Synbiotics and the mucosal barrier in critically ill patients

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Purpose of review

Outcome in severe and critical illnesses is strongly related to premorbid conditions: the strength of the mucosal barriers, the innate immune system, and the built-in resistance to disease. Early risk factors and determinants of poor outcome are factors such advanced age; impaired premorbid health status, especially diabetes and high body mass index (obesity); and immunosuppressive treatments. Combined supplementation of bioactive fibers and lactic acid bacteria (synbiotics) directly and indirectly influences several of these factors.

Recent findings

Determinants for poor outcome are degree of oxidative stress, neutrophil activation, and infiltration of tissues, especially in the lungs. Attempts at early reduction of the exaggerated inflammatory storm and limitation of further impairment of the immune function are always given the highest priority. The supply of live lactic acid bacteria and plant fibers can dramatically reduce the hyperinflammation and also the infiltration by neutrophils of organs such as the lungs. New and efficient autopositioning and regurgitation-resistant feeding tubes provide instruments for the early supply of enteral nutrition with immune-boosting antioxidants and synbiotics.

Summary

A meticulous choice of probiotic lactic acid bacteria is recommended because only a small minority of the lactic acid bacteria survive the harsh environment of the upper gastrointestinal tract, ferment strong semiresistant fibers such as inulin, and have the ability to control inflammation and eliminate unwanted pathogens, such as antibiotic-resistant microorganisms and *Clostridium difficile*.

Keywords

critical illness, enteral nutrition, feeding tubes, lactic acid bacteria, mucosal barrier, plant fibers, prebiotics, probiotics, synbiotics

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Abbreviations

ICU intensive care unit
LAB lactic acid bacteria

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Introduction

Pharmaceutical medicine has, despite edge-cutting progress in some fields, failed to prevent or reduce both chronic disease and acute critical illness. Today, chronic disease constitutes 46% of the global disease burden and 59% of global mortality, and its incidence increases from year to year worldwide, especially in the third world. Critical illnesses are also on the rise: each year more than 750 000 Americans are treated in intensive care units (ICUs), and almost a quarter of a million die of such illnesses. These figures also increase from year to year. It is important to remember that most often it is those with chronic illness and signs of dysfunction of the innate immune system who are the victims of critical illness.

Our knowledge of the innate immune system and its function and our understanding of resistance to disease has increased tremendously over the past 10 to 15 years. Solid evidence suggests that outcome after larger surgical operations as well as in medical emergencies is intimately associated with the premorbid health and strength of the innate immune system especially but is also associated with the speed and depth of deterioration in function during the early few hours. It is increasingly obvious that medicine should give more focus to measures that give early support to a failing immune system and enforce the individual's resistance to disease.

Lifestyle-related exaggerated inflammation

Approximately 75% of the immune cells of the body are localized to the gut, and almost all immune cells in the body, especially to those active in the chest, are conditioned in the gut. Recent development in genomics has made it possible to study and identify the effects on our genes of various food ingredients and has identified some with a strong association to increased inflammation and reduced barrier functions. Central to reduced resistance to disease are increased levels in the body of molecules such as nuclear factor-κB, cyclooxygenase-2, leukotreine oxygenase, and inducible nitric oxide synthase, to mention a few molecules often affected by various food ingredients.

The Western lifestyle with its reduced physical exercise, high levels of mental stress, and consumption of calorie-condensed foods with large amounts of bread and dairy products is clearly associated with elevated levels in the body of inflammatory markers. As an example, dairy products, especially milk (mostly from pregnant cows) is relatively rich in proinflammatory molecules: hormones, especially estrogens; growth factors, especially insulin-like growth factor-one; and various other stress-induced molecules. The consumption of bovine milk has also been shown both to release

inflammatory mediators, to increase intestinal permeability, and to induce leakage of molecules such as albumin and hyaluronan. Also, bread contains molecules with proinflammatory effects in a significant fraction of humans. Most fruits and vegetables, however, are rich in antioxidants and in antiinflammatory molecules.

Involvement of microbial genes

The collective genome of the microbes with which we live in symbiosis contains approximately 100 times more genes than the human genome *per se* [1••]. The microbiota is an important metabolic organ with great importance for the release and absorption of numerous antioxidants and nutrients; for the regeneration and growth of various human cells, particularly mucosal cells; for the function of the innate but also the adapted immune system; and for the control and reduction of various toxic and mutagenic agents and potentially pathogenic microorganisms – bacteria, viruses, and fungi. Recent observations suggest that the microbiota also has a profound regulatory effect outside the gut and is of significant importance for basic functions including the regulation of fat storage [1••,2••].

Nutrigenomics has the ability to identify both the beneficial and the damaging effects of food ingredients, pharmaceuticals, and other chemicals on both eukaryotic and prokaryotic genes. It has been known for centuries that diet can dramatically alter the microbial composition of the microbiota, but only recently have the consequences of that been fully appreciated. The widespread use of antibiotics has led to a reduced and malfunctioning immune system, which also contributes to the development of chronic diseases, particularly allergies and asthma. Antibiotic treatment not only destroys immune functions such as the phagic function of macrophages but also results in a long-term decrease of beneficial anaerobic flora, especially *Bifidobacterium*, *Lactobacillus*, and *Bacteroides* species, with implications for resistance to disease, especially infections.

It has been observed that flora are generally reduced in people with a Western type of lifestyle. A study from 1983 showed that *Lactobacillus plantarum*, a dominating lactic acid bacteria (LAB) among plant eaters, was found in only approximately 25% of omnivorous Americans and in approximately two thirds of vegetarian Americans [3]. A more recent study suggests that the normally most common colonic LAB of healthy individuals are present only in approximately 50% or less of healthy Scandinavians: *L. plantarum* in 52%, *L. rhamnosus* in 26%, and *L. paracasei* ssp *paracasei* in 17% [4]. No data support a recent change for the better.

Diversity in the microbiota for barrier function

The microbiota and the gut mucosa are intimately joined in the maintenance of a well-functioning barrier between the host and the external environment [5••,6•]. The barrier is suggested to be composed of three barriers in one: the physical barrier, the innate immune barrier, and the adaptive immune barrier. In the past, emphasis was mainly given to the physical barrier, but in more recent years it has shifted to the importance of the innate immune mechanisms, particularly the role of antimicrobial peptides such as defensins and, more recently, angiogenins as well [7].

Several plant fibers (prebiotics) and a few supplemented LAB (probiotics) have documented effects to improve both the function of the innate immune system and the physical barrier and to increase resistance to disease. The hope is that a combined supply of these components will have synergistic effects (more than additive effects) in boosting the immune system and enforcing the barrier functions. Products that combine prebiotics and probiotics are called synbiotics, and treatments using the combination are called synbiotic treatment.

The term 'defense by diversity' was coined in 1999 and seems applicable also to synbiotic treatment [8]. A recent study concluded that combining several fibers has more than additive effects on microbial ecosystem and immune responses [9•], and a recent review suggested that multispecies probiotics are superior to single-species probiotics to enhance growth, reduce antibiotic-associated diarrhea, prevent infections (*S. typhimurium*), and reduce pathogenic colonization (*E. coli*) [10•].

Supply of synbiotics important for barrier function

The choice of prebiotics and probiotics must be based on scientific evidence, as will be discussed below. This is especially important in the selection of LAB because most LAB have no effect or very limited effects on immune function and outcome. It is my opinion, based on extensive literature studies and personal experience, that the clinical effects vary from no effect or a modest effect to significant effects as one goes from single-strain to full flora replacement: single-strain probiotic < multistrain probiotic < or ~ single-strain/single fiber synbiotics < multistrain/multifiber synbiotics < total flora replacement [11,12•].

An example of a carefully selected synbiotic product is Synbiotic 2000, which was composed after extensive studies by microbiologists Åsa Ljungh and Torkel Wadström at Lund University, Sweden. The choice of LAB was made after studies of 350 human LAB and 180 LAB from growing plants and was based on the documented ability of the different LAB to produce proinflammatory and antiinflammatory cytokines; to produce bioactive proteins, including heat shock proteins; to transcribe nuclear factor-κB; and to release antioxidants [13,14]. The formulation contains four specific LAB, one from each of the four main genera of lactobacillus: 10^{10} of *Pediacoccus pentosaceus* 5–33:3, 10^{10} of *Leuconostoc mesenteroides* 32–77, 1.10^{10} of *L. paracasei*

subsp *paracasei* 19, and 10^{10} of *L. plantarum* 2362. This makes 40 billion LAB per dose, to which is added a mixture of four well-studied bioactive plant fibers: 2.5 g beta-glucan, 2.5 g inulin, 2.5 g pectin, and 2.5 g resistant starch, for a total of 10 g plant fibers (Medipharm, Kågeröd, Sweden, and Des Moines, Iowa, USA). A more recent study has also shown that these LAB, like some other probiotic bacteria such as *E. coli* Nissle, produce β-defensins especially and that these LAB seemingly have additive effects [15••].

Yogurt bacteria: no effect or modest effects

Attempts have been made in the past with a synbiotic composition that seems to have been composed at random without preclinical study of biologic activity; at least, no such documentation is provided. Two recent controlled studies describe the effects of a standard commercial product, TREVIS (Chr Hansen Biosystem, Denmark), consisting of LAB commonly found in dairy products: *L. acidophilus* LA5, *Bifidobacterium lactis* BP12, *Streptococcus thermophilus*, and *L. bulgaricus* combined with 7.5 g oligofructose [16••,17•]. Significant reductions in the number of potentially pathogenic organisms in the stomach but no influence on intestinal permeability and no clinical benefits were reported in critically ill patients (45 TREVIS-treated patients and 45 control individuals) [16••]. A similarly designed study was also performed in postoperative patients, 72 of whom received TREVIS and 65 of whom received placebo for 2 weeks. Nasogastric aspirate, mesenteric lymph nodes, and scrapings of the terminal ileum were obtained at surgery for microbiologic analysis. No significant differences were observed in septic morbidity and mortality (TREVIS, 12.1%; placebo, 10.7%; $P = 0.8$), septic complications (TREVIS, 32%; placebo, 31%; $P = 0.9$) gastric colonization (TREVIS, 41%; placebo, 44%; $P = 0.7$), systemic inflammation, or gut barrier function [18•].

Strong immunoenhancing effects from selected synbiotics

In a recently published study, a mixed group of 11 ICU patients were given two sachets of Synbiotic 2000 (diluted in 100 ml of sterile water) once daily for 3 days, and the numbers of *Lactobacillus* colonies were calculated before and after. Most of the patients had no LAB in the feces before treatment, but the levels of LAB returned to normal after the synbiotic treatment (Fig. 1) [19•]. The same group of researchers continued their studies and performed a more extensive study in 277 ICU patients; the study was recently concluded but unfortunately no analysis has yet been performed.

A prospective randomized double-blind trial was undertaken in 66 liver transplant recipients [20••]. All patients received enteral nutrition immediately postoperatively. A comparison was made between one group (A) receiving Synbiotic 2000 and another group (B) receiving only the fibers in the composition. Both treatments were well tolerated. The treatment started the day before surgery and continued for 14 days. In both groups, mainly mild or moderate infections occurred. The incidence of postoperative bacterial infections was significantly reduced by synbiotic treatment; being 48% (16 of 33 patients: urinary infections 12, cholangitis 2, chest infection 1, wound infection 1) in the fiber-only group and 3% (1 of 33 patients: urinary infection 1) in the Synbiotic 2000-treated group. Only one microbe (*Enterococcus fecalis*) was isolated in the Synbiotic 2000-treated group, in contrast to 17 in the fiber-only-treated group (*Enterococcus faecalis* 11, *Escherichia coli* 3, *Enterobacter cloacae* 2, *Pseudomonas aeruginosa* 2, *Staphylococcus aureus* 1). The total use of antibiotic therapy was significantly less in the Synbiotic 2000-treated group.

In a still unpublished study, patients with severe acute pancreatitis were supplemented for 14 days with either two sachets/day of Synbiotic 2000 (2×40 billion LAB/day, total 20 g) or fiber only (20 g). In this study, 9 of 33 patients (27%) in the Synbiotic 2000-treated group and 15 of 29 patients (52%) in the fiber-only-treated group experienced infections. A significant reduction ($P < 0.05$) in combined systematic inflammatory response syndrome and multiple organ failure was observed: 8 of 33 (24%) Synbiotic 2000-treated experienced the syndromes, compared with 14 of 29 (48%) of the fiber-only-treated patients (Olah A, personal communication).

Synbiotic treatment reduces oxidative stress and neutrophil infiltration

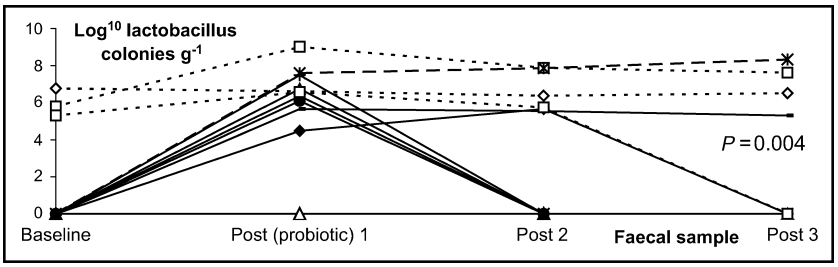
The big challenge in critical illness seems to be the exuberant inflammatory response that is evoked. Host phagocytic cells, predominantly neutrophils and macrophages, play a central role in both the containment of the insult and the ensuing tissue injury [21••]. The degrees of oxidative stress and of neutrophil activation seem to be the determining factor of outcome, and extensive neutrophil infiltration of the lungs but also other distant organs is a characteristic finding in patients dying in sepsis [22]. Pulmonary dysfunction is a most common complication in critical illness and will in the worst scenario lead to acute respiratory distress syndrome and eventually death. It occurs as the result of pulmonary accumulation of neutrophils with secondary damage of lung tissue through various neutrophil-released products such as reactive oxygen species, proteolytic enzymes, and eicosanoids, known to induce endothelial cell injuries, increased capillary permeability, and hypoxia. A recent but unpublished study suggests that the pulmonary infiltration with neutrophils can be totally prevented by an enteral supply of synbiotics and also intraperitoneal injection of live *Lactobacillus* in animals with induced sepsis (cecal ligation and puncture) (Ilkgul O, personal communication).

Choice of probiotics

Most of the LAB supplied orally will not survive the harsh environment of the upper gastrointestinal tract, the

Figure 1. Fecal *Lactobacillus* colony numbers before and after 3 days of supply of Synbiotic 2000.

Fecal context of LAB before (baseline) and, after various intervals of supplementation with LAB to critically ill. Published with permission from [19].



acidity of the stomach, and the large amounts of bile acids in the small intestine. When a few different *Lactobacillus* strains were orally administered in a concentration of 10^9, only one strain, *L. plantarum*, was recovered in the lower small intestine (ileostomy) in a concentration of 10^7 or greater [23]. The ability to induce immune response was, despite the loss of two logs, even greater than before administration, most likely owing to stress activation of the LAB during passage through the upper gastrointestinal tract. The ability to ferment prebiotic fibers is also of great importance to the choice of probiotics. When this ability was studied, only 8 of 712 LAB were tested and only 8 could ferment inulin type fiber: *L. plantarum* (several), *L. paracasei* subsp. p*aracasei*, *L. brevis,* and *Pediococcus pentosaceus* [24]. A recent study also suggested that only a few LAB have the ability to control pathogens such as *Clostridium difficile* [25••]. When the ability of 50 different LAB to control 23 different pathogenic *C. difficile* was studied, 27 strains were totally ineffective, 8 strains were antagonistic to some, and only 5 strains proved effective against all *C. difficile* strains: three *L. plantarum* strains and two *L. paracasei* subsp. *paracasei* strains [25••]. In various other studies, these LAB strains have also proved to be effective in inducing cellular immunity; stimulating production of suppressive cytokines (transforming growth factor-β, interleukin-10); suppressing CD4 T cells, Th2 activity, and splenocyte proliferation, and decreasing specific IgE and IgG1.

A narrow therapeutic window

Immune suppression with signs of hyperinflammation and of suppression of the expression of monocyte histocompatibility leukocyte antigen-DR is an early phenomenon in critical illness and is strongly associated with the subsequent development of septic complications and poor outcome [26,27••,28••]. There is much support for the existence of a narrow therapeutic window for modulation of the inflammatory cascade. Immunosupportive treatment must thus be instituted before the beginning of disease or injury if possible, otherwise from the earliest moment thereafter. Most experience supports the provision of enteral nutrition by nasojejunal tube when the patient cannot eat. I have for this purpose developed a special autopositioning and regurgitation-resistant tube with a built-in ability for placement without the assistance of radiology or endoscopy (Bengmark Flo-Care tube, Royal Numico-Nutricia group, Zoetermeer, The Netherlands). Successful intubation was early reported in patients with inhibited or delayed gastrointestinal motility (acute pancreatitis); the head of the tube had reached its optimal position in 10 of 10 patients in an average of 5.2 hours, and always within 24 hours [29]. Successful 24-hour placement was recently reported in patients with normal gastric emptying in 78% of patients using this tube, compared with 14% with the use of a standard straight tube (P = 0.041) and in 57% of patients with impaired gastric emptying (as occurs with most critically ill patients) compared with 0% of patients using standard tubes (P = 0.07) [30]. Successful insertion in 12 of 16 patients (75%) was reported in patients with acute pancreatitis, the tube reaching the ligament of Treitz within a median of 12 hours [31].

Conclusion

The ability of LAB to reduce inflammation can also be used to prevent various chronic diseases, most likely acute graft-versus-host disease as well. Increasing evidence suggests that flora play an important role in the initiation of acute graft-versus-host disease. Oral administration during the 7 days before and 7 days after transplantation of *L. rhamnosus* GG results in significantly reduced translocation of enteric bacteria, reduced acute graft-versus-host disease, and improved survival [32••].

New methods for the delivery of LAB will most likely be increasingly tried and will provide solutions for those unable to tolerate enteral feeding. Significant attenuation inflammation has also been observed after subcutaneous administration of live *Lactobacillus* to interleukin-10 knockout mice in conditions such as arthritis and colitis [33••]. The success with topical application by enemas in ulcerative colitis certainly stimulates further studies [34]. Significant reduction of inflammation and formation of inflammation-induced peritoneal adhesions has been observed in experimental animals after the intraperitoneal injection of probiotic bacteria (Ilkgul O, personal communication). Also, the inhalation of LAB offers an attractive

alternative, especially for critically ill patients. Probiotic, prebiotic, and synbiotic treatments have a huge potential. Bioecologic control is in its early infancy.

References and recommended reading

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 740).

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