

Review Article

Bioecological Control of Acute and Chronic Diseases: The Role of Pro-, Pre- and Synbiotics

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ABSTRACT

The incidence of acute and chronic diseases is increasing worldwide and especially in what has been called the third world. This development is strongly associated with Western lifestyle: lack of physical exercise, mental stress, use of tobacco and alcohol and consumption of refined and calorie-condensed foods, a lifestyle, which seems to "paralyze" the innate immune system and reduce resistance to disease. Increased level of general inflammation in the body is common for almost all diseases, and is likely to be a result of Western lifestyle. A microbial flora, which covers the surfaces of all living organisms, plants as well as animals, plays an important role in protecting the body surfaces and the whole body. The majority of the immune system is in the gut, and flora has a profound influence on its function. The flora exists on all human surfaces but the majority is to be

found in the large intestine, where it also contributes to the digestion of food ingredients and making them available for absorption. The flora is significantly reduced among Westerners, which might contribute to the reduced resistance to disease. Attempts are made to supplement beneficial bacteria, most often lactic acid bacteria (LAB). One can expect, as LAB are depending on access to plant fibers that more pronounced effects can be obtained by simultaneous administration of LAB and plant fibers, a formulation often referred to as synbiotics. Cutting-edge results have also been obtained when they are tried both in acute conditions such as perioperative treatment, in connection with larger abdominal operations, liver transplantation, acute severe pancreatitis, extensive trauma and in chronic diseases such as liver cirrhosis and chronic colitis.

AN EPIDEMIC OF CHRONIC DISEASES

Despite some breath-taking advances in medico-pharmaceutical and surgical treatment, are medical and surgical emergencies, as well as advanced medical and surgical treatments, still affected by an by unacceptably high morbidity and mortality? Sepsis is the most common medical and surgical complication, estimated in the US alone to annually affect as many as 751,000^[1,2], and cause death of approximately 215, 000 patients (29%)^[2], which makes sepsis the tenth most common cause of death in the country. It is especially alarming that both morbidity and mortality in critical illness (CI), especially when septic, is fast increasing and has done so for several decades. With a documented 1.5% rate of increase per year it might double within the coming 50 to 60 years. Presently available treatment options: antibiotics and antagonists/inhibitors of individual pro-inflammatory cytokines have not met early high expectations. Instead, these treatments have often instituted new complications and new

morbidity. Selective bowel decontamination *e.g.*, parallel parenteral and topical application of a handful of powerful antibiotics is no longer a treatment option. We seem, after more than 30 years of dedicated efforts to combat sepsis by the use of various combinations of antibiotics and more than 30 randomised clinical trials, ready to conclude that vigorous use of antibiotics, despite some observations of a modest decrease in incidence of chest infections, will not significantly reduce mortality in critically ill patients^[5]. Two recent multi-center studies document no effects of antibiotic treatment when used in severe acute pancreatitis^[6,7]. Cytokine inhibitors have most often failed when used in acute disease and critical illness^[8], but reported effects in chronic illnesses are somewhat more promising^[9]. Still side-effects and price constitute important obstacles, especially for long-term treatments.

It is well known that the majority of individuals, who end up in ICUs, are elderly and have one or several chronic illnesses and other signs of reduced

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resistance to disease. The epidemic of critical illness is strongly associated with an epidemic of chronic diseases (ChDs). World Health organization estimates that 46% of global disease burden and 59% of global mortality is due to ChDs; 35 million individuals die each year from chronic diseases, and the numbers are steadily increasing^[10]. Circumstantial evidence supports the association of ChDs to modern life style, stress, lack of exercise, abuse of tobacco and alcohol, and to the transition from natural unprocessed foods to processed, calorie-condensed and heat-treated foods.

OUTCOME ASSOCIATED WITH LIFE STYLE, ESPECIALLY INTAKE OF FOODS

The association between ChDs, CI and reduced intake of plant fibers, plant antioxidants together with increased consumption of industrially produced and processed dairy products, refined sugars and starch products is obvious. As examples; the per capita consumption of refined sugar has increased from about one pound per person per year in 1850 to about 100 lbs/person/year in the year 2000 and the per cow milk production from 2 - 50 quarts/day. Dairy products, especially milk (mostly from pregnant cows) are rich in proinflammatory molecules: hormones such as estrogens and growth factors such as IGF-1. Consumption of bovine milk has been shown to release inflammatory mediators, increase intestinal permeability and induce leakage of molecules such as albumin and hyaluronan. Heating up milk (pasteurization), and especially production of and storage of milk powder, produces large amounts of advanced glycation products (AGEs, Fig. 1) and advanced lipoxidation products (ALEs)^[11], known to induce and potentiate inflammation. This information is important as many enteral nutrition solutions are based on milk powder. Bread, especially from gluten-containing grains, is also rich in molecules with documented pro-inflammatory effects (see further: Bengmark)^[12-14].

OUTCOME ASSOCIATED WITH PREMORBID HEALTH

Signs of a failing immune system are often observed in those patients who later develop acute critical illness. About half of the patients, who develop sepsis, are in the age group of 65 years and above, and 48 % of the patients are neutropenic^[15,16]. Stress and hormones play an important role, and both flora and mucosal cells have important endocrine functions and produce as well as respond to hormones. The gastrointestinal (GI) tract contains 100 million neurons (which is equal to the number of neurons in the spinal cord) distributed through all its layers^[17] and they exert

strong effects on both immune cells and flora, affecting homeostasis of the immune system and resistance to disease. A series of experiment have demonstrated an upto 100, 000 times (5 logs of order) increase in growth of Gram-negative bacteria exposed to noradrenaline (see further: Lyte^[18]), which explains a relatively old observation of significantly higher blood levels of noradrenaline and adrenaline in patients, who develop severe septic conditions compared to patients with an uncomplicated postoperative course^[19]. Luminal release of noradrenaline is a strong inducer of increased virulence of luminal bacteria^[20] and much suggest that PPMs, normally indolent colonizers, under stress change their phenotype and become life-threatening pathogens^[21].

OUTCOME ASSOCIATED WITH IMMUNE DYSFUNCTION

Our knowledge and understanding of function of the innate immune system and resistance to disease has increased significantly during the last decade. Increasing evidence suggests that outcome after larger medical and surgical procedures and emergencies is intimately associated with pre-morbid health and the strength of the immune system, also reflected by the speed and depth of functional deterioration during the first few hours after trauma. A recent study suggests that in severe acute pancreatitis four variables: high age, chronic health status, need for mechanical ventilation and increase in serum creatinine during the first 60-72 hrs are strongly associated with poor outcome^[22].

OUTCOME ASSOCIATED WITH EXPOSURE TO CHEMICALS, INCLUDING PHARMACEUTICALS

Homeostasis is important for bodily functions and particularly for the immune system and resistance to disease. Modern man is also richly exposed to chemicals. The effects on immune functions of pharmaceuticals is often not known and appreciated as federal agencies do not regularly require testing of immune effects of new drugs. Evidence from experimental studies allows, however, the assumption that a large proportion of the pharmaceuticals used in medicine and particularly in the ICUs have depressive effects on the immune function. Chemical substances can, depending on dose, have both stimulatory and inhibitory functions, a phenomenon given the name of chemical hormesis and referred to as Arndt-Schultz law^[23]. A broad range of chemicals have also been shown to be immunostimulatory/preventive of morbidity in lower doses and immunoinhibitory/disease-inducing in larger doses. Several drugs have been shown to derange

macrophage functions, bactericidal efficacy and production and secretion of cytokines. For example, supply of antibiotics (150 mg/kg body weight of Mezlocillin, Bayer) has been shown to significantly suppress essential macrophage functions; chemiluminescence response, chemotactic motility, bactericidal and cytostatic ability and lymphocyte proliferation^[24].

OUTCOME ASSOCIATED WITH PROVIDED NUTRITION

In the past, it was largely ignored that the post-traumatic immuno-depression becomes significantly more pronounced when the regulatory functions of the gut and liver are by-passed through parenteral administration of nutrition. It was believed not long ago that parenteral infusion of large amounts of water, electrolytes, nutrients such as fat and sugar, would benefit the patient and improve outcome. Today, however, it is well known that supply, especially parenterally, of larger amounts of fluid and electrolytes^[25-27], fat^[28-30], sugar^[31,32] and nutrients^[33,34] leads to immune dysfunction, reduces resistance to disease and increases morbidity.

NEUTROPHIL FUNCTION IN FOCUS

Severe trauma, major surgery and severe sepsis will, parallel with a significant decrease in lymphocytes, induce a significant increase in circulating and tissue neutrophils.

A marked depression of innate cellular immunity with persistent decline in T-4 helper lymphocytes and elevation of T-8 suppressor lymphocytes is observed in patients after severe trauma^[35]. It is also suggested that a T-4/T-8 lymphocyte cell ratio of < 1 is a reliable sign of severe immuno-suppression and a prediction of complications such as multiple organ dysfunction syndrome, and this has been verified in patients with myocardial infarction, acute pancreatitis, multiple severe trauma and in oncology ICU patients^[36]. Parallel to the decrease in lymphocytes a significant increase in circulating neutrophils and accumulation in tissues of neutrophils will occur, often observed and reported in conditions such as shock, sepsis, major trauma, major burns and severe acute pancreatitis. Accumulating evidence suggest that tissue infiltration of neutrophils in trauma induces common post-trauma/post-operative dysfunctions such as paralytic ileus^[37,38], bone marrow suppression, endothelial cell dysfunction, and results in tissue destruction and organ failure, particularly in lungs^[39-41], intestines^[42], liver^[43] and kidney^[44]. Neutrophil infiltration of distant organs^[45], especially of the lungs^[46] are also characteristic findings in patients dying of sepsis, suggested to be a consequence of 'generalized auto-

destructive inflammation' more than twenty years ago^[45]. The extent of neutrophil infiltration is significantly aggravated by mechanical therapeutic efforts such as handling of the bowels during operation^[37], and ventilation of the lungs^[46]. It is also influenced by poor nutritional status, pre-existing immune deficiency, obesity, diabetes and high levels of blood sugar^[47] and is strongly associated with increased expressions in the body of molecules such as NF- B, COX-2, LOX and iNOS^[48,49]. A recent study emphasizes the role of suppressed apoptosis of circulating neutrophils and its association to increased activation of NF- B and reduced activity of caspases-9 and -3 in patients with clinical sepsis^[50].

THE LUNGS IN FOCUS

The most frequently observed and most often severe clinical manifestations of organ failure are seen in the lungs. In severe acute pancreatitis the organ systems most often involved in early (within 24 hrs) single organ failure are pulmonary (91%^[51], 81%^[52]), renal (4.5%^[51], 5%^[52]) and coagulation (4.5%^[51], 14%^[52]). Extensive neutrophil infiltration not only of the lungs but also other distant organs is a characteristic finding in patients dying of sepsis. The degree of oxidative stress and of neutrophil activation and infiltration, especially in the lungs, appears to be the main determining factor of outcome^[53]. The acute lung injury is characterized by alveolar capillary endothelial cell injury, increased capillary permeability and subsequent hypoxia, and accumulation of neutrophil-associated inflammatory products: reactive oxygen species, proteolytic enzymes, eicosanoids and various other mediators. Splanchnic hypoperfusion with endothelial cell injury, increased expression of intercellular adhesion molecule-1 (ICAM-1)^[54] and serine proteases released by the hypoxic pancreas^[55], mesenteric lymph, transported via the lymphatics and thoracic duct rather than portal vein^[54-56] are in addition to various cytokines^[57] suggested to initiate neutrophil-mediated tissue injuries, particularly in the lungs. Experimental studies have also shown that post-shock mesenteric lymph will activate the mechanisms leading to acute lung injury^[58], and that diversion of thoracic duct lymph will prevent trauma-hemorrhagic shock induced lung injury^[59]. It was recently demonstrated in experimental animals subjected to caecal ligation and puncture (CLP) that stress-induced neutrophil infiltration of the lung and subsequent tissue destruction can be effectively prevented by oral supplementation of a synbiotic cocktail. Asynbiotic formulation, Synbiotic 2000 Forte (see further below), administered orally before the trauma^[60] or a subcutaneous injection^[61]

of the four LAB in the cocktail prevented effectively both neutrophil accumulation and tissue destruction in the lungs (Fig. 2 A-C). The average neutrophil count in lung (average of five fields) after enteral administration of: mixture of LAB and bioactive fibers = 9.00 ± 0.44 (1), only LAB = 8.40 ± 0.42 (2), only bioactive fibers = 31.20 ± 0.98 (3) and placebo (non-fermentable fiber) 51.10 ± 0.70 (4). The corresponding values of myeloperoxidase (MPO) were 25.62 ± 2.19 (1), 26.75 ± 2.61 (2), 56.59 ± 1.73 (3) and 145.53 ± 7.53 (4). Similarly the changes in MDA were 0.22 ± 1.31 (1), 0.28 ± 3.55 (2), 0.48 ± 5.32 (3) and 0.67 ± 2.94 (4) and in nitric oxide 17.16 ± 2.03 (1), 18.91 ± 2.24 (2), 47.71 ± 3.20 (3) and 66.22 ± 5.92 (4). All differences between treatment groups and placebo were statistically significant ($p > 0.05$).

USE OF PRE-, PRO- AND SYNBIOTICS

Both prebiotic fibers and some probiotic bacteria, alone or in combination have demonstrated extraordinary efficacy to restore and maintain immunity and prevent complications. The following effects are reported after use of some specific LAB:

- reduce/eliminate potentially pathogenic micro-organisms (PPMs)
- reduce/eliminate various toxins, mutagens and carcinogens
- promote apoptosis
- synthesize/release numerous nutrient: antioxidants, growth, coagulation and other bioactive compounds
- modulate the innate and adaptive immune defence mechanisms (for further information - see Benchmark^[62-64])

More recent studies suggest that LAB

- promote/maintain gastrointestinal (GI) motility and prevent GI paralysis and postoperative ileus^[65-67]
- And has the ability to:
 - inhibit NF- B activation^[68-70]
 - inhibit constitutive synthesis of IL-8 and synthesis and secretion of IL-8 induced by TNF^[71,72]
 - inhibit COX-2 expression and restore the Cox-1/Cox-2 ratio^[73]

Some of these effects are produced by both live and dead LAB. However, the inhibition of synthesis and secretion of IL-8 is only induced by live LAB and not by bacterial lysate, heat-killed or gamma-irradiated LAB^[71]. Immuno-modulatory effects are also induced by microbial products, such as butyrate, propionate, pyruvate and sometimes also lactate and acetate. Butyrate and propionate for example decrease COX-2 expression by 85 and 72% respectively and increase COX-1 expression by 37 and 23% respectively, effects, which cannot be obtained with lactate or acetate^[72]. Of great interest in this connection are recent observations by Fink,

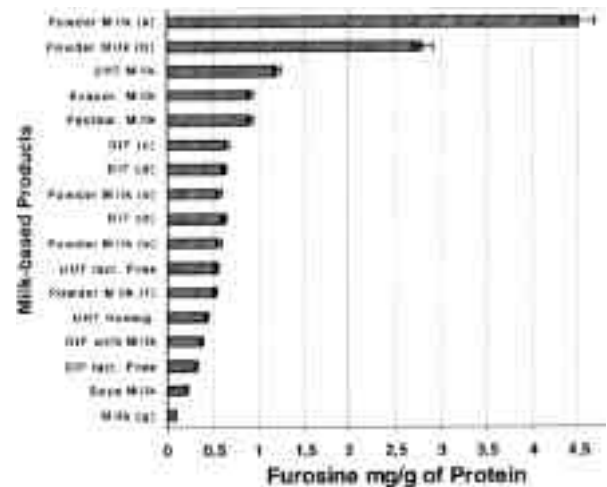


Fig 1: Content of AGE (furosine) in various milk-based products and soya-milk. a. Milk powder kept for two years at room temperature. b. Milk powder kept for one year at room temperature. c. DIF with whey plus casein. d. DIF with hydrolyzed whey. e. Milk powder kept for one year at 4° C. f. Fresh milk powder. g. Raw (whole) bovine milk. DIF= Dietetic Infant formulas, UHT = ultra heat treatment. (after Baptista et al)^[11].

who observed that supplemented pyruvate is an effective scavenger of ROS and exhibits strong anti-inflammatory effects: suppresses NF- B activation, reduces secretion of NO and proinflammatory cytokines, prevents intestinal translocation, reduces cardiac ischemia and improves kidney function^[73]. Cardioprotective effects have also been reported from intravenous administration of lyophilised LAB^[74].

LAB, which shows significant immunomodulatory effects *in vitro* and in animal experiments, will sometimes fail, when it comes to clinical trials. Only a small minority of existing LAB have been demonstrated to show strong clinical immunomodulatory abilities. Experience from clinical studies deem to indicate that the clinical efficacy varies from none to significant as one goes from single-strain probiotic to full flora replacement (enemas of feces): single-strain probiotic < multi-strain probiotic < or ~ single-strain/single fiber synbiotics < multi-strain/multi-fiber synbiotics < total fecal flora replacement^[75,76].

Prebiotics:

Prebiotics are substrates to be fermented by flora e.g., non-digestible food ingredients, mainly plant fibers, which undigested will reach the colon and food ingredients often referred to as colonic foods. Prebiotics are nutrients essential for supply of substrate and energy for both flora and the host, and essential for mucosal growth, water and electrolyte balance, and the body's resistance against invading pathogens. Thus far, only one clinical study has tried only prebiotics in critically

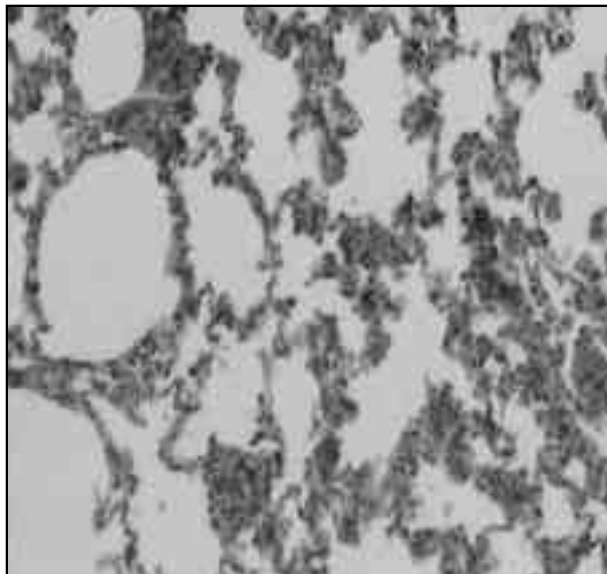


Fig. 2A

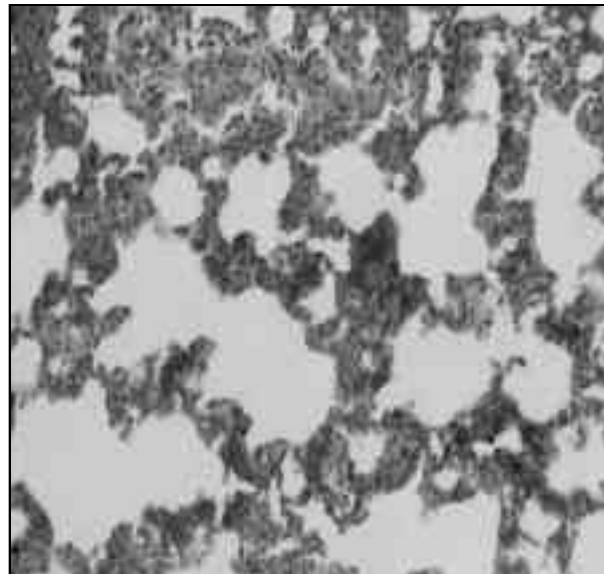


Fig. 2B

Fig. 2: Histological sections of rat lungs 24 hours after cecal ligation and puncture. A. After placebo treatment, B. After treatment with only bioactive fibers, C. After treatment with both bioactive fibers and live lactic acid bacteria (Synbiotic 2000) (Photo Dr Ozer Ilkgul Izmir, Turkey)

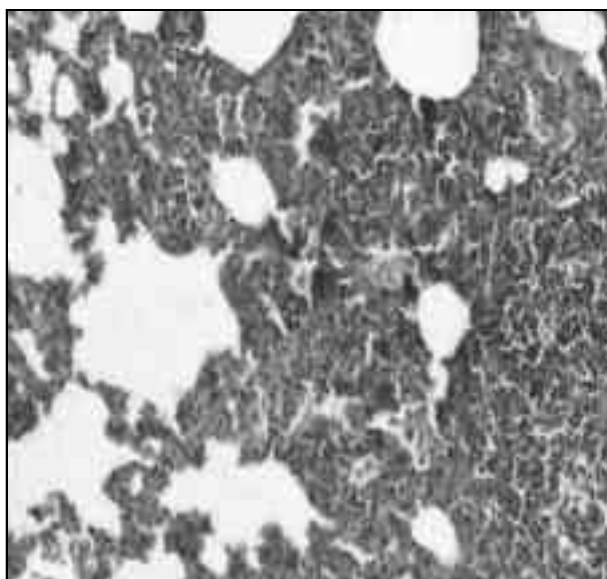


Fig. 2C

ill patients. Forty-one burn patients were randomized to receive during the first 15 days either 6 g of oligofructose/d or sucrose as placebo. No difference was observed between the groups in effect on lactulose/mannitol ratio or clinical outcome^[77].

Probiotics

Probiotics are live microorganisms supplied from outside the body, most commonly to the digestive tract. Most probiotics supplemented to humans, such as those provided with dairy products or sold in health stores, are not effective enough to be used in clinical medicine. Great

differences exist in the ability of LAB to survive the passage through the GI tract and to influence cytokine production after passage through the stomach and small intestine, as demonstrated in a study on ileostomy patients^[78]. Four different LAB species were compared: *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus* and *Bifidobacter animalis*. Out of the originally orally administered 108 cells/ml of each LAB, after the passage through the stomach and small intestine, only between 107 (*Lactobacillus plantarum*) and 102 (*Lactobacillus rhamnosus*) bacterial cells remained. Most of the strains tested showed, after passage through the small intestine, a significantly reduced or weak (especially *Lactobacillus rhamnosus*) ability to influence cytokine production, e.g., the state of inflammation. Also, the ability to ferment fiber is much dependent on the strain used - this is especially so for semi-resistant prebiotics such as oligofructans: inulin and phleins. When the ability of 712 different LAB to ferment oligofructans was studied, only 16/712 were able to ferment the phleins and 8/712 the inulin type fiber^[79]. Only four LAB species were able to ferment these fibers: *Lactobacillus plantarum* (several strains), *Lactobacillus paracasei* subspecies *paracasei*, *Lactobacillus brevis* and *Pediococcus pentosaceus*. The ability to control various pathogens is also strain-specific and limited to a few strains. When the ability of fifty different LAB to control 23 different pathogenic *Clostridium difficile* (CD) was tested, only five proved effective against all, eight were antagonistic to some, but 27 were totally ineffective^[80]. The five most effective strains were *Lb paracasei* subsp *paracasei* (2 strains) and *Lb plantarum* (3 strains).

BOVINE MILK NOT IDEAL AS CARRIER OF PROBIOTICS/SYNBIOTICS

It is important to recognize that cow's milk is not an ideal carrier of probiotics, especially for specific clinical use. In addition to its proposed role as risk factor for increased degree of inflammation in the body and development of ChDs^[81], bovine milk:

- In contrast to breast milk, does not contain any fibers or fiber-like molecules (only elephant milk contains as much as human milk). Complex fucosylated oligosaccharides characteristic of human milk, with structural similarities to immunomodulating cell surface glycoconjugates, which enforce GI immunity and stimulate growth of health-supporting gut microflora, do not exist in bovine milk^[82].

- Cow's milk releases inflammatory mediators, induce inflammation, and induce leakage of molecules such as albumin/hyaluronan, increases intestinal permeability and causes translocation /leaky gut^[83-88].

- Cow's milk is rich in advanced glycation products (AGEs), produced during the heating up/pasteurization process (Fig. 1)^[11,89]. It is particularly so for milk powder, a common ingredient in clinical nutrition formulas. There is a direct association between the dietary intake of AGEs and the level of markers of systemic inflammation^[90].

- Cow's milk is rich in free poly-unsaturated fats (PUFAs), seen also, in lower concentrations than those in fermented dairy products, to reduce the ability of LAB to adhere to mucous membranes and to grow^[91].

- The colonization rate (ability to adhere to the mucosa and replicate) of so called yoghurt bacteria is low (Examples: *lb casei* 2%, *lb Reuteri* 2% and *lb acidophilus* 0%)^[92].

- The LAB which can grow on milk substrates seem to lack clinical efficacy, as demonstrated in two recent controlled studies in postoperative and critically ill patients respectively. A standard commercial product (TREVIS[®], Ch Hansen, Denmark) containing *Lactobacillus acidophilus* LA5, *Bifidobacterium lactis* BP12, *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, mixed with 7.5 g oligofructose was supplied to 45 critically ill patients and 45 controls^[93] and to 72 elective abdominal surgery patients and 65 controls respectively^[94]. The ICU study reported significant reductions in number of PPMs in the stomach of the treated patients, but no influence on intestinal permeability nor any clinical benefits. The peri-operative study reported no differences in bacterial translocation, gastric colonization, or systemic inflammation, or septic complications (See further my commentary on the ICU study^[95]).

MULTI-STRAIN/MULTI-FIBER SYNBIOTICS IN CLINICAL TRIALS

Lund University microbiologists Åsa Ljungh and Torkel Wadström developed a multi-strain/multi-fiber synbiotic formula, which in recent years has been extensively used in clinical trials. The choice of LAB for the formulation was done after extensive studies of > 350 human^[96] and >180 plant microbial strains^[97] and especially their ability to produce bioactive proteins, transcribe NF- κ B, produce pro- and anti-inflammatory cytokines, produce antioxidants, and most important, to functionally complement each other. The formulation consists of a mixture of four bioactive LABs, one from each of the four main genera of lactobacillus; 10^{10} of *Pediococcus pentosaceus* 5-33:3, 10^{10} of *Leuconostoc mesenteroides* 32-77:1, 10^{10} of *Lactobacillus paracasei* subsp *paracasei* 19 and 10^{10} of *Lactobacillus plantarum* 2362, e.g. 40 billion LAB per dose, to which is added a mixture of four well studied bioactive plant fibers: 2.5 g betaglucan, 2.5 g inulin, 2.5 g pectin and 2.5 g resistant starch, a total of 10 g plant fibers. It is produced by Medipharm, Kågeröd Sweden and Des Moines, Iowa, USA under the name of Synbiotic 2000TM. One or two such doses are supplemented to the patients per day. In recent studies a Synbiotic 2000 FORTETM and a Probiotic 2000 FORTETM (no fiber added), containing 10^{11} of each of the four LABs, e.g., 400 billion LAB per dose are tried.

THE EFFECTS OF SYNBIOTIC 2000 THUS FAR INVESTIGATED IN THE FOLLOWING DISEASES

Acute pancreatitis:

Sixty-two patients with severe acute pancreatitis (SAP) (Apache II scores: Synbiotic 2000-treated = 11.7 ± 1.9 , controls = 10.4 ± 1.5) were given either two sachets/day of Synbiotic 2000TM (2 x 40 billion LAB/day and totally 20 g fibers) or the same amounts of fibers (20 g) as in Synbiotic 2000TM during the first 14 days after arrival to the hospital^[98]. 9/33 patients (27%) in the Synbiotic 2000-treated group and 15/29 patients (52%) in the fiber only-treated group developed subsequent infections. 8/33 (24%) Synbiotic 2000-treated and 14/29 (48%) of the fiber only-treated patients developed SIRS, MOF or both ($p < 0.005$)^[98].

Polytrauma:

In polytrauma patients two prospective randomized trials with Synbiotic 2000 and Synbiotic 2000 Forte respectively have been concluded. The first study compared in patients with acute extensive trauma: Synbiotic 2000 (40 billion LAB/d) with a soluble fiber, a peptide diet and supplementation of glutamine. Treatment with

Synbiotic 2000™ lead to a highly significant decrease in number of chest infections (4/26 patients - 15%) as compared to peptide diet (11/26 patients - 42%, $p < 0.04$), glutamine (11/32 patients - 34%, $p < 0.03$) and only fibers (12/29 patients - 41%, $p < 0.002$) (Spindler-Vesel A, personal communication). Also the total number of infections were significantly decreased; Synbiotic 2000^a 5/26 patients (19%), only fibers 17/29 patients (59%), peptide 13/26 patients (50%) and glutamine 16/32 patients (50%). In the second study sixty-five polytrauma patients were randomized to receive once daily for 15 days Synbiotic 2000 Forte (400 billion LAB + 10 gram of fibers, see above) or maltodextrine as placebo. Significant reductions were observed in number of deaths (5/35 Vs 9/30, $p < 0.02$), severe sepsis (6/35 Vs 13/30, $p < 0.02$), chest infections (19/35 Vs 24/30, $p < 0.03$), central line infections (13/32 Vs 20/30, $p < 0.02$) and ventilation days (average 15 Vs 26 days)^[99].

Abdominal surgery:

In a randomized controlled study forty-five patients undergoing major surgery for abdominal cancer were divided into three treatment groups: 1) Enteral nutrition (EN) + Synbiotic 2000 (LEN), 2) EN + only the fibers in the same amounts (20 g) as in Synbiotic 2000™ (FEN) and 3) a standard parenteral nutrition (PN). All treatments lasted for two preoperative and seven postoperative days. The incidence of postoperative bacterial infections was 47% with PN, 20% with FEN and 6.7% with LEN ($p < 0.05$)^[100]. Significant improvements were also documented in prealbumin (LEN, FEN), C-reactive protein (LEN, FEN), serum cholesterol (LEN, FEN), white cell blood count (LEN), serum endotoxin (LEN, FEN) and IgA (LEN). In another prospective randomized double-blind trial performed in 80 patients subjected to pylorus-preserving pancreatoduodenectomy (PPPD) received twice daily either Synbiotic 2000™ (2 x 40 billion LAB) or only the fibers in composition from the day before surgery and during the first seven postoperative days. A highly significant difference in infection rate ($p = 0.005$) was observed as only 5/40 patients (12.5%) in the Synbiotic 2000-treated group suffered infections (four wound and one urinary tract infection) versus 16/40 (40%) in the only fiber group (six wound infections, five peritonitis, four chest infections, two sepsis, and one of each of urinary tract infection, cholangitis and empyema) (Rayes N, et al, personal communication). The infecting microorganism in the Synbiotic treated group were *Klebsiella pneumoniae* (two patients), *Enterobacter cloacae* (two patients), *Proteus mirabilis* (one patient) and *Enterococcus faecalis/faecium* (one patient) and in the

only fiber group *Enterobacter cloacae* (eight patients), *Enterococcus faecalis/faecium* (seven patients), *Escherichia coli* (seven patients), *Klebsiella pneumoniae* (two patients), *Staphylococcus aureus* (two patients), and *Proteus mirabilis* (one patient). Statistically significant differences between the groups were also observed in use of antibiotics (mean: Synbiotic 2000; 2 ± 5 days, only fibers; 10 ± 14 days).

Chronic liver disease and liver transplantation:

Fifty-eight patients with liver cirrhosis suffering so called minimal encephalopathy were randomized into three treatment groups: Group 1 (20 patients) received Synbiotic 2000 (40 billion LAB), Group 2 (20 patients) received the same amount of the fibers in Synbiotic 2000 and Group 3 (15 patients) received placebo (non-fermentable, non-absorbable fiber - crystalline cellulose)^[101]. A significant increase in intestinal LAB flora was observed after one month of supplementation in the Synbiotic-treated group, but not in the other two groups. Intestinal pH was significantly reduced in both treatment groups but not in the placebo-treated group. Significant decreases in faecal counts of *Escherichia coli*, *Staphylococcus* and *Fusobacterium*, but not in *Pseudomonas* and *Enterococcus*, and significant decreases in ammonia/s, endotoxin/s ALT/s and bilirubin/s (original level 252 ± 182) were observed in the Synbiotic 2000-treated group (84 ± 65 , $p < 0.01$) and in the only fiber-treated group (110 ± 86 , $p < 0.05$) while it remained unchanged in the placebo group. The improvements in liver function were accompanied by significant improvements in psychometric tests and in the degree of encephalopathy. Later, studies by the same group of investigators did also show significant improvements in liver blood flow and indocyanine clearance in patients supplemented for one week with Synbiotic 2000^[102]. These results offers great hope that synbiotic treatment to patients on a waiting list for liver transplantation, would help prevent septic episodes, improve liver function, and promote an improved outcome.

Sixty-six patients were randomized to either receive Synbiotic 2000 or only the fibers in Synbiotic 2000 in connection with human orthotopic liver transplantation. The treatment was started on the day before surgery and continued for 14 days after surgery. During the first postoperative month only one patient in the Synbiotic 2000-treated group (3%) showed signs of infection (urinary infection) compared to 17/33 (51%) in the patients supplemented with only the four fibers^[103]. The infecting organisms in the Synbiotic-treated group were *Enterococcus faecalis* in one patient and in the only fiber-treated group were *Enterococcus faecalis/faecium* -11, *Escherichia coli* -3, *Enterobacter*

cloacae-2, *Pseudomonas aeruginosa*-2 and *Staphylococcus aureus* in one patient. The use of antibiotics was on an average 0.1 ± 0.1 d in the synbiotic-treated patients and 3.8 ± 0.9 d in the only fiber-treated group.

Inflammatory bowel disease:

Daily rectal instillations with Synbiotic 2000 reconstituted in saline were given to ten patients with distal colitis over two weeks. One patient withdrew after one week. The remaining patients showed dramatic improvements in various disease scores during the three weeks of observation; episodes of diarrhea (2.4 ± 0.8), visible blood in stool (2.2 ± 0.8), nightly diarrhea (0.5 ± 0), urgency (1.9 ± 1.0) and consistency of stool (1.1 ± 0.8)^[104]. Two patients reported significant bloating and wind but no other adverse or side effects were reported.

TREATMENT-RESISTANT CONDITIONS

Treatment with Synbiotic 2000 has failed in two types of diseases:

General Intensive Care patients:

Two large studies have been performed in a general intensive care population; one with Synbiotic 2000 and one with Synbiotic 2000 FORTE. Synbiotic 2000 (40 billion LAB) was given to 162 patients and only fiber to 168 patients. No difference was observed in frequency of MODS or in hospital mortality^[106]. In another study, Synbiotic 2000 FORTE was supplemented to 130 patients twice a day throughout the whole ICU stay (2 x 400 billion LAB) and compared to 129 patients supplemented with a cellulose based placebo. No statistical difference was demonstrated between the groups in the incidence of VAP (9 and 13%, $p = 0.31$). The rate of ventilator-associated pneumonia (VAP) per 1000 ventilator days was 13 and 14.6% ($p = 0.73$) and hospital mortality 27 and 33%, ($p = 0.32$), respectively^[106].

Inflammatory bowel disease - Crohn's Disease:

Two studies with negative outcome have also been performed in patients with Crohn's disease. Sixty-three patients after an initial treatment with infliximab were randomized to receive daily either Synbiotic 2000 or crystalline cellulose as placebo^[107]. Median time to relapse was 9.8 and 10.1 months respectively. Twenty patients in another study were supplied Synbiotic 2000 and nine patients with crystalline as placebo following surgery for Crohn's disease. Seven patients in the Synbiotic-treated group and two in the placebo group completed the scheduled 24 month treatment^[108]. No difference in either endoscopic picture or rate of clinical relapse was found between patients. The so called

Rutgeerts' disease scores after three months of treatment were 0.6 ± 0.8 in the Synbiotic-treated group and 0.8 ± 1 in the placebo group (NS).

CONCLUSIONS

The novel treatment of Synbiotics is still in its early infancy. It is clear that, although sometimes dramatic effects are observed after synbiotic treatment, there are conditions, which do not seem to respond to such treatment. A lot of evidence suggests that one of the main effects of synbiotic treatment is reduction of both acute and chronic inflammation. However, some indications suggest that synbiotic treatment is not an effective tool to restore suppressed immunity, which might explain the observation that synbiotic treatment, as well as probiotic treatment is more effective when supplemented pre-trauma/operation and/or immediately or early after trauma/operation. There is presently no solid explanation why seemingly synbiotic treatment is very effective in chronic liver disease, but is unable to reduce local inflammation and degree of disease in a chronic bowel disease such as Crohn's disease.

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