The Gut as the Motor of Multiple Organ Dysfunction in Critical Illness

Nathan J. Klingensmith, MD, Craig M. Coopersmith, MD*

KEYWORDS

• Sepsis • MODS • Gut • Intestine • Critical illness

KEY POINTS

- The gut is composed of an epithelium, adaptive immune system, and microbiome. Each plays a crucial role in the maintenance of health and the pathophysiology of critical illness.
- Toxic mediators travel through mesenteric lymphatics, causing remote inflammatory injury. Preclinical trials have demonstrated that ligation of the lymph duct can prevent lung injury caused by gut-derived factors.
- Gut integrity is compromised in critical illness with increases in apoptosis and permeability. Multiple preclinical studies have demonstrated that targeting gut epithelial integrity results in improved survival in critical illness.
- The microbiome can alter its behavior based on environmental cues. Preventing bacteria from becoming virulent or reprogramming them to a nonvirulent phenotype may revolutionize the treatment of gut-derived sepsis.
- Outside of enteral nutrition, no treatment targeting the gut is currently widely used in the intensive care unit. Multiple techniques for modulating the microbiome are of potential interest as therapeutics.

OVERVIEW

The gut has been hypothesized to be the motor of multiple organ dysfunction syndrome (MODS) for the past quarter century.[1–3] Whereas initial theories of gut and critical illness suggested that hyperpermeability resulted in bacterial translocation into the systemic circulation, the reality is significantly more complex than was hypothesized originally. All elements of the gut—the epithelium, the immune system, and the microbiome—are impacted by critical illness and can, in turn, propagate a pathologic host

This work was supported by funding from the National Institutes of Health (GM104323, GM095442, GM072808, GM109779 and GM113228).
Department of Surgery, Emory Critical Care Center, Emory University School of Medicine, Atlanta, GA, USA
* Corresponding author. 101 Woodruff Circle, Suite WMB 5105, Atlanta, GA 30322.
E-mail address: cmcoop3@emory.edu

Crit Care Clin 32 (2016) 203–212
http://dx.doi.org/10.1016/j.ccc.2015.11.004 criticalcare.theclinics.com
0749-0704/16/$ – see front matter

response. Further, alterations in the gut can lead to both local and distant insults, via alterations in homeostatic processes and defense mechanisms as well as release of toxic mediators into both the mesenteric lymph and the systemic circulation. Although considerable effort has been put into directly targeting the gut for therapeutic gain in critical illness, the results to date have been modest. This review focuses on the cellular and molecular underpinnings of how the gut functions as the motor of MODS, as well as clinical ways in which the gut can, at least in part, be potentially manipulated for therapeutic gain.

THE GUT IN HEALTH

Epithelium

The gut contains a single layer epithelium with a myriad of important functions. It provides a large surface area—estimated to be approximately 32 m^2 or one-half the size of a badminton court[4]—for use in nutrient absorption and preventing entrance of pathogens from its lumen. Microscopically, the gut is in a state of constant renewal from the multipotent stem cells near the crypt base. These give rise to daughter cells, which then give rise to 4 major intestinal cell types: (a) enterocytes, which absorb nutrients and make up greater than 90% of intestinal epithelial cells, (b) mucus-producing goblet cells, (c) hormone-producing enteroendocrine cells, and (d) defensin-producing Paneth cells that protect intestinal stems cells and play a role in intestine–microbiota interactions.[5] Unlike other cells in the gut that migrate upward along the villus, Paneth cells migrate downward toward the crypt base. The journey from cell birth, differentiation, and migration along the villus to cell loss via either apoptosis or luminal sloughing of intact cells takes only 5 to 7 days in a healthy human.

Immune System

The intestine is the largest lymphoid organ of the body.[6] It contains 4 immune cell compartments: Peyer's patches, the lamina propria, mesenteric lymph nodes, and intraepithelial lymphocytes. Peyer's patches come in contact with luminal antigens and direct antigen-presenting cells to the mesenteric lymph nodes. This sets off the immune differentiation of T and B cells in the draining nodes. The highly complicated gut mucosal immune system plays a myriad of roles in host defense including (but not limited to) antigen recognition, presentation, amplification of antigen-specific response, and production of cytokines and chemokines.[7]

Microbiome

There are 10 times more bacterial cells in a human than host cells—100 trillion bacteria to 10 trillion human cells.[8] Under normal conditions, there is a well-tolerated symbiotic relationship between the human host and its microbiome, which has a robust diversity, with the predominant species being *Bacteroides* and *Firmicutes*. With the recent explosion in our (still nascent) understanding of the microbiome, it has become apparent that the diversity of an individual's microbiota depends on a wide variety of factors starting from the type of birth they underwent (vaginal or Cesarean section) to the diet they eat to their age to even the pets they have.[9]

PRECLINICAL INSIGHTS INTO THE ROLE OF THE GUT AS THE MOTOR OF MULTIPLE ORGAN DYSFUNCTION SYNDROME

The Gut Lymph Hypothesis

Given the overwhelming number of bacteria that reside in the intestine, the initial hypothesis for why the gut is the motor of MODS was whole bacteria translocation that

spread via portal circulation. Although bacterial translocation clearly occurs in some preclinical models of critical illness,[10] human data have generally remained inconclusive or not supportive of this as a common phenomenon seen in critically ill patients, although it likely occurs in select pathophysiologic conditions.[11,12] A search for how intestine-derived mediators caused distant injury led to the gut–lymph hypothesis. This theory postulates that toxic mediators from the gut travel through mesenteric lymphatics toward the lung where they cause remote injury. Several lines of investigation support the importance of the gut-derived lymph as being physiologically important. When the mesenteric lymph duct is ligated in multiple models of critical illness, lung injury and neutrophil activation are abrogated or prevented and, importantly, mortality is diminished or prevented.[13,14] Additionally, when mesenteric lymph from rats undergoing trauma/hemorrhagic shock is injected into nonmanipulated rats, the rats receiving the injection develop lung injury similar to shock rats.[15] Of note, gut-derived lymph typically does not contain intact bacteria, endotoxin, or cytokines but rather contains protein or lipid factors that stimulate Toll-like receptor 4, leading to activation of inflammatory neutrophils in the lung. Although not a part of the gut–lymph hypothesis, it has also been shown that gut-specific deletion of Mttp (a protein required for chylomicron assembly) improves survival in septic mice subjected to *Pseudomonas aeruginosa* pneumonia,[16] although aged animals with the identical genetic knockout have lower survival when subjected to the same insult.[17]

Apoptosis

Cell death via apoptosis is an evolutionarily conserved process that is important for normal development and function. However, gut epithelial apoptosis seems to be detrimental after the onset of sepsis. Both preclinical mouse models of sepsis and autopsy studies of patients who died in the intensive care unit (ICU) demonstrate a marked upregulation in gut epithelial apoptosis compared with those who die without sepsis.[18,19] Gut-specific overexpression of the antiapoptotic protein Bcl-2 has been shown to decrease sepsis-induced intestinal epithelial apoptosis and importantly improve survival in murine models of both cecal ligation and puncture and *P aeruginosa* pneumonia.[20,21] Notably, this beneficial effect of Bcl-2 overexpression is abrogated in septic mice with cancer, suggesting that alterations in the host response caused by comorbidities can impact gut apoptosis.[22]

There is evidence that cross-talk exists between the intestinal epithelium and immune system in sepsis that results in changes in gut epithelial apoptosis. Although the presence or absence of lymphocytes does not impact gut epithelial apoptosis under basal conditions, sepsis-induced gut epithelial apoptosis is significantly higher in $Rag^{-/-}$ mice (which lack lymphocytes) than wild-type mice, suggesting that lymphocytes play an antiapoptotic role in the gut epithelium that is, unmasked in sepsis.[23] Subset analysis demonstrates that $CD4^{+}$ T cells are responsible for the antiapoptotic effect of the adaptive immune system on the gut epithelium. In addition, when Bcl-2 is overexpressed in myeloid cells, there is a decrease in the amount of gut epithelial apoptosis after sepsis, in addition to improved survival.[24]

Hyperpermeability

The intestinal epithelium consists of only a single layer of cells that is responsible for maintaining a permaselective barrier that, in a simplistic description, is charged with keeping out the bad and letting in the good. It performs these functions via cell–cell intramembrane protein interactions within the tight junction.[25] There are several families of intramembrane proteins (claudins, occludin, tricellulin, junctional adhesion molecule), as well as intracellular connector proteins (zonula occludins, myosin light

chain) that link the tight junction to the intracellular cytoskeleton and allow for modulation of the space.[26,27] Alteration of this space can lead to changes in intestinal permeability, and there is significant evidence that intestinal permeability is increased after sepsis and MODS.[28,29]

There is increasing preclinical evidence that targeting tight junctions directly or indirectly might have beneficial effects in critical illness. When myosin light chain kinase is activated, it phosphorylates the myosin light chain causing contraction of the cytoskeleton, increasing the intercellular space and thereby increasing permeability. Inhibiting myosin light chain kinase in mice in the setting of binge alcohol ingestion and burn injury decreases bacterial translocation and intestinal cytokine production to levels seen in sham animals, associated with a prevention in injury-induced alterations in tight junction expression and localization.[30,31] A broader strategy involves targeting global intestinal integrity. Epidermal growth factor is a cytoprotective peptide that exhibits trophic and healing effects on the intestinal mucosa. When mice are given systemic epidermal growth factor after the onset of either cecal ligation and puncture or *P aeruginosa* pneumonia, they have improved or normalized permeability, apoptosis, proliferation, and villus length. Importantly, this is associated with a significant improvement in survival, even if the drug is initiated 24 hours after the onset of sepsis.[32,33] This improvement in survival seems to be mediated through the gut as transgenic mice with enterocyte-specific overexpression of epidermal growth factor have the same improvement in intestinal integrity and survival after sepsis as those that receive systemic epidermal growth factor.[34]

Altering the Microbiome

There is increasing recognition that microbes are not inherently good or bad, but rather alter their behavior based on their environment. Bacteria that are present in someone's healthy microbiome for decades can became virulent if environmental cues suggest an advantage to them. Further, simply the presence of bacteria that can cause fatal disease does not inherently implicate them as being pathologic. For example, *P aeruginosa* injected into the cecum of mice undergoing a sham operation and subsequently removed can be injected into the peritoneum of a control mouse without causing any disease. In contrast, if *P aeruginosa* is injected in the cecum of mice subjected to a nonlethal partial hepatectomy and subsequently removed and injected into a control mouse peritoneum, the resulting mortality is 100%.[35] The ability of bacteria to sense host stress, their own environment, and surrounding bacterial density and alter their virulence in response has profound clinical implications.[36,37] This is because microbial identification without attention to its virulence may not be sufficient for treating critically ill patients while the simple presence of bacteria is not inherently harmful. In addition, virulent bacteria can potentially cause MODS without systemic dissemination. Thus, a potential complementary approach to improving the antibiotic pipeline and preventing antimicrobial resistance is to prevent bacteria from becoming virulent or reprogramming them to a nonvirulent phenotype. A preclinical example of this is seen with administration of a nonantibiotic, high-molecular-weight polymer that protects mice inoculated with typically virulent organisms from mortality by altering their phenotype.[38] A further example of the how the host response is altered by the microbiome can be seen when studying germ free mice, which are raised in microisolator cages and lack an endogenous microflora. When germ-free mice are given *P aeruginosa* pneumonia, they have a significantly higher mortality compared with wild-type mice[39]; however, germ-free mice subjected to hemorrhagic shock or ischemia–reperfusion injuries have an improved survival compared with mice with intact, normal gut microflora.[40,41]

GUT FAILURE IN CRITICALLY ILL PATIENTS

Clinical Diagnosis of Gut Failure

Symptoms of gut failure in the ICU are nonspecific and are not included currently in severity scoring symptoms such as the Sequential Organ Failure Assessment score. A recent prospective multicenter study of 377 patients in the ICU requiring mechanical ventilation sought to determine whether 6 gastroenterological symptoms, namely, high gastric residual volumes, absent bowel sounds, vomiting/regurgitation, diarrhea, bowel distension, and gastrointestinal bleeding, could predict patient outcome.[42] None of the symptoms[43] alone was an independent predictor of mortality. However, when 3 or more symptoms were present at day 1 of ICU stay, there was a 3-fold increase in the risk of mortality.

Additionally, analysis of patient stool samples has shown promise in predicting outcomes. In a study of nearly 500 stool samples from an ICU cohort with sepsis, it was determined that when fecal pH goes up or down by 1, the incidence of bacteremia more than triples and mortality more than doubles.[44] Further, a decrease in obligate and facultative anaerobes has been shown to correlate with increased risk of mortality in patients with the systemic inflammatory response syndrome, whereas a depleted or single pattern fecal stain for bacteria is associated with a greater risk of mortality in MODS compared with a diverse pattern.[43]

Although not commonly used clinically, biomarkers have shown significant promise in diagnosing gut failure. The concentration of plasma citrulline is a marker of enterocyte functional metabolic mass, so decreased serum citrulline is a potential marker of intestinal damage. Further, intestinal fatty acid-binding protein is localized in enterocytes and is released after enterocyte damage, so an increase in this protein is also a potential marker of intestinal damage. The importance of both citrulline and intestinal fatty acid binding protein was recently shown in a series of more than 100 medical intensive care unit patients, of which 15% had septic shock and 20% had acute respiratory distress syndrome.[45] Increased intestinal fatty acid binding protein on ICU admission was associated with catecholamine support, higher lactate, higher Sequential Organ Failure Assessment score, and higher International Normalized Ratio, whereas decreased citrulline was associated with higher intraabdominal pressure, greater C-reactive protein concentration, and more frequent antibiotic use. Alterations in both were associated with greater 28-day mortality. Of note, 2 additional studies found increased serum intestinal fatty acid binding protein in patients with acute mesenteric ischemia.[46,47]

TARGETING THE MICROBIOME

Clinical strategies aimed at augmenting, decreasing, or transplanting the microbiome are all used in clinical practice to varying degrees. Despite the widely varying intellectual basis for each of these as a potential therapeutic, each has shown some potential benefit, although their efficacy and potential unwanted side effects remain incompletely understood.

Probiotics, Prebiotics, and Synbiotics

Because microbial diversity has been shown to be associated with outcomes in critical illness, the concept of augmenting "good" bacteria and restoring microbial ecology is potentially beneficial with the goal of restoring a normal, diverse flora. This can be done in a number of complementary ways: (a) probiotics are exogenous live organisms, (b) prebiotics are nondigestible nutrients that stimulate commensal bacterial growth, and (c) synbiotics are a combination of probiotics and prebiotics. The theoretic benefit of each of these is multifactorial, including local release of antimicrobial

factors, maintenance of gut barrier integrity, competition for epithelial adherence, prevention of bacterial translocation, and modulation of the local immune response.[48] Two recent meta-analyses of probiotics in more than 1000 patients in the ICU demonstrate a decrease in the incidence of ventilator-associated pneumonia, with one showing a decreased length of stay.[49,50] No alteration in mortality was noted. It should be noted that the largest trial of probiotics to date showed increased mortality (16% vs 6%) in 296 patients with severe pancreatitis.[51] However, this trial has been heavily criticized,[52] and does not seem to be representative of other studies of probiotics. Multiple questions remain before augmenting the microbiome gaining widespread usage as a strategy to improve outcomes in the ICU. These include what (if any) the optimal probiotic agent is, if combinations of agents are more beneficial, if synbiotics are superior to probiotics alone, what the ideal "dose", is and what the long-term safety profile is.

Selective Decontamination of the Digestive Tract

In contrast with augmenting the microbiome, selective decontamination of the digestive tract (SDD) seeks to preferentially minimize pathogenic enteral bacteria. The goal of this practice is to eradicate oropharyngeal and intestinal carriage of pathogenic microorganisms without adversely impacting the remaining microbiome on either the patient level or the ICU level. SDD includes 3 components: (a) 4 to 5 days of parenteral antibiotics (cefotaxime in previously healthy patients, combination therapy or antipseudomonal cephalosporin in patients with chronic disease), (b) nonabsorbable enteral antibiotics given via nasoenteric tube given throughout the ICU stay, and (c) pastes or gels applied to the oropharynx.[53] It should be noted that the term "selective" is a bit of a misnomer, because this approach targets both normal and abnormal flora, and does not cover multiple low-level pathogens.

For a practice that is used rarely worldwide (with certain exceptions), the data on SDD are both robust and impressive. In fact, it is a great paradox that the sheer volume of studies on this practice might be greater (and more supportive) than in almost any aspect of critical care, yet this has not translated to a change in clinical practice. Specifically, there have been more than 60 randomized controlled trials and more than 10 metaanalyses on SDD in more than 15,000 patients, demonstrating a reduction of lower airway infection of 72% and bloodstream infection by 37%.[54,55]

Given this significant literature, why is SDD not used more commonly used? The answer relates exclusively to concerns related to the development of antibiotic resistance. Although the majority of studies examining this issue have not demonstrated the development of resistance (although a few have), these have generally been performed in ICUs that have low levels of antibiotic resistance at baseline.[56] With increasing attention being paid to antibiotic stewardship and resistance worldwide, the fear that widespread antibiotic usage for preventive purposes will induce new and difficult or impossible to treat "superbugs" has limited adoption of SDD. Further, with an increased understanding of the importance of microbial health and diversity, it is currently unclear how these are impacted by the use of SDD in critically ill patients.

Fecal Transplant

There has recently been an explosion of interest in fecal microbiota transplant, where stool from a healthy donor is given to a recipient with the goal of restoring the microbiome to its homeostatic state seen in health. Although multiple indications are currently being studied, the most convincing data are in recurrent *Clostridium difficile* infection, where cure rates are 3 times higher than seen with conventional medical therapy without apparent side effects.[57] To date, fecal transplant is not typically

used in critically ill patients because antibiotic use (which is common in the ICU) would immediately change the microbial components of a patient's stool (either from donor or recipient) after the transplant.

Nutrition

Although a comprehensive review of nutritional support is outside the scope of this review, it is worth emphasizing the importance of nutritional support in the ICU, as one of the major roles of the healthy intestine is to absorb nutrients. Enteral nutrition is preferable to parenteral nutrition because enteral nutrition has beneficial effects on gut-associated lymphoid tissue and mucosal health, and not does have the increased risk of infection associated with parenteral nutrition. Enteral nutrition should be initiated within 48 hours of ICU admission if possible.

REFERENCES

1. Mittal R, Coopersmith CM. Redefining the gut as the motor of critical illness. Trends Mol Med 2014;20(4):214–23.
2. Clark JA, Coopersmith CM. Intestinal crosstalk: a new paradigm for understanding the gut as the "motor" of critical illness. Shock 2007;28(4):384–93.
3. Carrico CJ, Meakins JL, Marshall JC, et al. Multiple-organ-failure syndrome. The gastrointestinal tract: the "motor" of MOF. Arch Surg 1986;121(2):196–208.
4. Helander HF, Fandriks L. Surface area of the digestive tract - revisited. Scand J Gastroenterol 2014;49(6):681–9.
5. Clevers HC, Bevins CL. Paneth cells: maestros of the small intestinal crypts. Annu Rev Physiol 2013;75:289–311.
6. Galperin C, Gershwin ME. Immunopathogenesis of gastrointestinal and hepatobiliary diseases. JAMA 1997;278(22):1946–55.
7. Schulz O, Pabst O. Antigen sampling in the small intestine. Trends Immunol 2013;34(4):155–61.
8. Defazio J, Fleming ID, Shakhsheer B, et al. The opposing forces of the intestinal microbiome and the emerging pathobiome. Surg Clin North Am 2014;94(6):1151–61.
9. Xu Z, Knight R. Dietary effects on human gut microbiome diversity. Br J Nutr 2015;113(Suppl):S1–5.
10. Earley ZM, Akhtar S, Green SJ, et al. Burn injury alters the intestinal microbiome and increases gut permeability and bacterial translocation. PLoS One 2015;10(7):e0129996.
11. Moore FA, Moore EE, Poggetti R, et al. Gut bacterial translocation via the portal vein: a clinical perspective with major torso trauma. J Trauma 1991;31(5):629–36.
12. Purohit V, Bode JC, Bode C, et al. Alcohol, intestinal bacterial growth, intestinal permeability to endotoxin, and medical consequences: summary of a symposium. Alcohol 2008;42(5):349–61.
13. Badami CD, Senthil M, Caputo FJ, et al. Mesenteric lymph duct ligation improves survival in a lethal shock model. Shock 2008;30(6):680–5.
14. Deitch EA. Gut-origin sepsis: evolution of a concept. Surgeon 2012;10(6):350–6.
15. Senthil M, Watkins A, Barlos D, et al. Intravenous injection of trauma-hemorrhagic shock mesenteric lymph causes lung injury that is dependent upon activation of the inducible nitric oxide synthase pathway. Ann Surg 2007;246(5):822–30.
16. Dominguez JA, Xie Y, Dunne WM, et al. Intestine-specific Mttp deletion decreases mortality and prevents sepsis-induced intestinal injury in a murine model of Pseudomonas aeruginosa pneumonia. PLoS One 2012;7(11):e49159.

17. Liang Z, Xie Y, Dominguez JA, et al. Intestine-specific deletion of microsomal triglyceride transfer protein increases mortality in aged mice. PLoS One 2014;9(7):e101828.
18. Hiramatsu M, Hotchkiss RS, Karl IE, et al. Cecal ligation and puncture (CLP) induces apoptosis in thymus, spleen, lung, and gut by an endotoxin and TNF-independent pathway. Shock 1997;7(4):247–53.
19. Hotchkiss RS, Swanson PE, Freeman BD, et al. Apoptotic cell death in patients with sepsis, shock, and multiple organ dysfunction. Crit Care Med 1999;27(7):1230–51.
20. Coopersmith CM, Stromberg PE, Dunne WM, et al. Inhibition of intestinal epithelial apoptosis and survival in a murine model of pneumonia-induced sepsis. JAMA 2002;287(13):1716–21.
21. Coopersmith CM, Chang KC, Swanson PE, et al. Overexpression of Bcl-2 in the intestinal epithelium improves survival in septic mice. Crit Care Med 2002;30(1):195–201.
22. Fox AC, Breed ER, Liang Z, et al. Prevention of lymphocyte apoptosis in septic mice with cancer increases mortality. J Immunol 2011;187(4):1950–6.
23. Stromberg PE, Woolsey CA, Clark AT, et al. CD4+ lymphocytes control gut epithelial apoptosis and mediate survival in sepsis. FASEB J 2009;23(6):1817–25.
24. Iwata A, Stevenson VM, Minard A, et al. Over-expression of Bcl-2 provides protection in septic mice by a trans effect. J Immunol 2003;171(6):3136–41.
25. Bischoff SC, Barbara G, Buurman W, et al. Intestinal permeability–a new target for disease prevention and therapy. BMC Gastroenterol 2014;14:189.
26. Cunningham KE, Turner JR. Myosin light chain kinase: pulling the strings of epithelial tight junction function. Ann N Y Acad Sci 2012;1258:34–42.
27. Odenwald MA, Turner JR. Intestinal permeability defects: is it time to treat? Clin Gastroenterol Hepatol 2013;11(9):1075–83.
28. Fink MP. Intestinal epithelial hyperpermeability: update on the pathogenesis of gut mucosal barrier dysfunction in critical illness. Curr Opin Crit Care 2003;9(2):143–51.
29. Fredenburgh LE, Velandia MM, Ma J, et al. Cyclooxygenase-2 deficiency leads to intestinal barrier dysfunction and increased mortality during polymicrobial sepsis. J Immunol 2011;187(10):5255–67.
30. Chen C, Wang P, Su Q, et al. Myosin light chain kinase mediates intestinal barrier disruption following burn injury. PLoS ONE 2012;7(4):e34946.
31. Zahs A, Bird MD, Ramirez L, et al. Inhibition of long myosin light-chain kinase activation alleviates intestinal damage after binge ethanol exposure and burn injury. Am J Physiol Gastrointest Liver Physiol 2012;303(6):G705–12.
32. Clark JA, Clark AT, Hotchkiss RS, et al. Epidermal growth factor treatment decreases mortality and is associated with improved gut integrity in sepsis. Shock 2008;30(1):36–42.
33. Dominguez JA, Vithayathil PJ, Khailova L, et al. Epidermal growth factor improves survival and prevents intestinal injury in a murine model of pseudomonas aeruginosa pneumonia. Shock 2011;36(4):381–9.
34. Clark JA, Gan H, Samocha AJ, et al. Enterocyte-specific epidermal growth factor prevents barrier dysfunction and improves mortality in murine peritonitis. Am J Physiol Gastrointest Liver Physiol 2009;297(3):G471–9.
35. Babrowski T, Romanowski K, Fink D, et al. The intestinal environment of surgical injury transforms Pseudomonas aeruginosa into a discrete hypervirulent morphotype capable of causing lethal peritonitis. Surgery 2013;153(1):36–43.

36. Zaborina O, Lepine F, Xiao G, et al. Dynorphin activates quorum sensing quinolone signaling in pseudomonas aeruginosa. PLoS Pathog 2007;3(3):e35.
37. Wu L, Estrada O, Zaborina O, et al. Recognition of host immune activation by Pseudomonas aeruginosa. Science 2005;309(5735):774–7.
38. Zaborin A, Defazio JR, Kade M, et al. Phosphate-containing polyethylene glycol polymers prevent lethal sepsis by multidrug-resistant pathogens. Antimicrob Agents Chemother 2014;58(2):966–77.
39. Fox AC, McConnell KW, Yoseph BP, et al. The endogenous bacteria alter gut epithelial apoptosis and decrease mortality following Pseudomonas aeruginosa pneumonia. Shock 2012;38(5):508–14.
40. Ferraro FJ, Rush BF Jr, Simonian GT, et al. A comparison of survival at different degrees of hemorrhagic shock in germ-free and germ-bearing rats. Shock 1995;4(2):117–20.
41. Fagundes CT, Amaral FA, Vieira AT, et al. Transient TLR activation restores inflammatory response and ability to control pulmonary bacterial infection in germfree mice. J Immunol 2012;188(3):1411–20.
42. Reintam BA, Poeze M, Malbrain ML, et al. Gastrointestinal symptoms during the first week of intensive care are associated with poor outcome: a prospective multicentre study. Intensive Care Med 2013;39(5):899–909.
43. Shimizu K, Ogura H, Hamasaki T, et al. Altered gut flora are associated with septic complications and death in critically ill patients with systemic inflammatory response syndrome. Dig Dis Sci 2011;56(4):1171–7.
44. Osuka A, Shimizu K, Ogura H, et al. Prognostic impact of fecal pH in critically ill patients. Crit Care 2012;16(4):R119.
45. Piton G, Belon F, Cypriani B, et al. Enterocyte damage in critically ill patients is associated with shock condition and 28-day mortality. Crit Care Med 2013;41(9):2169–76.
46. Thuijls G, van WK, Grootjans J, et al. Early diagnosis of intestinal ischemia using urinary and plasma fatty acid binding proteins. Ann Surg 2011;253(2):303–8.
47. Guzel M, Sozuer EM, Salt O, et al. Value of the serum I-FABP level for diagnosing acute mesenteric ischemia. Surg Today 2014;44(11):2072–6.
48. Theodorakopoulou M, Perros E, Giamarellos-Bourboulis EJ, et al. Controversies in the management of the critically ill: the role of probiotics. Int J Antimicrob Agents 2013;42(Suppl):S41–4.
49. Bo L, Li J, Tao T, et al. Probiotics for preventing ventilator-associated pneumonia. Cochrane Database Syst Rev 2014;(10):CD009066.
50. Barraud D, Bollaert PE, Gibot S. Impact of the administration of probiotics on mortality in critically ill adult patients: a meta-analysis of randomized controlled trials. Chest 2013;143(3):646–55.
51. Besselink MG, van Santvoort HC, Buskens E, et al. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. Lancet 2008;371(9613):651–9.
52. Expression of concern–Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. Lancet 2010;375(9718):875–6.
53. Silvestri L, van Saene HK. Selective decontamination of the digestive tract: an update of the evidence. HSR Proc Intensive Care Cardiovasc Anesth 2012;4(1):21–9.
54. Petros AJ, Silvestri L, van Saene HK, et al. 2B or not 2B for selective decontamination of the digestive tract in the surviving sepsis campaign guidelines. Crit Care Med 2013;41(11):e385–6.

55. Silvestri L, de La Cal MA, van Saene HK. Selective decontamination of the digestive tract: the mechanism of action is control of gut overgrowth. Intensive Care Med 2012;38(11):1738–50.
56. de Smet AM, Kluytmans JA, Blok HE, et al. Selective digestive tract decontamination and selective oropharyngeal decontamination and antibiotic resistance in patients in intensive-care units: an open-label, clustered group-randomised, crossover study. Lancet Infect Dis 2011;11(5):372–80.
57. Drekonja D, Reich J, Gezahegn S, et al. Fecal microbiota transplantation for Clostridium difficile infection: a systematic review. Ann Intern Med 2015;162(9):630–8.