplements is the greatest attention given to betaglucans (oat gum) pectin, resistant starch, glycomannan, algal fibres and various oligosacharides. Many of these prebiotics are not only precursors for fermentation, they are also very bioactive in their own capacity. Pectin, as an example, is a strong antioxidant, important mucosa protectant (pseudomucin) but also carrier and protectant of flora from the mouth to the large intestine. Resistant starch (amylose maize) has recently been shown to be a good vehicle for transportation of LAB through the "acid and bile acid rich" upper GI tract. *Bifidobacterium* spp has been found to strongly adhere to starch granules resulting in an increased survival of supplemented probiotic bacteria through the upper GI tract and a six-fold increase in its recovery in feces (6**).

It has in the last decade become clear that a group of non-digestible oligosaccharides (NOD) play an important nutritional role (7*). These fibres, which comes from plants such as artichoke, onions, garlic, banana, soya and other beans etc - see further (!) - are today eaten in insufficient amounts (1-4 g/day) by most Westerners, and especially by children. It was recently agreed by a European consensus conference that NOD has a great potential to improve health[9] as they affect several mechanisms:

- increase composition and metabolic activity of the intestinal microflora (prebiosis)
- stimulate bowel habits
- stimulate mineral (especially Ca and Mg) absorption, and might postpone osteoporosis

It is likely that several hundred thousand, if not million, of synbiotic compounds are released by microbial fermentation and subsequently absorbed. Among these are various short chain and other fatty acids, amino acids, peptides, polyamins, carbohydrates, vitamins, and numerous antioxidants and phytosterols. Only of plant flavonoids are more than 4000 identified, of carotenoids about six hundred, and some of these have an antioxidant potential ten times as strong as that of vitamin C and E. Furthermore, the fermentation process in the gut releases a whole series of growth factors, coagulation factors and various signal molecules such cytokine-like bacteriokines – see further Bengmark(1-5).

Documented molecular effects

Probiotics, but also prebiotics, have when orally supplied, been proven to induce a whole series of molecular effects – see table 1. It is well documented that pre- and probiotics reduces the leakage into the body of microbes and toxins from the gut, but also that they prevent overgrowth of the numerous potentially pathogenic micro-organisms, normally harboured by the gut. Several mechanism have been suggested:

I. *Interference in adhesion of the pathogen.*
A: Competing by carbohydrate (mannose) or lipid receptors receptors.
Some *Lactobacillus e.i. L. plantarum* has been shown to adhere via carbohydrate (mannose) adhesion mechanisms, e.g. the same receptors as for Gram-negative bacteria such as *E. coli, Enterobacter, Klebsiella, Salmonella, Shigella, Pseudomonas* and *Vibrio cholerae*(9). Therefore by competing for the same receptors these bacteria may prevent infection caused by Gram negative bacteria.

B. Increase in mucin
Another defence mechanism against adherence of pathogens to the intestinal wall is the production of the mucins MUC2 and MUC3 (10). Both mucins show expression in Goblet cells in the large and small intestine, but MUC2 is suggested to be the major secreted mucin component of the colon (11). An increase in MUC2 and MUC3 mRNA expression was recently demonstrated in vitro when mucosal cells were incubated with *Lactobacillus plantarum 299v* (12), and resulting in an inhibition of adherence of pathogenic *Escherichia coli* to HT29 intestinal cells.
C. Production of cytokine-like molecules – bacteriokines.

D. Alteration of the physical environment - production of free radicals, low pH, organic acids, hydrogen peroxide etc.

II. *Effect on macrophage function.*
Supply of oral antibiotics was already fifty years ago shown to increase the susceptibility to infections (13). It has later been shown that administration of antibiotics such as mezlocillin at 150 mg/kg results in suppression of macrophage function as demonstrated by studies of chemiluminescence response, chemotactic motility, bactericidal and cytostatic ability (14). This defects in macrophage function is significantly reconstituted by supply of low molecular weight peptides obtained from indigenous gastrointestinal tract microflora species such as *Bacteroides sp., Clostridium sp., Propionebacterium sp.* and from *Lactobacillus*(15). Other studies demonstrate that supply of live or nonviable bacteria or bacterial wall components such as peptidoglucans stimulates macrophage recruitment and function (16). Cell-free extracts of both *Bifidobacterium longum* and *Lactobacillus acidophilus* have been shown to significantly enhance phagocytosis both of inert particles and viable *Salmonella*(17).

III. *Elimination of toxins*
Several studies show strong effects of various lactobacilli to significantly reduce and sometimes eliminate various toxins. Several lactobacillus (18*) and bifidobacteria (19) strains have proven to non-covalently bind and sequestrate, both in vitro and in vivo, very potent endotoxins such as aflatoxin B and *E coli* endotoxin. In addition supplementing *Lactobacillus* has been shown to reduce endotoxemia and severity of experimental alcoholic liver disease (20).

Clinical effects of probiotics
Table 2 summarizes the documented clinical effects of external supply of probiotics. It should be emphasised that most of the experience is mainly and sometimes only obtained from experimental animals. There seem to be no condition is which LAB (and fibres) have been as extensively tried in humans as in diarrhoea of various kinds, varying from rather simple tourist diarrhoea to severe and life-threatening conditions such as antibiotic-associated and radiotherapy-induced diarrhoea. Several excellent reviews have been published in recent years (21*,22*,23*,24*). It clear from all these studies that LAB provides a simple, inexpensive and effective tool, with no documented side effects, to be used both in prevention and treatment of all forms of diarrhoea. It is also obvious that LAB are effective in controlling diarrhoea of both bacterial and viral origin, but seem to be slightly more effective in virus-induced diarrhoea. This is promising, as an increasing number of infections today both in connection with extensive surgery such as transplantation and severe chronic disease such as HIV are of viral origin. But all LAB are not equally efficient.

Probiotics in infant diarrhea

Several millions of children die each year in diarrhoeal dehydration (25*). A larger European multi-center trial in children one month to three years of age was recently reported (26**). One hundred and forty children were randomly allocated to oral rehydration and placebo, another 147 children to oral rehydration and daily supply of 10^{10} cfu of *Lactobacillus* GG. Clinical signs of diarrhoea lasted 58.3±27.6 hours in the LAB-treated group to be compared to 71.9±35.8 hours (p=0.03) in the placebo group. Diarrhoea lasted in rotavirus-positive children treated with LAB 56.2±16.9 hours compared to 76.6±41.6 in the control group (p=0.008) (26).

The same lactobacillus was tried with the aim to prevent diarrhoea in a placebo-controlled trial performed in 204 undernourished Peruvian children, age 6 to 24 months (27*). The treatment was given during 15 months. The lactobacillus-treated children had fewer episodes if diarrhoea (5.21 episodes/child and year compared to 6.02 in the placebo group, p=0.028). The therapeutic gain, as pointed out by du Pont (28*) and others, must be regarded as modest. It is likely that use of other and more potent LAB, or combinations of LAB, would lead to more significant therapeutic success.

A study of 1237 newborn Columbian children with risk of developing severe diarrhoea (inpatients and transfer patients) and receiving prophylactically during one week or until they were discharged a daily supply 250 million live *Lactobacillus acidophilus* and 250 million live *Bifidobacterium infantis* was recently reported (29**), and the outcome compared to the outcome for similar children treated during the year before. The incidence of necrotizing enterocolitis was with probiotic prophylaxis reduced to one third (18 vs 47, p<0.0005) in the inpatient group, and by half (19 vs 38, p<0.03) in the patients transferred from other hospitals (which most likely came late under treatment) (29). No complications could be attributed to the use of probiotic preparations even in very sick newborn children, weighing in average 2600 gr (range < 1000 to >4000 g), of which one third suffered from severe conditions such as sepsis, pneumonia or meningitis. It was incidentally observed that the LAB-treated children suffered significantly less diaper dermatitis.

Probiotics in antibiotic-associated diarrhea

Diarrhoea is a common side effect of antibiotic therapy (21,30). Up to 40% of children receiving broad-spectrum antibiotics experience diarrhoea (31). Given the large numbers of pediatric patients, who receive antibiotic therapy each year, preventing even a proportion of the cases of antibiotic-associated diarrhoea may have a large impact. The efficiency of *Lactobacillus* GG (LGG) to prevent diarrhoea was tried in a series of 202 antibiotic-treated children. 25 placebo-treated (26%) and 7 LGG-treated developed diarrhoea (32*). The mean duration of diarrhoea was 4.7 days in the LGG group vs 5.88 days in the placebo group. Again, the efficacy of the treatment is not impressive, and as pointed out by Saavendra, “the reduction of 1 day of two liquid stools over a 10 day period in a child might be questioned” (33**).

Probiotics in inflammatory bowel disease (IBD)

We observed in the early nineties that humans with inflammatory bowel disease have a reduced LAB flora (34). We also observed in experimental animals with induced colitis that the inflammation could be significantly reduced by supply of pre- and probiotics in combination (synbiotics) (35). Subsequently it has been convincingly demonstrated that the concentrations of endogenous *Lactobacillus* and *Bifidobacteria* are significantly reduced in patients with active Crohn´s disease, ulcerative colitis, pouchitis as well as in experimental colitis (36,37**). A recent study quantified and characterized changes to a species level for

aerobic and anaerobic bacteria from colonic biopsies in ulcerative colitis (UC) (38**). A significant quantitative decrease in growth of Lactobacillus spp in colitis biopsies was observed. Total aerobic speciation revealed 32 different subspecies of which only 18 were found in UC. Anaerobic speciation revealed in average 4.7 subspecies in UC patients compared to 6.7 in controls. An incidental finding was that *Bacteroides thetaiotaomicron* could be identified in 8/10 UC biopsies compared to 4/10 controls, an observations, which significance remains to be explored.

Numerous experimental studies and some clinical observations suggest that the contents of the intestinal luminal environment are responsible for the initiation and/or perpetuation of inflammatory bowel disease. Germ-free animals, which are conventionalized, suffer an acute but self-limited colitis. An induction of small intestine-specific genes of the cryptdin family and of colon-specific amyloid A1 gene was observed during this process (39**), an observation which could lead to a better understanding of the role of luminal bacteria in the generation and amplification of mucosal inflammation.

A cocktail called VSL#3 consisting in four lactobacillus strains, three bifidobacteria strains plus *Streptococcus salivarius* ssp *thermophilus* (5×10^{11} cells/g), and most probably chosen at random without any further documentation of the molecular/immonological effects for each of the LAB was recently tried in an uncontrolled study in patients with ulcerative colitis (40*) . The patients were given 3 gram a day during one year and 15/20 patients remained in remission, one was lost to follow up and 4/20 had signs of relapse. The same LAB cocktail was also tried in a small controlled study in patients with pouchitis (41**). Twenty patients served as controls, all showed remission within 9 months. In sharp contrast to this did only 3/20 patients develop remission during the same time period, when supplied with VSL#3 probiotic cocktail (41). These results are surprisingly good and thought-provocative. They are most likely better than what is so far achieved by any conventional treatment, an assumption supported by a recent systematic review of the literature (42*) concluding that this far" metronidazole is is an effective treatment for active chronic disease" (odds ratio 12.34) but "oral probiotic therapy with VSL#3 for maintaining remission" (odds ratio 15.33).

Although the scientific basis for treatment of IBD seems reasonable and attractive, it must me emphasised that it is far too soon to recommend routine use of probiotics in IBD. Further studied are much warranted. Also prebiotics without additional supply of probiotics seem also to alleviate colitis symptoms (43). The good results obtained in the two small studies cited above seem to suggest that combination of several LAB might have stronger clinical effects in IBD than use of single-bacteria treatments. It is also tempting to anticipate that a cocktail consisting in LAB, where each of the bacteria has been chosen with the regard to their documented metabolic and immunological effects, should eventually be even more successful. The ideal treatment remedy will probably be complex, and much remains before the most suitable prebiotic(s), and the most effective probiotics have been identified.

Probiotics in *Helicobacter pylori* infections

It was suggested already more than ten years ago lactic acid produced by *Lactobacillus acidophilus* is able to inhibit *Helicobacter pylori* (44). 100 mM was suggested to be enough to effectively inhibit growth, an amount verified also by later investigations. A recent study tested the antibacterial activity of seventeen strains of lactobacilli against ten different strains of *H. pylori* (45**).All lactobacillus strains were able to inhibit *H. pylori*, but the effect was lost if pH was adjusted to 6.0. However, the effect of *Lactobacillus acidophilus* CRL 639 remained even after pH was adjusted. The effect seemed rather than being related to pH to be

related to the release of a proteinaceous compound, with autolysin effects. Gasparini and his group in Rome have in the recent two years given significant contributions to the effects of LAB in helicobacter infections. One hundred and twenty *H. pylori* patients were randomised to, in addition to a 7-day triple therapy (Rabeprozole, Chlarithromycin, Amoxicillin), receive either placebo or a lyophilised and inactivated culture of *Lactobacillus acidophilus*. The eradication rate was significantly improved by supplementation of the LAB: 52/59 patients (88 %) vs 42/58 patients (72 %) (p=0.03) (46**). The effects of live *Lactobacillus* GG was investigated in a subsequent study and performed in a similarly sized material of patients receiving identical triple therapy. This study reports improved tolerability (reduced antibiotic-induced bloating, diarrhoea and taste disturbances), but in contrast to the effect of *Lactobacillus* acidophilus (46) no improvement in eradication rate from the use of live *Lactobacillus* GG (47*,48*).

Daily oral consumption of 4x50 ml of the supernatant from a whey-based *Lactobacillus acidophilus* (La1) culture, combined with either omeprazole or placebo, was reported to show a significant reduction in breath test both with and without supply of omeprazole, immediately as well as six weeks after the treatment episode (49*). It should be remembered that whey is extraordinarily rich in immunologically active and anti-infectious substances. It is thus, this far not clear whether the observed effects are due to the *lactobacillus* used, the whey or the combination of both.

Synbiotics in intensive care patients.
Modern surgery is, despite significant advances in surgical techniques, far from safe. The incidence of the three leading causes of complications and sequelae; infections, thrombosis and adhesion formation seem to remain unchanged during the last fifty years. It is been calculated that about 2 million Americans (6 % of the hospital patients) suffer each year from nosocomial infections, and most of the patients have reduced immune functions, and half of the patients are over the age of 65 (50). Infections are especially common in neutropenic patients (48 %), after transplantation (appr. 50 %) and after extensive operations such as liver or pancreas resections (appr. 33 %), but the infection rates are also unacceptably high after gastric and colonic resections (appr 20 %). The mortality in acute conditions such as severe pancreatitis is increased four times when the pancreatic tissue has become infected (appr 40 %) with anaerobic gut bacteria (51), which occurs after 2 weeks in one third of the patients and after 3 weeks in as many as two thirds of the patients.

ICU patients acquire nosocomial infections at a much greater rate than patients elsewhere in the hospital. For ICU patients the risk is as much as 5 to 10 times greater than for those on general medical wards (52,53). The most representative data on nosocomial infection rates are provided by the National Nosocomial Infections Surveillance (NNIS) system in the USA but similar systems are increasingly introduced in most Western countries. The major types of infection found in the European Prevalence of Infection in Intensive Care (EPIC) study (54) were pneumonia/lower respiratory infection (64.7%), urinary tract infection (17.6%) and blood stream infection (12%). An American study found four major systems to be frequently involved: respiratory tract (31%), urinary tract (24%), blood stream (16%) and surgical sites (8%) (55).

A. In extensive surgery
Antibiotics seem not to solve the problem of surgical and intensive care sepsis. In addition antibiotic resistance has evolved as an increasing problem. It is thus of the greatest interest to find alternative ways to control infections. Three important studies with use of synbiotics e.g.

pro- and prebiotics (*Lactobacillus plantarum* 299 combined with oat and inulin fibre) to prevent sepsis are currently under publication (table 3). A prospective, randomised placebo controlled study was performed in abdominal surgery patient at the University of Berlin (Rayes N, Hansen S, Boucsein K et al. submitted manuscript). Thirty patients received *Lactobacillus plantarum* 299 in a daily dosis of 10^{10} (+fibres) and were compared to 30 patients receiving fibres + inactivated heat-killed *Lactobacillus plantarum* 299. Another thirty received parenteral nutrition (PN). The rate of complications were for the various groups: PN 30 %, heat-inactivated lactobacilli 17% and active lactobacilli 13 %. Infections developed in 3/30 (10%) patients in each of the two treated groups vs 9/30 (30%) in the PN group (p=0.001). Furthermore, significantly more antibiotics were administered in the PN group. The difference in sepsis rate was even larger when the subgroup of patients having more extensive surgery (gastric and pancreatic surgery) were separately analysed: live *Lactobacillus plantarum* 7 % (1/15), heat-inactivated *Lactobacillus plantarum* 17 % (3/17) and parenteral nutrition 50 % (8/16).

B. In liver transplantation

A similar study was also performed by the same Berlin-based group in patients undergoing liver transplantation (Rayes, N, Hansen, S., Boucsein K et al. submitted manuscript). The synbiotic treatment was in this study randomised against selective bowel decontamination (SBD). Four of 31 patients (13 %) in the group receiving active *Lactobacillus plantarum* and fibre, 11/32 (34 %) in the group receiving inactivated *Lactobacillus plantarum* and fibre and 15/32 (48 %) in the group treated by SBD developed infection within 30 days (p=0.017).

C. In severe acute pancreatitis

A randomised study in a material of patients with severe pancreatitis recently concluded did also demonstrate a significant reduction in sepsis (Oláh, A. personal communication) in synbiotic-treated patients: the infection rate was with supplied live *Lactobacillus plantarum* and fibre 4.5 % (1/22) to be compared to heat-inactivated *Lactobacillus plantarum* and fibre; 30.4 % (7/23), $p<0.05$. The rate of operation was also significantly reduced with prophylaxis with LAB and fibre ; 4.5 % (1/22) vs 26.1 % (6/23), $p<0.05$.

The dramatic reduction in surgical and ICU sepsis obtained in these three studies is very promising. This far it has been necessary to give the synbiotic treatment in parallel with conventional antibiotic treatment. It cannot be excluded that if antibiotics was not supplied even better results could be expected. Furthermore, as stated above, there are reasons to believe that simultaneous use of several pro- and prebiotics could contribute to further improvement in outcome.

Prospect for the future.

There are fundamental differences between various lactic acid bacteria. Genetically greater differences are observed between various LAB than between fishes and humans. The expected clinical effects from use of various LAB are most varying and conclusions can never be made from one strain to another. Generally milk-fermenting LAB are bioactively less powerful than fibre-fermented LAB such as *Lb plantarum, Lb paracasei, and Lb casei.* The majority of the clinical studies have this far been performed with *Lb rhamnosus GG,* but often with rather modest clinical effects (26, 27, 32, 47 48). The clinical efficiency of LAB such as *Lb Casei* Shirota and *Lb plantarum* appears to be more pronounced, but the most pronounced effects seem to be achieved through use of mixtures of several probiotic LAB (29,40 41), most likely in combination with several prebiotic fibres. Experience both in infants and in adults, in IBD and in ICU/surgery seem to suggest that in the future mixtures of several pro-

and prebiotics, e.g. synbiotics, will offer the best options successful control of disease. A combination of four bioactive LAB and four bioactive fibres, called Synbiotic 2000, presently tried around the world, seem to offer interesting possibilities.

Pro- and prebiotics should not be regarded as a panacea for everything. However, consumption of large amounts of lactic acid bacteria and fibre was an important part our diet during millions of years, and it is only in the recent 50 to 100 years that such comsumption has been dramatically reduced. It cannot be excluded that this has negative influences on our health, and that increased supply of LAB and fibre could be important for our health for everyone. In addition such treatment will provide beneficial effects in numerous groups of sick patients. Table 4 summarizes suggested indications for the use of pre-, pro- and synbiotics. However, the majority of these indications are so far unproven, and research in the form of well planned and well-controlled studies are much warranted.

Calorie and nitrogen balance is no longer a priority issue in short-term clinical nutrition such as peri-operative and ICU nutrition. Hyper-alimentation is no longer in general practice. As pointed out in my latest review for this journal (56), early or even better, uninterupted enteral nutrition offers unique possibilities to up-regulate the immune functions and prevent sepsis (57,58). Uninterrupted enteral nutrition in combination with peri- and intraoperative supply of synbiotics is a promising option for future perioperative mangement of patients (59).

References

1 Bengmark, S. Prospect for a new and rediscovered form of therapy: Probiotic and phage. In PW Andrew, P Oystron, GL Smith, DE Stewart-Tull (Eds) Fighting Infection in the 21st century. Blackwells, 2000, pp 97-132

2 Bengmark, S. Gut and the immune system: Enteral nutrition and immunonutrients. In SIRS, MODS and MOF – systemic inflammatory response syndrome, multiple organ dysfunction syndrome, multiple organ failure – pathophysiology, prevention and therapy,ed A.E. Baue, E. Faist, D. Fry.(Eds) New York: Springer, 2000, pp 408-424

3 Bengmark, S. Refunctionalizaton of the gut. In SIRS, MODS and MOF – systemic inflammatory response syndrome, multiple organ dysfunction syndrome, multiple organ failure – pathophysiology, prevention and therapy. In A.E. Baue, E. Faist, D. Fry. (Eds). New York: Springer, 2000, pp 435-446

4 Bengmark S. Bacteria for optimal health. Nutrition, 2000;16:611-615.

5 Bengmark S. Use of Pro-, Pre- and Synbiotics in the ICU – Future options. In: Shikora SA, Martindale RG, Schwaitzberg SD (Eds). Nutritional Considerations in the Intensive Care Unit – Science, Rationale and Practice. To be published ASPEN 2002

6 ******Wang X, Brown IL, Evans AJ, Conway PL. The protective effects of high amylose maize (amylomaize) starch granules on the survival of *Bifidobacterium* spp in mouse intestinal tract. J Appl Microbiol 1999;87:631-639 **a unique study which shows significant protection of ingested bacteria during transportation through the stomach and intestine by simultaneously ingested fibres.**

7 *****Loo JV, Cummings J, Delzenne N et al. Functional food properties of nondigestible oligosacccharides: a consensus report from the ENDO project (DGXII AIRII-CT94-1095). Brit J Nutr 1999;81:121-132 **a consensus report on bioactivities of non-digestible oligosacharides.**

8 Cummings JH, Roberfroid MB, Anderson H et al. A new look at dietary carbohydrate: chemistry, physiology and health. Eur J Clin Nutr 1997;51:417-423

9 Adlerberth I, Ahrné S, Johansson ML, et al. A mannose-specific adhesion mechanism in *Lactobacillus plantarum* conferring binding to the human colonic cell line HT-29. Applied and Environmental Microbiology 1996;62:2244-2251
10 Forstner JF, Forstner GG . Gastrointestinal mucus. In Physiology of the Gastrointestinal Tract (3rd ed) Edited by LG Johnson. New York: Raven, 1994, pp 1255-1283
11 van Klinken BJ, Tytgat KMAJ, Buller HA et al. Biosynthesis of intestinal mucins:MUC1, MUC2, MUC3 and more. Biochem Soc. Trans 1995;23:814-818
12 Mack DR, Michail S, Wei S et al. Probiotic inhibit enteropathogenic *E. coli* adherence in vitro by inducing intestinal mucin gene expression. Am J Physiol 1999;276(Gastrointest. Liver Physiol 39):G941-G950
13 Freter R. The fatal enteric cholera infection in guinea pig achieved by inhibition of normal enteric flora. J Infect Dis 1955;97:57-65
14 Roszkowski, K., Ko, K. L., Beuth, J., Ohshima, Y., Roszkowski, W., Jeljaszewics, J., Pulverer, G. Intestinal microflora of BALB/c-mice and function of local immune cells. Zeitschrift für Bakteriologie und Hygien 1988;270:270-279
15 Pulverer G, Ko HL, Roszkowski W et al. Digestive tract microflora liberates low molecular weight peptides with immunotriggering activity. Zentralblatt für Bakteriologie 1990;272:318-327
16 Kilkullen, J. K., Ly, O. P., Chang, T. H., Levenson, S. M., Steinberg, J .J. Nonviable *Staphylococcus aureus* and its peptidoglucan stimulate macrophage recruitment, angiogenesis, fibroplasia and collagen accumulation in wound rats. Wound, Repair and Regeneration1998;6:149-156
17 Hatcher, G. E., Lamprecht, R. S. Augmentation of macrophage phagocytic activity by cell-free extracts of selected lactic acid-producing bacteria. Journal of Diary Science 1993;76:2485-2492
18 ******Haskard C, Binnion C, Ahokas J Factors affecting the squestration of aflatoxin by *Lactobacillus rhamnosus* strain GG. Chemico-Biological Interactions 2000;128:39-49 **demonstrates significant LAB-induced reduction of the carcinogen aflatoxin**
19 Oatley JT, Rarick MD, Ji GE, Linz JE Binding of Aflatoxin to Bifidobacteria in vitro. J Food Protect 2000;63:1133-1136
20 Nanji AA, Khetty U, Sadrzadeh SMH. *Lactobacillus* feeding reduces endotoxemia and severity of experimental alcoholic liver disease. Proc Soc Exp Med Biol 1994;205:243-247
21 *****Heyman M. Effect of lactic acid bacteria on diarrheal diseases. J Am Coll Nutr 2000;19:S137-S146 **excellent review on the effects of LAB in diarrhoeal diseases.**
22 *****De Roos NM, Katan MB. Effects of probiotic bacteria on diarrhea, lipid metabolism, and carcinogenesis: a review of papers published between 1988 and 1998. Am J Clin Nutr 2000;71:405-411 **excellent review on general effects of LAB.**
23 *****Hove H, Borgaard H, Brobech Mortensen PB. Lactic acid bacteria and human gastrointestinal tract. Eur J Clin Nutr 1999:53:339-350 **a review focussing on gastrointestinal effects of LAB.**
24 *****Reid G. Probiotics in the treatment of diarrheal diseases. Current Infectious Disease Report 2000;2:78-83 **excellent review on the use of probiotics in diarrhoeal diseases.**
25 *****Rhoads M. Management of acute diarrhea in infants. J Parent Ent Nutr (JIPEN) 1999;23:S18-S19 **excellent review focussing on the pediatric aspect of diarrhoea**
26 ******Guandalini, S, Pensabene L, Zikri MA et al. *Lactobacillus* GG administered in oral rehydration solution to children with acute diarrhea: a multicenter European study. J Pediatr Gastr Nutr 2000;30:54-60 **well performed multicenter study showing**

modest but significant effects of LAB (*Lactobacillus GG*) in children with acute diarrhoea.

27 *****Oberhelman RA, Gilman RH, Sheen P et al. A placebo-controlled trial of *Lactobacillus* GG to prevent diarrhea in undernourished Peruvian children. J Pediatr 1999;134:15-20 **a controlled study in third world children showing modest but statistically significant reduction in diarrhoea with supply of *Lactobacillus* GG**

28 *****DuPont HL. Prevention of diarrhea by the probiotic *Lactobacillus* GG. J Pediatr 1999;134:1-2 *** a critical discussion of the experience with *Lactobacillus* GG in children with diarrhoea.**

29 ******Hoyos AB. Reduced incidence of necrotizing enterocolitis associated with enteral administration of *Lactobacillus acidophilus* and *Bifidobacterium infantis* to neonates in an intensive care unit. Int J Infect Dis 1999;3:197-202 **an important pioneer study in large material of neonates. Shows despite the lack of randomisation a clear potential for probiotics in neonatals.**

30 Bartlett JG. Antibiotic-associated diarrhea. Clin Infect Dis 1992; 15:573-81

31 Elstner CL, Lindsay AN, Book LS, et al. Lack of relationship of *Clostridium difficile* to antibiotic-associated diarrhea in children. Pediatr Infect Dis 1983; 2:364-6

32 *****Vanderhoof JA, Whitney DB, Antonson DL et al. *Lactobacillus* GG in the prevention of antibiotic-associated diarrhea in children. J Pediatr 1999;135:564-568 **a well peformed study in 188 patients showing modest, but statistically significant reduction in stool numbers and increase in stool consistency.**

33 ******Saavendra JM. Probiotics plus antibiotics; regulating our bacterial environment. J Pediatr 1999;135:535-537 **a critical analysis of the effects of LAB in diarrhoeal disease, written by one the the pioneers in the field**

34 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;54:248-255

35 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;28:155-162

36 Favier C, Neut C, Mizon C et al. Fecal β-D-galactosidase and bifidobacteria are decreased in Crohn´s disease. Dig Dis Sci 1997;42:817-822

37 ******Sartor RB. Microbial factors in the pathogenesis of Crohn´s disease, ulcerative colitis and experimental intestinal inflammation. In Kirsner JG (ed). Inflammatory bowel diseases. 5 ed, Philadelphia, Saunders 1999:153-178 **a state of the art review on inflammatory bowel disease, including the experience with LAB as treatment.**

38 ******Pathmakanthan S, Thornley JP, Hawkey CJ. Mucosally associated bacteria flora of the human colon: quantitative and species specific differences between normal and inflamed colonic biopsies. Microb Ecolog Health Dis 1999;11:169-174 **an excellent study further documenting the reducting in LAB in patients with inflammatory bowel disease.**

39 ******Ogawa H, Fukushima K, Sasaki I et al. Identification of genes involved in mucosal defense and inflammation associated with normal enteric bacteria. Am J Physiol Gastrointest Liver Physiol 2000;279:G492-G499 **unique study identifying the genes involved in mucosal defense.**

40 *****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;13:1103-1108 **experience with a cocktail of LAB in a small non-controlled material of patient with ulcerative colitis.**

41 ******Gionchetti P, Rizello F, Venturi A et al. Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. Gastroenterology 2000;119:305-309 **Significant improvement in prevention of recurrence by supply of an LAB cocktail in a small controlled study.**
42 *****Sandborn WJ, McLeod R, Jewell DP. Medical therapy for induction and maintenance of remission in pouchitis: a systemic review. Inflammatory Bowel Diseases 1999;5:33-39 **an excellent state of the art review on pouchitis.**
43 Meijer HP, Welters CF, Heineman E et al. Enteral inulin does not affect epithelial gene expression and cell turnover within the ileoanal pouch. Dis Colon Rectum. 2000;43:1427-34
44 Bhatia SJ, Kochar N, Abraham P. *Lactobacillus acidophilus* inhibits growth of *Campylobacter pylori* in vitro. J Clin Microbiol 1989;27:2328-2330
45 ******Lorca GL, Wadström T, Fond de Valdez G, Ljungh Å. *Lactobacillus acidophilus* autolysins inhibit *Helicobacter pylori* in vitro. Current Microbiology 2001;42:39-44 **interesting study showing specific strain-specific? autolysin activity by *Lactobacillus acidophilus* on *Helicobacter pylori.***
46 ******Canducci F, Armuzzi A, Cremonini F et al. A lyophilised and inactivated culture of *Lactobacillus acidophilus* increases *Helicobacter pylori* eradication rates. Aliment Pharmacol Ther 2000;14:1625-1629 **pioneer study showing significant potentiating effects of LAB in treatment of *Helicobacter pylori* infected patients with triple therapy.**
47 *****Armuzzi A, Cremonini F, Ojetti V et al. Effect of *Lactobacillus* GG supplementation on antibiotic-associated gastrointestinal side effects during *Helicobacter pylori* eradication therapy: a pilot study. Digestion 2001;63:1-7 **shows reduction in side effects, but no improvement in eradication of *Helicobacter pylori* by supply of *Lactobacillus* GG with triple therapy.**
48 *****Armuzzi A, Cremonini F, Bartolozzi F et al. The effect of oral administration of *Lactobacillus* GG on associated gastrointestinal side effects during *Helicobacter* eradication. Aliment Pharmacol Ther 2001;15:163-169 **shows reduction in side effects, but no improvement in eradication of *Helicobacter pylori* by supply of *Lactobacillus* GG with triple therapy.**
49 *****Michetti P, Dorta G, Wiesel PH et al. Effect of whey based culture supernatant of *Lactobacillus acidophilus* (jonsonii) La1 on *Helicobacter pylori* infections in humans. Digestion 1999;60:203-209 **an interesting piece of work showing inhibition of *Helicobacter pylori* by the combination of *Lactobacillus Acidophilus* and whey.**
50 Swartz NN Hospital-acquired infections: diseases with increasingly limited therapies. Proc Natl Acad Sci 1994:91: 2420-2427
51 Isenmann R, Büchler MW. Infection and acute pancreatitis. Brit J Surg 1994;81:1707-1708
52 Brawley RL, Weber DJ, Samsa GP et al. Multiple nosocomial infections; an incidcence study. Am J Epidemiol 1989;130:769-780
53 Weber DJ, Raasch R, Rutala WA. Nosocomial infections in the ICU; the growing importance of antibiotic-resistant pathogens. Chest 1999;115:34S-41S
54 Vincent JL, Bihari DJ, Suter DM et al. The prevalence of nosocomial infection in intensive care units in Europe. Results of the European prevalence of infection in intensive care (EPIC) study. JAMA 1993;274:639-644
55 Weinstein RA. Epidemiology and control of nosocomial infection in adult ICU. Am J Med 1991;91 suppl:179-184
56 Bengmark S. Gut microenvironment and immune function. Current Opinion in Clinical Nutrition and Metabolic Care 1999;2:83-85

57 Bengmark S, Andersson R, Mangiante G. Uninterrupted perioperative enteral nutrition. Clin Nutr 2001;20:11-19
58 Bengmark S. Aggressive peri- and intraoperative enteral nutrition –Strategy for the future. In Shikora SA, Martindale RG, Schwaitzberg SD: Nutritional Considerations in the Intensive Care Unit – Science, Rationale and Practice. Aspen 2001
59 Bengmark S. Use of Pro-, Pre- and Synbiotics in the ICU – Future options. In Shikora SA, Martindale RG, Schwaitzberg SD: Nutritional Considerations in the Intensive Care Unit – Science, Rationale and Practice. Aspen 2001

Table 1.

PROBIOTICS-claimed molecular effects.

General:
- Produces nutrients and antioxidants
- Produces growth and coagulation factors
- Activates the MALT system
- Modulates Th1/Th2 response
- Promotes antioxidant actions
- Controls potentially pathogenic microorganisms (PPMs)
- Reduces production of endotoxins
- Reduces mutagenicity

Humoral:
- Stimulates IgA production
- Inhibits IgE production
- Stimulates NO production
- Modulates cytokine response

Cellular:
- Stimulates macrophage function
- Stimulates NK cell activity
- Promotes growth and regeneration
- Promotes apoptosis

Table 2
PROBIOTICS-claimed clinical effects

General:

Reduces the incidence and severity of sepsis in Intensive Care Units
Reduces the incidence and severity of sepsis in major surgery
Delays onset of diabetes (only animal studies available)
Reduces extent of tumour growth and number of metastases*

Intestine:

Prevents or reduces duration of diarrhoea (rotavirus)
Prevents or reduces *Clostridium difficile* infections
Induces remission of inflammatory bowel disease
Prevents recurrence of ulcerative colitis manifestations
Reduces symptoms in irritable colon
Reduces the incidence of colonic cancer*

Stomach:

Prevents or reduces *Helicobacter* infections*

Pancreas:

Prevents or reduces septic manifestations in pancreatitis*

Liver:

Reduces clinical manifestations, mortality and extent of cellular damage in toxic liver injury*

Skin and body surfaces:

Reduces atopic eczema manifestations in children
Reduces biofilm

- *= only animal studies available

Table 3
SYNBIOTICS IN SURGICAL/INTENSIVE CARE PATIENTS
Rate of infections:

	Abdominal surgery	Gastric+ pancreatic surgery	Liver transplantation	Acute pancreatitis
Author	Rayes et al (57)	Rayes et al (57)	Rayes et al (58)	Olah et al (59)
Year	2000	2000	2000	2000
Total Parenteral Nutrition	30%(9/30)	50%(8/16)		
Selective Bowel Decontamination			48%(15/32)	
Inactivated LAB + FIBER	10%(3/30)	17%(3/17)	34%(11/32)	30%(7/23)
LAB + FIBER	10%(3/30)	7%(1/15)	13%(4/31)	4.5%(1/22)
Statistical Significance	$p<0.0001$	$p<0.0001$	$p=0.017$	$p=0.05$

Table 4.
TREATMENT WITH PRE-, PRO- AND SYNBIOTICS – Suggested use

Clinical nutrition:

To supply antioxidants and nutrients to persons and patients who cannot eat normally, do not eat the recommended amount of fresh fruits and vegetables, are on total parenteral nutrition or on enteral nutrition with factory-produced artificial nutritional formulas with or without residue.

Allergology:

To reduce allergic manifestations

Immunology:

To stimulate the immune system in immuno-depressed patients

To reduce early rejection in transplant patients

Intensive care:

To reduce morbidity in critically ill patients, especially those on immuno-depressing antibiotics and other pharmaceuticals

Topically applied around entrances through the skin of foreign material such as venous or arterial lines, drainage and tracheostomy tubes and voice prostheses in order to avoid biofilm development and infection

Neonatology:

To prevent or reduce development of topic diseases

To prevent and reduce rate and severity of infections premature and newborns

Gastroenterology:

To prevent and reduce diarrhoea

To treat antibiotic-associated diarrhoea

To prevent and reduce *Helicobacter* infections

To suppress or cure *Clostridum difficile* infections

To reduce symptoms and prevent recurrence in inflammatory bowel disease

Hepato-pancreatology:

To prevent infection in biliary obstruction

To prevent infection in toxic liver injuries

To prevent secondary infections in liver cirrhosis and portal hypertension

To improve liver blood flow in portal hypertension and hereby reduce incidence of variceal bleedings

To reduce hepatic encephalopathy

To prevent sepsis in acute and chronic pancreatitis

Hematology:

To prevent complications in patients with severe hematological diseases

Rheumatology:

To reduce further development and symtoms of rheumatoid arthritis

Nefrology:

To prevent infections in patients om hemo or CAPD dialysis

Oncology:

To prevent cancer development

To limit progress of malignant growth

To improve quality of life in cancer patients

Stomatology:

To prevent and reduce stomatitis in critically ill and cytostatic-treated patients.

To prevent infection in connection with teeth extraction or replacement of mercury-containing fillings

Surgery:

To reduce surgical morbidity: sepsis, thrombosis, adhesion formation

Gynecology:

To reduce bacterial vaginosis and sexually transmitted diseases incl. HIV

To reduce complications such as premature membrane rupture and preterm labor

Infectious diseases:

To reduce morbidity and improve quality of life in HIV/Aids patients