The role of bacterial translocation in sepsis: a new target for therapy

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Abstract: Sepsis is a leading cause of death in critically ill patients, primarily due to multiple organ failures. It is associated with a systemic inflammatory response that plays a role in the pathogenesis of the disease. Intestinal barrier dysfunction and bacterial translocation (BT) play pivotal roles in the pathogenesis of sepsis and associated organ failure. In this review, we describe recent advances in understanding the mechanisms by which the gut microbiome and BT contribute to the pathogenesis of sepsis. We also discuss several potential treatment modalities that target the microbiome as therapeutic tools for patients with sepsis.

Keywords: bacterial translocation, dysbiosis, gut microbiome, sepsis

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Introduction
Sepsis is a life-threatening organ dysfunction associated with a dysregulated host response to infection.1 It is a leading cause of death due to multiple organ failure (MOF) in critically ill patients.2 Sepsis affects 300 million people annually and is a cause of death in more than 200,000 patients, making it the tenth most common cause of death in the United States.3 The gut microbiome is associated with the pathogenesis of sepsis. Figure 1(a) shows a schematic diagram of several variables involved in the mechanism underlying sepsis development. Both bacteria-associated and host-related factors contribute to the development of gut barrier dysfunction and bacterial translocation (BT) in sepsis.4–7 In this review, we describe some of the recent advances in understanding the mechanisms through which the gut microbiome contributes to the pathogenesis of sepsis and discuss potential therapeutic measures for sepsis that target the microbiota.

Dysbiosis is an imbalance of the gut microbiota in which the composition or function of the normal bacteria colonizing the gut is disrupted.8

The cause of dysbiosis is multifactorial and involves a combination of genetic, dietary, stress, and disease factors (Figure 1(a)). Several genetic disorders were described in association with dysbiosis.9–11

Dysbiosis is associated with an impaired ability to maintain mucosal membrane function, contributing to systemic inflammation.12 When dysbiosis occurs, toxins, bacterial endotoxins, bacteria, or debris can leak from the gut, along with food particles containing proteotoxins, such as gluten, casein, and zein, and heat-induced molecules, such as advanced glycation end products and advanced lipoxidation end products.12

BT in sepsis
The gut is a source of systemic infection in critically ill patients due to increased BT associated with compromised barriers. Intestinal bacteria can be disseminated systemically via BT, moving through the mesenteric lymph nodes (MLNs) or activating the gut immune system.13,14 The intestinal barrier and BT dysfunction are involved in MOF, a complication of sepsis due to activation of the immune system and the secretion of pro-inflammatory cytokines.15–18 In BT, bacteria move through transcellular passages between enterocytes under the control of membrane pumps and via paracellular pathways due to the disruption of tight junctions.19,18 These mechanisms are potentiated by the intestinal barrier dysfunction, which is composed of physical, biochemical, and immunological factors. Endotoxins and antigens are transported from the gut into the circulation.20–22
Gut-origin sepsis is a process in which bacteria and bacteria-associated products incite a systemic response. These systemic responses are responsible for other clinical manifestations in critically ill patients with syndromes, such as acute respiratory distress syndrome (ARDS) and multiple organ dysfunction syndrome (MODS).\textsuperscript{23–25} BT can induce an inflammatory response, resulting in cytokine-mediated systemic inflammatory response syndrome (SIRS) and MODS.\textsuperscript{15} The mechanisms associated with the development of SIRS and MODS are linked with bacterial mediators leaving the gut termed ‘danger particles’ or pathogen-associated molecular patterns (PAMPs). PAMPs stimulate the innate immune system.\textsuperscript{26}

**Figure 1.** (a) Variables associated with the development of sepsis. Bacterial factors, including bacteria and host factors, include age, ethnicity, the immune system, diet, background diseases, and medications. Each factor represents a potential target for therapeutic interventions. The roles of dysbiosis and BT in the pathogenesis of sepsis are highlighted. (b) A schematic presentation of several potential therapeutic measures for sepsis, focusing on gut dysbiosis. A shift between a normal/healthy/low sepsis risk microbiome and an altered microbiome and a sepsis microbiome or that the altered microbiome (‘dysbiosis’) increases the risk of sepsis.
Both viable bacteria and PAMPs can translocate and exert immunomodulatory effects that lead to sepsis. These factors lead to increased expression of nuclear factor kappa light-chain enhancer of activated B cell (NF-κB)-associated inflammatory transcription factors, and the excess production of inflammatory cytokines associated with end-organ damage.

**Dysbiosis: malfunctioning of the gut microbiota and bacteria-dependent factors that determine virulence**

Patients in the early stages of sepsis manifest differences in their microbiome composition. These patients show lower counts of *Bifidobacterium* and *Lactobacillus* species, which protect the normal gut microbiota, and higher counts of pathogenic facultative anaerobes, such as *Staphylococcus* sp. and *Pseudomonas aeruginosa* sp. Similar changes have been described in patients post laparotomy. Mice injected intraperitoneal with a fecal solution collected from C57BL/6 (B6) cecum developed polymicrobial sepsis, with a high mortality rate and significant bacterial dissemination. Hepatic dysfunction and systemic pro-inflammatory responses were noted, and their cytokine profiles, among them interleukin (IL)-1β, IL-6, and tumor necrosis factor-alpha (TNF-α), were correlated with the severity of sepsis.

Gram-negative bacteria release lipopolysaccharide (LPS) and other virulence factors that induce an inflammatory response. They trigger the translocation of NF-κB to the nucleus, resulting in the upregulation of target adhesion molecules and cytokines, including IL-6, leading to leukocyte recruitment via increased E-selectin and intercellular adhesion molecules. Immunegenic bacterial proteins, including proteins involved in cell adhesion, fimbria, oxidoreductase activity, proteolysis, antimicrobial resistance, and ion transport, were expressed in humans with *Salmonella Paratyphi* bacteremia and sepsis.

**The interplay between the gut microbiome and the gut immune system**

Sepsis and MOF result from recognizing virulent species by the innate immune system. In sepsis, viable and non-viable pathological bacterial products translocate and promote a self-perpetuating circle of dysfunctional immune activation and systemic inflammation. The innate immune system plays a role in regulating the composition of the microbiome, and BT occurs more frequently in immunocompromised patients. Immune deficiencies and immunosuppressive therapies are associated with quantitative and qualitative changes in the intestinal microbiota, mucosal barrier permeability, and BT. Bloodstream infections that occur in patients with febrile neutropenia following cytotoxic chemotherapy are caused by BT. Common gastrointestinal organisms are more prevalent in patients with neutropenia, suggesting that the BT of gut organisms causes central line-associated bloodstream infections in the setting of neutropenia.

In an animal model of sepsis, death was linked to a component of the innate immune system, the node-like receptor (NLR) apoptosis-inhibitory protein (Naip) 5-NLR4 (Naip5-Nlrc4) inflammasome. The immune system is activated via pathways involving toll-like receptors (TLRs) and NF-κB. TLRs are transmembrane proteins that recognize microbial pathogens via the motifs mentioned above and invoke an intracellular signaling cascade that culminates in the activation of macrophages and the secretion of pro-inflammatory cytokines, promoting MODS.

In individuals with an impaired gastrointestinal barrier, LPS stimulates inflammatory reactions involved in the pathogenesis of sepsis. LPS originating in the intestinal lumen reaches the MLNs and circulatory system, causing endotoxia, which leads to systemic inflammation. Immune suppression via a process in the MLNs affects the level of LPS in the abdominal aortic blood, thereby determining the degree of endotoxin translocation. LPS can be eliminated in humans by inactivating LPS via LPS-binding molecules, enzymes that degrade LPS, or modifying the LPS-responding target cells. Primary or secondary defects in any of these can induce SIRS.

In mice with cecal ligation and perforation (CLP), a model of polymicrobial sepsis, perforation of the cecum allows the release of fecal material into the peritoneal cavity to generate an exacerbated immune response. In this model, LPS was positively correlated with the percentage of regulatory
T lymphocytes and negatively correlated with the ratio between pro- and anti-inflammatory cells. Studies have shown that the microbiome affects the immune system. Dysbiosis was shown to be associated with elevated plasma endotoxin levels and a higher rate of BT in the liver in rats with acute rejection following orthotopic liver transplantation. Acute rejection is accompanied by a shift in gut microbiota composition toward *Bacteroides* and *Ruminococcus*. Bacteremia is a frequent complication of allogeneic hematopoietic stem cell transplantation. These patients develop a shift in the bacterial populations of the gut and reduced microbial diversity. Commonly encountered organisms, including *Enterococcus*, *Streptococcus*, and *Proteobacteria*, account for more than 30% of the microbiota. Enterococcal domination increased the risk of vancomycin-resistant *Enterococcus bacteremia*, and Proteobacterial domination increased the risk of Gram-negative rod bacteremia. The antibiotic treatment further affects the microbiota and the associated risk for sepsis. The risk of sepsis within 90 days after discharge from a previous hospital stay by type of antibiotic received during the previous stay was recently determined. The study showed that the risk of sepsis-associated exposure to our high-risk antibiotics was 65% higher than those without antibiotic exposure and was higher with increased quantities of antibiotics during hospitalization.

Within hours, the SIRS in sepsis shifts to an adaptive anti-inflammatory state with immunosuppression. This anti-inflammatory phenotype is characterized by diminished expression of pro-inflammatory cytokine genes in response to TLR stimulation with bacterial LPS. Translation of TNFα and IL-6 mRNA in endotoxin-adapted human monocytes is suppressed by a microRNA (miRNA)-based mechanism. TLR4-induced miRNA-146 modifies the subcellular localization of RNA-binding motif protein 4 (RBM4), resulting in the assembly of a translation-suppressor complex that disrupts the synthesis of TNFα and IL-6. Knockout of miRNA-146a results in serine-309 phosphorylation of RBM4 and its nuclear re-localization, which restores the TLR4-dependent synthesis of TNFα and IL-6, suggesting that miRNA-146a has a role in limiting an excessive acute inflammatory reaction.

Matrix metalloproteinase 7 (MMP7) is associated with activating alpha-defensins and broad-spectrum antimicrobial, anti-inflammatory peptides produced by Paneth cells in the gut. MMP7 protects mice from LPS-induced intestinal permeability and mortality. LPS induces the activation of MMP7 in the small intestine, degranulation of Paneth cells, and promotes intestinal permeability. MMP7(−/−) mice are resistant to LPS-induced death, which is correlated with reduced cytokine levels and a decrease in LPS-induced BT to MLNs. Alpha-defensins stimulate IL-6 release from macrophages and ileum explants in a TLR4-independent manner.

Follistatin-like protein 1 (FSTL-1) is overexpressed under inflammatory conditions characterized by elevated IL-1β. FSTL-1 activates the mitochondrial electron transport chain increasing adenosine triphosphate (ATP) production. It is an essential potentiator of the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome. Serum concentrations of FSTL-1 are increased in patients with bacterial sepsis and mice administered LPS.

**The interplay between the gut microbiome and the gut-associated nervous system affects BT**

The enteric nervous system (ENS), which comprises multiple small ganglia that operate discretely but maintain the ability to communicate with each other to form the myenteric and submucosal neural plexuses, is crucial for the proper functioning of the intestine. Enteric neurons express TLRs, suggesting that bacterial metabolites impact the ENS. Data from studies of neurodegenerative diseases and their relationship with the ENS and microbiome showed that the ENS functions like a relay system between the immune system, brain, and microbiome. In addition, the microbiome was shown to support the postnatal establishment of the ENS and play a role in maintaining homeostasis.
and central nervous system impacts inflammation. The interplay among the epithelial cells, mast cells, resident macrophages, glial cells, neurons, and smooth muscle cells within the gut wall involves intracellular signaling pathways, TLRs, and neuroactive substances, such as nitric oxide, prostaglandins, cytokines, chemokines, growth factors, tryptases, and hormones.

Intestinal ischemia and hyperdynamic circulation are associated with low systemic vascular resistance and altered gut permeability

Primary or secondary circulatory changes are associated with BT. During intestinal ischemia, inorganic phosphate, urea, and threonic acid were upregulated, while stearic acid, arabinose, xylose, glucose, and ribose acid were downregulated. These molecular changes resulted from decreased gut microbiota metabolism, altered intestinal absorption, impaired renal function, and increased oxidative stress, which affected gut permeability.

Sepsis and cirrhosis are characterized by a hyperdynamic state of low systemic vascular resistance and the release of multiple mediators. The similarity between the two conditions is related to the translocation of common bacterial products. Permeability changes and BT influence intra-abdominal hypertension (IAH), IAH-induced enterogenic endotoxemia, and subsequent MOF and alter the histology of the colonic mucosa, the expression of tight junction proteins, mucosal permeability, and the pro-oxidant–antioxidant balance. Acute exposure to elevated intra-abdominal pressure alters intestinal permeability and the pro-oxidant-antioxidant balance, intestinal mucosal injury, and subsequent gut-derived sepsis. The gut-lymph hypothesis of MODS suggests that it occurs in high-risk patients due to gut injury and systemic spread of non-microbial, tissue-injurious factors that reach the systemic circulation via the intestinal lymphatics.

External factors impacting the gut microbiome

i. Various environmental insults alter the gut microbiome. Age, ethnicity, the immune system, and diet also regulate the intestinal microbiota. Mortality due to sepsis is linked to weak immune defense systems and an artificial environment associated with mechanical ventilation and the use of tubes, drains, intravascular lines, artificial nutrition, and extensive synthetic chemical drugs. These factors can reduce or eliminate the gut microbiota, impair immune function, increase systemic inflammation, and contribute to poor outcomes.

ii. The ‘gut-origin sepsis’ hypothesis evolves from the notion that under stress, such as sepsis, trauma, burn, or shock, infectious complications originate from the gut microbiota. Following surgical trauma, dysregulated metabolic reactions and infections can occur, including hyperglycemia, insulin resistance, increased hepatic glucose production, and muscle protein breakdown, and these changes can become self-destructive, leading to further metabolic damage. In survivors of significant burns, the number of beneficial bacteria, such as obligate anaerobes and Bifidobacterium, initially decreased but increased as their condition improved. In contrast, in non-survivors, these bacteria decreased as gut failure and sepsis progressed. Several pathogenic bacteria, such as Pseudomonas aeruginosa, and fungi, such as Candida, increased only in non-survivors, whereas short-chain fatty acids, such as propionic and butyric acids, decreased. Intestinal surgery in children disrupts the normal intestinal microbiota and barrier function, predisposing them to SIRS.

iii. Dietary patterns, including diets containing high fructose and high fat, and nutritional deficiencies affect intestinal permeability. A western-style diet contributes to endotoxemia by causing changes in the intestinal barrier and altering the composition of the microbiota. Approximately, 75% of the food consumed in Western diets does not benefit the microbiota of the lower gut. Much of the Western diet include refined carbohydrates, which are absorbed in the upper part of the GI tract. Food that reaches the large intestine is of limited value and contains fewer minerals, vitamins, and other nutrients essential for maintaining the microbiota. Such a diet can lead to a microbiome of reduced size and diversit.

There are characteristic differences in the composition and activity of the gut microbiota between
lean and obese individuals. In obese people, a high-fat diet contributes to low-grade inflammation and the development of non-alcoholic fatty liver disease. In obese animal models caused by feeding either a high-fructose or high-fat diet, increased gut permeability, low-grade endotoxemia, and fatty liver were observed. Germ-free mice did not develop obesity and liver damage. Following a fat diet, LPS can translocate from the gut into the circulatory system via direct diffusion due to increased gut permeability or be absorbed by enterocytes. Obese patients with high postprandial hypertriglyceridemia increased serum LPS levels and chylomicron fraction following fat overload. Postprandial LPS levels correlate with triglyceride levels. LPS binding to TLR4 activates an inflammatory cascade that alters insulin signaling. Chronic exposure to LPS contributes to weight gain and the development of type 2 diabetes mellitus (T2DM), and obese and diabetic individuals had increased serum LPS levels. An increase in the number of gram-negative bacteria in the gut microbiota reduced gut mucosal integrity, and consumption of a high-fat diet increased serum LPS levels.

iv. The gastrointestinal tract is affected by antibiotics. Antibiotic treatment damages several mechanisms related to maintaining the gut barrier and homeostasis. In animals, antibiotic use created histopathological lesions, such as desquamation and epithelial tissue destruction. Prior antibiotic exposure was associated with increased mortality in Gram-negative bacteria-induced sepsis. The bacterial communities inhabiting the gut exist in a delicate balance, and antibiotics damage this balance by eliminating several key communities and promoting the colonization of antibiotic-resistant bacteria by exerting stress and eliminating sensitive strains. Antibiotic treatment is associated with intestinal colonization and dominance of orally acquired antibiotic-resistant E. coli. The spread of antibiotic-resistant bacteria has been attributed to impaired innate mucosal defenses caused by antibiotics. Antibiotics modify the host immune system by altering the gut bacterial metabolites used for signal transmission from the microbiome to the gut immune system. Short-chain fatty acids (SCFAs), produced by bacteria through fermentation, have broad effects on enterocytes and play roles in various processes, including maintaining epithelial integrity, Treg differentiation, and the inflammatory response. Antibiotics hinder this.

The commensal microbiota is essential for keeping an intact intestinal barrier and for gut expression of non-defensin proteins. The stimulation of TLR promotes the expression of non-defensin proteins and can reverse the antibiotic-related reduction of gut defense. Antibiotic-associated dysbiosis of the gut predisposes subjects to infections. It also underlies the systemic dissemination of antibiotic-resistant and commensal enterobacteria by promoting transcytosis across the layers of the gut epithelium.

Antibiotic treatment reduced the bactericidal-killing activity of the gut mucosa. It also amplified the translocation of Klebsiella pneumoniae and lowered the expression of non-defensin proteins. TLR stimulation following antibiotic treatment increased the binding activity of NF-κB to DNA and the bactericidal activity in the gut mucosa. Germ-free mice showed significant decreases in non-defensin proteins and the intestinal defense against pathogen translocation.

Therapeutic measures that target the gut microbiome

Most systemic effects arising from dysbiosis are attributed to the metabolites produced by commensal gut bacteria, which are essential for immune system function. Despite extensive research on this topic, no therapeutic intervention aimed at microbiome alteration has been implemented to treat or prevent sepsis. However, several therapeutic measures to alter the gut microbiome to improve the prognosis of patients with sepsis are being explored. Several measures used for affecting gut and gut dysbiosis are described below.

Figure 1(b) shows a schematic representation of several potential therapeutic measures for sepsis.

Antibiotics. In sepsis, antibiotics act as a double-edged sword. Although they are a cornerstone of sepsis treatment, their effect on the microbiome may have detrimental consequences. Targeted antibiotics that alter the microbiome have been studied. Azithromycin is a macrolide that inhibits
bacterial protein synthesis and reduces biofilm formation. This antibiotic accumulates in phagocytes and is delivered at high concentrations to sites of infection. In chronic inflammatory disorders, it was shown to exert immunomodulatory effects on immune and epithelial cells by modulating the NF-κB inflammatory pathway, mucin release, surface receptor expression, macrophage phenotypes, and autophagy. At the mucosal level, azithromycin decreased mortality and ventilator dependency in patients with sepsis-associated ARDS. In patients with severe sepsis, azithromycin increased intensive care unit (ICU)-free days.

Selective digestive tract decontamination (SDD) and selective oropharyngeal decontamination (SOD) using enteral non-absorbable antimicrobials eradicated gram-negative bacteria, Staphylococcus aureus, and yeast colonization while leaving the anaerobic microbiome intact. Using SDD in ICU settings reduced the incidence of respiratory tract infections, colonization of antibiotic-resistant bacteria, and improved survival. Despite the evidence of SDD efficacy, multiple studies have fueled controversy, and SDD is not used in clinical practice because it may promote the emergence of antibiotic-resistant strains. However, a Cochrane review suggested that a combination of topical and systemic prophylactic antibiotics reduces overall mortality and respiratory tract infections.

**Probiotic, prebiotic, and synbiotic treatments.** Recovery of the microbiome is essential for preventing long-lasting immune suppression after the initial bout of sepsis has ended. Probiotic, prebiotic, and synbiotic treatments have been explored for maintaining and repairing the gut microbiota. These treatments may prevent and improve the prognosis of patients with sepsis.

Probiotics are viable, non-pathogenic microorganisms that confer benefits to the host by modifying the gut microbiota, promoting the local release of antimicrobial factors, maintaining gut barrier integrity, competing for epithelial adherence, preventing BT, and modulating the local immune response. Probiotic-associated changes in the gut microbiota are correlated with disease outcomes. The properties of probiotics are strain-specific, and beneficial effects have been observed for certain strains of *Lactobacillus, Bifidobacterium, Saccharomyces, Enterococcus sp., Streptococcus sp., Pediococcus sp., Leuconostoc, Bacillus,* and *E. coli.* Probiotics are well tolerated and are believed to prevent disease progression by competing with native bacteria for nutrients and binding loci, producing bacteriocins that kill pathogens, increasing the production of IgA, improving mucosal immunity, and suppressing systemic inflammation. In an animal pancreatitis model, the host-specific ileal microbiota was replaced with an ‘acute pancreatitis-associated microbiota’, which was reversed by administering probiotics. The microbiome of animals that received probiotics contained an abundance of a bacterial phylotype related to *Clostridium lituseburens*, which was correlated with reduced bacterial overgrowth, improved pancreatic pathology, and reduced pro-inflammatory cytokine levels.

The use of probiotics in critically ill patients has yielded mixed results. Several studies have shown that probiotics are associated with decreases in susceptibility to antibiotic-associated diarrhea, *Clostridium difficile*, ventilator-associated pneumonia, necrotizing enterocolitis (NEC), sepsis, MOF syndrome, and a shortened infection duration. However, a meta-analysis did not show a beneficial effect on mortality or length of ICU stay. Probiotic use in immunocompromised patients or patients with a leaky gut was associated with increased fungemia and bacteremia. The use of *Lactobacillus rhamnosus* decreased ventilator-associated pneumonia in ICU patients. Probiotic administration prevented necrotizing enterocolitis (NEC) and decreased mortality in preterm infants; however, the level of evidence was deemed insufficient to recommend it as a routine treatment. A recent large-scale multicenter trial did not show any benefit in using probiotics as a measure for decreasing ventilator-associated pneumonia or other infections among patients in ICU. However, probiotics were beneficial in selected patient populations, requiring an individualized approach for sepsis.

Prebiotics are nutrients degraded by the gut microbiota that alter the function and composition of the microbiome. These nutrients are non-digestible carbohydrates fermented by intestinal microbes, resulting in energy transfer. Digestion of these nutrients produces signaling
metabolites, such as SCFAs and peptidoglycan, which affect the innate immune system. Prebiotics can reduce inflammation, endotoxemia, and cytokine levels, thereby improving insulin resistance and glucose tolerance. A diet rich in prebiotics is beneficial for managing T2DM for its positive impact on intestinal microbiota modulation. Prebiotics, such as fermentable dietary fiber, increase glucagon-like peptide-1 and peptide YY and decrease ghrelin levels. In patients with T2DM, a macrobiotic diet improved fasting blood glucose, plasma lipid fractions, and plasma insulin levels. Different prebiotic molecules were tested in mouse models of sepsis, with positive results. Desaminotyrosine (DAT) maintained mucosal immune homeostasis and protected against barrier integrity. DAT attenuated dextran sodium sulfate-induced mucosal inflammation, and it protected mice treated with endotoxin to induce septic shock. In an animal model, Xuanbai Chengqi decoction (XBCQ), a traditional Chinese medicine formulation, was protective against pulmonary infections, improved gut barrier function by increasing the amounts of occludin, a mucosal protein, improved the diversity of the gut microbiota, attenuated inflammatory markers in the lungs, and improved survival. Finger millet arabinoxylan (FM–AX), a non-starch polysaccharide extracted from cereals, prevented metagenomic alterations in the cecum and endotoxia in mice fed a high-fat diet. In preterm infants, prebiotics decreased the incidence of sepsis, mortality, and length of hospital stay.

Synbiotic therapy employs a combination of probiotics and prebiotics and reduces septic complications. Perioperative use of synbiotics reduced the incidence of bacteria in MLNs and blood. Synbiotic nutrition reduced the incidence of postoperative sepsis in an elective general surgery setting. A randomized controlled study in mechanically ventilated patients with sepsis showed that synbiotic treatment reduced ventilation-associated pneumonia but had no effect on mortality. A meta-analysis of 13 randomized controlled trials including 962 patients who received synbiotics or probiotics showed a reduced incidence of postoperative sepsis in both groups. Subgroup analysis did not detect a reduction in the occurrence of urinary tract infections, pneumonia, or wound infections following surgery. A randomized trial evaluating a synbiotic preparation of *Lactobacillus plantarum* and fructooligosaccharide in 4,500 healthy term infants showed a 40% decrease in the composite outcome of lower respiratory tract infection, sepsis, and death.

Several factors may have contributed to the lack of success of these approaches, including an unsuitable choice of probiotic species, low doses, and its use as a complement to antibiotic therapy rather than an alternative treatment. In such approaches, the supplemented lactic acid bacteria were killed before reaching their target organs. At present, the routine use of these methods in critically ill patients is not recommended.

**Fecal microbial transplantation.** Fecal microbial transplantation (FMT) is a method for altering the gut microbiota that may be superior to other methods due to the delivery of other organisms, viruses, fungi, and other metabolites in addition to commensal bacteria. FMT has been proven efficacious in treating recurrent and refractory Clostridium difficile (CDI) and several other dysbiosis-related conditions. In a rat model of sepsis, FMT reduced mortality by 50%, prevented intestinal injury and mucosal atrophy, had antioxidant and anti-inflammatory effects, and inhibited NF-κB. Animal models restored bacterial communities in cecal crypts, replenished the gut epithelium, and protected gut stem cells. There are several barriers to using FMT in critically ill patients treated with broad-spectrum antibiotics, as the treatment can inhibit proliferation of the transplanted bacteria. Therefore, the use of FMT in human subjects to treat sepsis remains anecdotal.

**Anti-LPS measures.** Endotoxin from gram-negative bacteria, LPS, activates TLRs and stimulates the inflammatory processes in sepsis and other conditions. Several LPS-binding molecules have been studied for their potential to prevent or alleviate excess inflammatory responses. The role of LPS in gut-derived sepsis was studied in neutropenic rats fed a preparation of *P. aeruginosa* to induce sepsis. The animals were administered colostrum or hyperimmune colostrum produced by vaccinating pregnant cows against a conserved portion of LPS. The use of both regular and antibody-enriched colostrum was associated with increased survival and decreased amounts of bacteria in extra-intestinal sites. Anti-LPS antibodies reduce the inflammatory response that induces metabolic disease by altering the ‘leaky’ mucosal membrane and removing endotoxin. Anti-inflammatory LPS (A-LPS), produced by certain microbes, such as Bacteroides,
was shown to help prevent and treat LPS-induced inflammatory response by altering the balance between A-LPS and pro-inflammatory LPS (P-LPS). The results of therapeutic interventions aimed at removing LPS by immunoglobulins or extracorporeal means are controversial, and human trials for sepsis have confirmed positive effects on several secondary parameters but not on morbidity or survival.

**Dietary measures.** Dietary adjustment is a relatively simple way of altering the intestinal microbiota. Dietary changes can modulate the composition of the gut microbiota and improve gut mucosal integrity, decrease the occurrence of endotoxemia and postprandial inflammatory effects, and lead to adequate insulin signaling. Parenteral nutrition, elemental enteral nutrition (EN), and eco-immuno-nutrition (EIN) hastened the recovery of patients with severe pancreatitis and alleviated the associated SIRS. EN decreased endotoxin, TNF-α, IL-6, and BT levels and enhanced IL-10 expression. Pancreatic sepsis, MOF, and mortality were lower in the EN and EIN-treated groups. EN improved the intestinal mucosal barrier, promoted mucosal repair, and stabilized the intestinal microbiome. Early enteral nutrition (EEN) in patients with sepsis inhibited an excessive immune response, shortened the duration of mechanical ventilation, and the hospital stay, but had no effect on 28-day mortality.

Dietary fiber has anti-inflammatory properties. Fiber supplementation improved outcomes by inducing microbial changes that regulate inflammatory metabolites. Fiber supplementation in critically ill patients increased the abundance of SCFA-producing bacteria without increasing the occurrence of diarrhea or abdominal distention. Immune cells utilize large amounts of glutamine, most of which derives from the degradation of muscle protein. In a model using samples from healthy children, glutamine promoted HSP70 and TNF-α release. The effects depend on the degree of inflammation, and glutamine administration to patients with sepsis enhanced immune competence and improved the catabolic phase of septic patients with malnutrition.

**Immune modulatory and anti-inflammatory therapies.** Immunomodulatory drugs indirectly affect the gut microbiota, contributing to their beneficial effects on sepsis. In germ-free mice subjected to inflammatory stimulation, mice treated with gut microbiome samples collected from patients treated successfully with methotrexate for rheumatoid arthritis showed a lesser immune response. However, the clinical use of steroids and other immunosuppressive agents in patients with sepsis is controversial. Immune modulatory therapy with a combination of dexamethasone and thymosin alpha1 altered dendritic cell (DC) function and enhanced the resistance of endotoxemic mice to BT and secondary infections, improving their outcomes. Mice treated with this combination showed improved survival, a decrease in BT to extra-intestinal organs, and an enhanced ability to eradicate secondary infections by reversing changes in DC during endotoxemia. Failure to respond to steroid therapy in sepsis is caused by decreased expression and function of glucocorticoid receptors (GRs), and GR expression and translocation were decreased in septic animals. Leflunomide has immunomodulatory and anti-inflammatory effects, which mitigate the host response to BT, and leflunomide-mediated prevention of protein and lipid peroxidation was observed in septic bowel tissue in rats.

Thymic stromal lymphopoietin (TSLP), a member of the IL-2 cytokine family, has multiple effects on immune cells. The short form of TSLP is linked to the homeostasis of the gut barrier in a healthy state. However, its long form is overexpressed in an inflammatory state, whereas the short form is downregulated. Most studies showed a decreased inflammatory response, the clinical outcomes varied.

Amino-procalcitonin (NPCT) is a peptide derived from the prohormone procalcitonin. NPCT underlies the pathogenesis of acute lung injury in sepsis and is a risk factor for mortality. Treatment with anti-NPCT reduced the inflammatory cytokine expression during sepsis and prevented the nuclear NF-κB translocation in tissues while increasing the anti-inflammatory cytokine IL-10 in mice. The humanized anti-PcrV IgG2a anti-gen-binding fragment KB001 inhibited toxin translocation, with demonstrated safety and a favorable pharmacokinetic profile, and thus has potential as a nonantibiotic strategy to reduce inflammation and damage in *P. aeruginosa*-related pneumonia.
Colchicine, an anti-inflammatory drug, is an inhibitor of mitosis and microtubule assembly.\textsuperscript{146–149} By binding to soluble non-polymerized tubulin heterodimers, it forms tight colchicine-tubulin complexes, thereby inhibiting the polymerization of microtubules.\textsuperscript{150} Microtubules are involved in the function of both adaptive and innate immune systems, making them potential therapeutic targets for immune modulation.\textsuperscript{149} Colchicine inhibits microtubule rearrangement and interferes with the function of the immune system. It inhibits the recruitment, migration, and chemotaxis of neutrophils. Colchicine reduces neutrophil deformability by lowering microtubule levels, thus altering the ability of neutrophils to migrate via small pores, which is crucial for extravasation in response to inflammatory signals.\textsuperscript{151} The colchicine-tubulin complex attenuates macrophage NLRP inflammasome arrangement and activation.\textsuperscript{152}

A new artificial intelligence-based system is being developed for analyzing data on the function of microtubules of gut cells and data on microbiota metabolites, ENS, and the gut immune cells. The system will provide a method for improving the response to the currently used measures targeting BT and dysbiosis to reduce the hyperinflammatory response associated with sepsis, similar to other disorders.\textsuperscript{153–160}

**Summary**

The human gut is a complex organ involved in multiple physiological processes. The gut immune system and microbiota play essential roles in health and disease,\textsuperscript{51,154,161–167} and their roles in sepsis have been established. BT and several other microbiota-linked mechanisms contribute to sepsis and subsequent MOF development. Bacterial-associated, host and environment-dependent mechanisms are involved in the pathogenesis of sepsis. These findings provide potential new therapeutic approaches for sepsis. In these types of therapies, the microbiome is targeted, directly or indirectly, to restore its homeostatic states, thus reducing the damage caused by the aggravated inflammatory processes that ensue. Since the treatment for sepsis has not changed dramatically in recent years, and the morbidity and mortality rates remain high, there is a need for new therapeutic targets.

**Abbreviations**

BT: bacterial translocation; MOF: multiple organ failure; MLN: mesenteric lymph nodes; ARDS: acute respiratory distress syndrome; LPS: lipopolysaccharide; SIRS: systemic inflammatory response syndrome; MODS: multiple organ dysfunction syndrome; SBP: spontaneous bacterial translocation; PAMPs: pathogen-associated molecular pattern; IL: interleukin; TNFα: tumor necrosis factor-alpha; NF-κB: nuclear factor kappa-light-chain enhancer of activated B cell; TTSS: type III secretion system; OMVs: outer membrane vesicles; TLR: Toll-like receptor; CLP: cecal ligation and perforation; TMED7: transmembrane emp24 protein transport domain containing 7; miRNA: microRNA; MMP7: Matrix metalloproteinase 7; FSTL-1: follistatin-like protein 1; NLRP: nod-like receptor family, pyrin domain containing 3; ENS: enteric nervous system; IAH: intra-abdominal hypertension; SCFA: short-chain fatty acids; EN: enteral nutrition; EIN: eco-immuno-nutrition; HSP: heat shock protein; DC: dendritic cells; GR: glucocorticoid response; NPCT: amino-procalcitonin

**Author contributions**

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