The intestinal microenvironment in sepsis

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Abstract

The gastrointestinal tract has long been hypothesized to function as “the motor” of multiple organ dysfunction syndrome. The gastrointestinal microenvironment is comprised of a single cell layer epithelia, a local immune system, and the microbiome. These three components of the intestine together play a crucial role in maintaining homeostasis during times of health. However, the gastrointestinal microenvironment is perturbed during sepsis, resulting in pathologic changes that drive both local and distant injury. In this review, we seek to characterize the relationship between the epithelium, gastrointestinal lymphocytes, and commensal bacteria during basal and pathologic conditions and how the intestinal microenvironment may be targeted for therapeutic gain in septic patients.

Key terms
sepsis; microbiome; immune system; intestine; epithelium; gut

1. Introduction

Sepsis is life threatening organ dysfunction caused by a dysregulated host response to infection (1). Sepsis continues to be the leading cause of mortality in the intensive care unit. An estimated 900,000 to 3 million people develop sepsis in the United States annually (2), and mortality from septic shock is greater than 40% (3). Despite significant advancements in our understanding of the pathophysiology of sepsis (4), treatment of sepsis is limited to antibiotics, aggressive fluid resuscitation, vasopressor administration, and supportive care (5), and no targeted therapeutics for sepsis are approved for usage in patients (4).

The gastrointestinal tract has long been hypothesized to play an integral role in the pathophysiology of sepsis, by acting as a motor that both drives and perpetuates multiple organ dysfunction (6–12). The original concept of gut-derived sepsis proposed that the altered inflammatory milieu induced by overwhelming infection leads to intestinal hyperpermeability, allowing for translocation of intact bacteria from the lumen of the gut...
into previously sterile locations, which, in turn, drives sepsis and perpetuates the inflammatory response. Although this construct has significant intellectual appeal, reality has proven to be significantly more complex. While there is clear evidence of bacterial translocation in specialized clinical scenarios (such as in neutropenic patients), bacteremia is not routinely detected in patients with a leaky gut barrier. Further, in a classic study of trauma patients – who typically have intestinal hyperpermeability -- catheters placed in the portal venous system demonstrated intact bacteria less than ten percent of the time (13). This does not mean that the intestine has no role in the pathophysiology of critical illness, but suggests that a fuller understanding of the intestinal microenvironment is required to understand the multiple ways that it may be perturbed in sepsis.

Under basal conditions, the components of the intestinal microenvironment act in concert to maintain a symbiotic, mutually beneficial relationship. Protection of this microenvironment is imperative not only to the host but also to the bacteria themselves, and is achieved through a balance of intestinal integrity, anti-inflammatory and inflammatory responses, and a diverse composition of bacteria. However, when this balance becomes skewed in sepsis, it results in a breakdown of the alliance between the host and its bacterial colonizers, resulting in transformation and selection of virulent pathogens and a robust -- and maladaptive -- inflammatory and anti-inflammatory response. Together, this allows propagation of disease, and importantly this feed-forward pathway leading to worsening organ dysfunction can occur largely independent of the inciting factor. As such, not only can the gut play a role in mediating intra-abdominal sepsis, but it can also play an equally important role in propagating sepsis from extra-abdominal sepsis as well. In addition, common therapeutic or prophylactic interventions initiated during sepsis such as antibiotics, proton-pump inhibitors, and parenteral nutrition can all alter microbial composition and, as such, can alter the gastrointestinal environment in ways that we are only beginning to understand. This review aims to characterize the intestinal microenvironment in both health and sepsis (and other forms of critical illness as appropriate), which is required for development of treatment modalities aimed at maintaining and restoring homeostasis within the gut.

2. The intestinal microenvironment in health

The intestine plays a critical role in host health by absorbing food; however, this is far from its only role. Broadly speaking, the intestine is made up of three components: a) a single cell layer epithelium, b) the microbiome that lives within the gut lumen and contains as many microbes as there are host cells in the entire body, and c) an immune system which helps preserve the fragile balance between the host and microbial communities, allowing for mutually beneficial co-existence.

2.1 The intestinal epithelium

The small intestine is lined by a single cell epithelium. Although narrow in terms of width, the specialized structure of the intestinal epithelium is actually comprised of a surface area of half of a badminton court (14). The epithelium plays a vital role in health through a) being the primary location through which food is absorbed in the body, b) host defense (both
physical and chemical) against invading microorganism within the gut lumen, c) providing a semi-permeable barrier between the host and the gut lumen, and d) hormone secretion.

Pluripotent intestinal stem cells reside near the base of the crypts, which then divide into daughter cells. The majority of these cells migrate upwards to the villus where they differentiate into absorptive enterocytes (which make up 85% of the surface area of the intestinal epithelia), mucus-producing goblet cells and hormone-producing enteroendocrine cells. When cells reach the villus tip, they either are exfoliated whole into the lumen or die by apoptosis. This entire journey from cell birth to migration/differentiation to death takes less than one week. A small number of epithelial cells also migrate downward from the stem cell region where they differentiate into defensin-producing Paneth cells.

2.2 The microbiome

The gut lumen contains the intestinal microbiome, a population of approximately 40 trillion bacterial cells (15). While the general term microbiome refers to all microbes living within the host (including the skin, mouth and lungs as well as the gut), the majority of bacterial species and diversity in the microbiome reside within the intestine. Historically, it was thought that the host and the commensal bacteria lived fairly independent lives, albeit in the same geographic neighborhood. However, as our understanding of the microbiome grows, it has become readily apparent that both host and microbiome greatly influence each other, and perturbations in this relationship play a significant role in multiple acute and chronic diseases (16;17).

Notably, the human microbiota contains as many cells as are present in the entire human host, with over 1,000 different species residing within the intestinal lumen (18). The diversity within this is astounding, with the microbiome containing over two million microbial genes (15). The relationship between the human host and the microbiome begins to develop upon exit of the newborn from the birth canal, and, as such, vaginal microbes are the original source of the commensal bacteria that will ultimately compose the microbiome (19). While not completely understood, the early interactions between these microbes and the neonate’s immune system initiate the development of a symbiotic relationship that allows for acceptance of commensals to occur. For example, IgA present in breast milk acts to limit immune activation in response to foreign antigen and oligosaccharides and allows for the expansion of certain populations of beneficial bacteria including *Bifidobacterium* (20). Disruption of this process is thought to be associated with development of diseases associated with epithelial barrier dysfunction, such as asthma (21). Notably, the composition of the microbiome is altered by a variety of factors including breast milk composition, introduction of solid foods, and antibiotic exposure in early infancy; however, by 2 to 3 years of age, the microbiome composition stabilizes (22).

There are four dominant phyla that comprise the entire human microbiome, but the majority of intestinal bacteria fall within two: Firmicutes and Bacteroidetes (23;24). The relationship between these two phyla undergoes significant alterations between infancy and adult life. An increase in the relative abundance of Firmicutes compared to Bacteroidetes is found in the elderly, thought likely secondary to diet and other environmental factors (24). An increase in
the Firmicutes to Bacteroidetes ratio has also been associated with the development of metabolic syndromes, including type 2 diabetes and obesity (25).

2.3 The mucus layer

Mucus plays an important role in mediating barrier defense by preventing bacteria, digestive enzymes, and other toxic mediators from coming into contact with the gut epithelium. The hydrophobic properties of mucus, resulting from negatively charged glycoproteins released from epithelial goblet cells, greatly limits the ability of positively charged, water-soluble toxic molecules to traverse the surface (26). While originally thought to be a static structure, the mucus layer is actually dynamic and altered by a variety of factors. For instance, studies in germ free mice have demonstrated a significant delay in formation of an adequate mucus layer associated with microbial composition shifts after colonization (27), suggesting a relationship between bacterial populations and the properties and functionality of mucin glycoproteins.

The properties and development of the epithelial mucus layer differ greatly based upon its location within the gastrointestinal tract. The mucus within the small intestine is comprised of a single semi-permeable layer, allowing for passage of some anti-bacterial peptides. Clearance of larger and potentially pathogenic molecules occurs through the shedding of detached mucus, which is then excreted with other fecal matter (27). In contrast, mucus in the colon consists of two distinct layers where the inner layer is entirely impermeable to luminal bacteria (28). Notably, genetically altered mice that lack the Muc2 gene, a gene that regulates production of mucin glycoproteins, do not possess this inner bacteria-free layer and develop spontaneous intestinal inflammation demonstrating the importance of its function in regulating intestinal homeostasis (29).

2.4 The intestinal immune system

While host-microbial interactions play crucial roles in health, this relationship can easily turn hostile. As such, the host requires constant surveillance of its inner microbial world to assure that pathogenic bacteria are not able to drive an inflammatory response that might be expected when the host encounters antigen it recognizes as foreign. Part of host defense to microbial invaders comes from the epithelium, in the form of physical barriers such as tight junction proteins between epithelial cells and mucous produced by goblet cells. In addition, a vital component of intestinal host defense comes from the local intestinal immune system, which has both surveillance and effector arms. The intestine is home to one of the largest immune organs found in the human body, with over 80% of the total body’s lymphocyte population residing in the gut (30). There are several compartments of immune cells within the gastrointestinal tract associated lymphoid tissue. These include intraepithelial lymphocytes, lamina propria lymphocytes, Peyer’s Patches, and mesenteric lymph nodes. Both Peyer’s Patches and mesenteric lymph notes are organized lymphoid aggregates; however only the latter are connected to systemic lymphoid systems via drainage channels. Peyer’s Patches act in concert with epithelial cells to participate in the induction of local immune responses to luminal antigen by mediating antigen presenting cells/T cell interactions (12) and by the release of cytokines by activated T cells.
2.5 Interactions between the microbiome and the intestinal immune system

While the immune system plays a critical role in surveillance of the microbiome and threat response when required, the relationship is not one sided, as bacteria are also involved in maintaining their mutually beneficial relationship with the host. Proteins known as bacteriocins produced by bacteria within the intestinal microbiome stimulate Paneth cells at the base of the crypts to produce antimicrobial peptides, which work to prevent overgrowth of potentially pathogenic bacteria (31).

Additionally, bacteria influence secondary lymphoid organ development as well as adaptive immune cell responses and proliferation. Germ free mice that lack an endogenous microflora have significantly smaller Peyer’s Patches, fewer CD4+ T cells and limited IgA production within the gastrointestinal tract (32), suggesting that commensal bacteria play an important role in the development of the local immune system. Additional, T cell responses in germ free mice are nearly absent, indicating that the microbiome not only plays a role in the development of immune cells but also in their function (12).

The interactions between the microbiome and the immune system are complex. Commensal bacteria have antigenic properties that influence the local immune system and in particular, stimulate T cell development into certain cellular subsets to maintain homeostasis. These T helper cellular subsets, most notably Th17 cells and T regulatory (T\textsubscript{reg}) cells, have been implicated in protecting the intestine from pathogenic insults as well as immune-mediated damage in the setting of unregulated inflammation. Th17 cells function to limit bacterial colonization during times of tissue damage and prevention of enteric infections by recruiting neutrophils to the intestinal barrier and in assisting in the clearance of pathogens. T\textsubscript{reg} cells, defined as FOXP3\textsuperscript{+}CD25\textsuperscript{+}CD4 cells, work to dampen Th17 responses and hinder excessive inflammation in the setting of a mounted immune response that can cause tissue damage. Failure to regulate inflammation in the setting of commensal antigen can result in chronic disease processes such as inflammatory bowel disease (33).

Notably, expansion of Th17 cellular populations has been linked to certain commensal bacteria, particularly segmented filamentous bacteria. Germ free mice have reduced populations of Th17 cells within the lamina propria (34), showing the importance of bacteria in developing local immune cell populations. In addition, although not a predominant species in the healthy intestine, Bacteroides fragilis, a Gram-negative bacterium, has been shown to be involved in development of T\textsubscript{reg} cells. This organism is surrounded by a capsular polysaccharide complex with zwitterionic properties, creating a strong immunomodulatory effect. The most notable of these is polysaccharide A (PSA). Mice given PSA-deficient Bacteroides fragilis develop defective colonic tissue colonization secondary to excessive Th17 production (25). Additionally, germ free mice that are colonized solely with PSA\textsuperscript{+} Bacteroides fragilis have CD4\textsuperscript{+} T cell clonal expansion, specifically with an increase in T\textsubscript{reg} cells, suppressing inflammation by dampening Th17 responses. PSA has also been shown to influence dendritic cell functionality through TLR2 signaling to prime T cell responses to maintain antigenic tolerance (35). Dendritic cells stimulate production of IFN\textgamma\textsuperscript{+} IL-10\textsuperscript{+} CD4\textsuperscript{+} cells, an immunosuppressive T helper cell phenotype that helps to control inflammation in the setting of persistent enteric antigen exposure (36).
**Clostridium** has also been shown to be integral to not only T<sub>reg</sub> cell development within colonic tissues but also to its suppressive function and ability to protect against autoimmune disease. In a germ free murine model colonized with 46 strains of *Clostridium*, elevated numbers of T<sub>reg</sub> cells were found specifically within the colonic lamina propria (37). Additionally, this environment was rich in TGF-β, a cytokine that plays a significant role in differentiation of CD4<sup>+</sup> T cells into T<sub>reg</sub> cells, as well as IL-10 expression, indicating suppressive capabilities of T<sub>reg</sub> cells. Notably, mice inoculated with *Clostridium* at an early age have an attenuated response to oxazolone-induced colitis when they become adults, indicating the importance of bacterial antigen in developing immune tolerance that can protect from inflammatory pathology in later life.

Firmicutes -- one of the predominant phyla of bacteria in the intestinal lumen -- have also been implicated in modulating immune responses. Segmented filamentous bacteria interact with T cells directly to induce pro-inflammatory cellular differentiation with a polarization towards a Th17 phenotype. Oral gavage of segmented filamentous bacteria into germ free mice results in development of arthritis through amplification of Th17 responses both locally within the lamina propria and systemically via splenic lymphocytes that can travel to distant sites and cause inflammatory destruction (34;38).

IgA also plays an important role in mucosal immunity. Recognition of enteric pathogens via dendritic cells results in production of pathogen-specific IgA within Peyer’s Patches. Upon repeat exposure to commensal bacteria, IgA can alter global bacterial composition, cell motility and bacterial gene expression, preventing adhesion to the epithelial cell wall (39). Furthermore, IgA coats bacteria wherein commensal bacteria express relatively low levels of IgA coating and more virulent pathogens express higher levels. Bacteria with higher coating of IgA have been implicated in murine and human models of colitis (40), and germ free mice colonized with bacteria heavily coated with IgA demonstrate increased vulnerability to colitis (25). Additionally, T<sub>reg</sub> cells induce antigen specific IgA class switching (19), further highlighting the importance of IgA in cultivating a homeostatic environment.

### 2.6 Nutrition and the intestinal microenvironment

The composition of host diet impacts all components of the intestinal microenvironment. Dietary fiber content alters the ratio of Firmicutes to Bacteroidetes, and a diet low in fiber reduces the diversity of bacterial species located within the gastrointestinal tract (41). Further, carbohydrates and proteins present within the host diet are metabolized by colonic bacteria resulting in the production of a variety of metabolites, including short chain fatty acids (SCFAs) (42). The most abundant of these are butyrate, propionate, and acetate (43). These not only provide fuel for local colonocytes, but also influence local lymphocyte function. Through stimulation of G protein-coupled receptors GPR41 and GPR43, SCFAs modulate epithelial cellular function and exert immunomodulatory effects (44). SCFAs have been implicated in neutrophil recruitment, T cell proliferation (45) and secretion of inflammatory cytokines, as well as interference in dendritic cell and T cell interactions. Both butyrate and propionate have been implicated in the proliferation of T<sub>reg</sub> cells, which helps to maintain intestinal homeostasis (46). Acetate is also involved in activation of GPR43,
which ultimately leads to increased IL-18 production and promotion of intestinal barrier integrity (44).

3. Intestinal failure in sepsis

On a macro scale, gut failure is common in the intensive care unit. Critically ill patients often have a constellation of symptoms including absent bowel signs, diarrhea, abdominal distension, vomiting, poor intestinal motility with resultant high tube feeding residuals, stress ulceration with resultant gastrointestinal bleeding and intra-abdominal hypertension. While none of these symptoms in isolation is associated with mortality, having three of these on day one in an intensive care unit stay is associated with a three-fold increase in mortality (47). Biomarkers of gut failure have also been described. Elevated intestinal fatty acid binding protein (a marker of enterocyte damage) and decreased citrulline (a marker of enterocyte mass) have both been associated with disease severity and mortality in the intensive care unit (48;49).

On a mechanistic level, these gross abnormalities are observed because all elements of the intestinal microenvironment are perturbed in sepsis. The loss of the adaptive homeostatic intestinal milieu leads to a myriad of local perturbations, which can rapidly lead to systemic propagation of disease. Although many of these alterations will be discussed in isolation below, it is important to emphasize that crosstalk occurs within the intestinal microenvironment and between the intestine and extra-intestinal tissues, such that a perturbation in one component of the gut in sepsis has a high likelihood of impacting cells and tissues outside of its local environment (Figure 1).

3.1 Intestinal permeability

Sepsis (and critical illness in general) induces significant dysfunction in the intestinal barrier with resultant hyperpermeability (50–53). This allows luminal contents (intact microbes, microbial products) to escape their natural environment where they can cause either local or distant injury. In preclinical models of sepsis, alterations in tight junctions occur as early as one hour after the onset of sepsis, and intestinal hyperpermeability persists for at least 48 hours after the onset of sepsis (52). This is associated with increased claudin 2 and junctional adhesion molecule A as well as decreased claudin 5 and occludin in the small intestine of both cecal ligation and puncture (CLP) as well as Pseudomonas aeruginosa pneumonia. Colonic tight junctions are also impacted by CLP, with alterations in cellular localization of claudins 1, 3, 4, 5, and 8, and upregulation of claudin 2 (54). In addition, a marked decrease in zonula occludens-1 is seen only in pneumonia (and not CLP) suggesting that the mechanisms underlying barrier dysfunction may be mediated via a combination of a common host response and a more specific response modulated by the source of sepsis (52). Intestinal hyperpermeability is also related to chronic co-morbidities as mice with alcohol use disorder prior to the onset of CLP have a further increase in permeability, associated with decreased zonula occludens-1 and occludin expression (53).

The intestinal tight junction is also closely associated with the peri-junctional actin-myosin ring. Myosin light chain kinase (MLCK) phosphorylates the myosin regulatory light chain, resulting in contraction of the actin-myosin ring, which, in turn, increases paracellular
permeability (55). MLCK activation is a common finding seen with bacterial infection (56;57) and is associated with increases in IL-6, TNF and IL-1β that further activate MLCK in a feed forward mechanism, in part via altered ZO-1 and occludin (58–60). Notably, inhibition of MLCK in a murine model of burn injury results in improved barrier function and tight junction rearrangement (61) while a similar strategy results in a decrease in bacterial translocation and intestinal cytokine production in a model of binge ethanol followed by burn injury (58).

3.2 Intestinal epithelial apoptosis

Although low-level apoptosis occurs in both the intestinal crypt and villus tip under basal conditions, intestinal epithelial apoptosis is markedly upregulated in sepsis in both patients and preclinical models of sepsis. This further reduces the effectiveness of the intestinal barrier and also alters the local inflammatory milieu. Murine models of sepsis have demonstrated upregulation of epithelial cell death activated by both death-receptor and mitochondrial pathways (62). Notably, the pathways of intestinal apoptosis appear to be model dependent. Mice with Methicillin-resistant Staphylococcus aureus pneumonia-induced sepsis have increased intestinal epithelial apoptosis with an increase in the expression of the proapoptotic proteins Bid and Bax and the antiapoptotic protein Bcl-xL in the mitochondrial pathway as well as increased Fas ligand and decreased Fas, FADD, pFADD, TNF-R1, and TRADD in the receptor-mediated pathway (63). In contrast, while Pseudomonas aeruginosa pneumonia induces a similar increase in intestinal epithelial apoptosis, this is associated with increased Bcl-2 and TNF-R1 and decreased Fas. Notably, preventing sepsis-induced gut epithelial apoptosis in transgenic mice by overexpression of the anti-apoptotic protein Bcl-2 leads to a marked survival advantage following both CLP and Pseudomonas aeruginosa pneumonia (64;65). Notably, age appears to play a significant in gut apoptosis in sepsis. Murine models of sepsis have demonstrated significantly increased mortality in aged mice compared to younger animals (66–68), and aging is associated with increased gut epithelial apoptosis (69;70).

Another strategy that has been found to be beneficial in improving outcome in preclinical studies is the administration of epidermal growth factor (EGF). EGF given systemically improves survival in both CLP and pneumonia, even if started 24 hours after the septic insult (71;72). Notably, when EGF is expressed solely in villus enterocytes in transgenic mice, mortality is improved from both CLP and pneumonia, suggesting that the intestine is sufficient (and possibly necessary) to confer the survival advantage conferred by this agent (73). Unlike Bcl-2 which is purely an anti-apoptotic protein, EGF has multiple effects on gut integrity. EGF normalizes intestinal epithelial apoptosis with decreases in Bid, FADD and p21 cip/waf following CLP. In addition, EGF causes a normalization of crypt proliferation (which ordinarily decreases in sepsis) as well as a normalization of villus length. EGF also improves intestinal permeability via a decrease in the pore forming tight junction mediator claudin 2. Notably, the effects of EGF are complicated as systemic EGF improves permeability in septic mice with pre-existing alcohol use disorder via increased levels of the tight junction mediators claudin-5 and junctional adhesion molecule A (74).
A complementary approach demonstrating the importance of intestinal epithelial apoptosis and permeability involved transgenic mice lacking functional Nuclear Factor kappa B (NFκB) in their intestinal epithelium (75). When sepsis is induced in these animals via CLP, both intestinal epithelial apoptosis and permeability are greater than is seen in wild type septic animals, associated with an increase in claudin 2 levels. This is also associated with elevated levels of both pro and anti-inflammatory cytokines. Notably, mortality is higher in animals lacking NFκB in the intestinal epithelium than wild type mice following the onset of sepsis, potentially mediated by TNF as mortality and intestinal integrity are both improved in transgenic animals treated with anti-TNF antibodies.

3.3 Dysbiosis

Changes in intestinal permeability and apoptosis have the potential to alter outcome in sepsis, both by altering inflammation and increasing the likelihood that bacteria and bacterial products have access to local and distant spaces they would not ordinarily be able to reach. In and of itself, this poses a threat. However, the composition of the microbiome is dramatically altered in sepsis to greatly increase its pathogenicity, significantly amplifying the risk posed by increased permeability and apoptosis.

The microbial contents of the intestine are determined by three factors—introduction of bacterial species via the oropharynx, elimination of microbes via fecal matter, and the regulation and proliferation of bacterial species within the GI tract (25). During times of stress, these processes are altered for a variety of reasons, inevitably shifting the composition of the microbiome. Additionally, clinical therapies not aimed at the microbiome – such as utilization of antibiotics, proton-pump inhibitors and parenteral nutrition – can result in destruction or alteration of commensal bacteria. This combination of primary changes on the microbiome induced by sepsis and secondary effects on the microbiome as a result of therapeutics targeted elsewhere results in an environment that promotes overgrowth of pathogenic species.

This transition has been termed “dysbiosis” (76), wherein the microbiome is converted into the “pathobiome” (23;77). It is highlighted by a) a loss of microbial diversity, b) dominance of pathogenic microorganisms (such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus*, *Staphylococcus aureus*, and *Klebsiella*), and c) alterations in bacteria present to become more virulent (78;79). These changes have been shown to occur within hours of a variety of pathological insults, including sepsis, trauma, and burns (80). Further, compared to healthy adults and children, patients in the pediatric intensive care unit (average age 2.9) have decreased alpha diversity in the gut with enrichment of gut pathogens such as Enterococcus and Staphylococcus and depletion of commensals such as *Faecalibacterium* and *Ruminococcus* (81).

The mechanism of the transition from a healthy microbiome to one where virulent pathogens predominate has not been entirely elucidated. However, there is compelling data to suggest that transition plays an important role in determining host outcome. An ingenious example of this was recently described by Alverdy et al (82). Although *Pseudomonas aeruginosa* is a common nosocomial infection, it rarely if ever infects immunocompetent patients. As an example of the virulence (or lack thereof) of this microorganism in health, *Pseudomonas*
*P. aeruginosa* was injected into the cecum of mice subjected to sham surgery. This bacteria was then harvested and implanted into the peritoneum of uninjured mice, where it did not cause mortality. In contrast, when the identical *Pseudomonas aeruginosa* was injected into the cecum of mice subjected to 30% hepatectomy, then harvested and implanted into the peritoneum of uninjured mice, all recipient animals died. Since the microorganism injected at the beginning of the experiments was identical, the best explanation for the difference in lethality is that some factor or factors in the host environment induced the microbes to become more virulent.

A possible explanation for this highlights the central role of phosphate. Phosphate is an important nutrient for bacterial proliferation and growth and, as such, can significantly affect bacterial survival. Phosphate depletion within the intestine frequently occurs in the setting of malabsorption, parenteral nutrition, or liver failure, and has been associated with the presence and proliferation of more virulent strains of *Pseudomonas aeruginosa* (83). In the setting of low levels of phosphate, PA-I lectin/adhesion, a quorum sensing protein that plays a role in mucosal adherence and intestinal barrier dysfunction, is upregulated by *Pseudomonas aeruginosa* (84). Notably, restoration of phosphate via phosphate-containing polyethylene glycol polymers administered via rectal enema is associated with the preservation of the core microbiome and importantly prevents mortality induced by multiple highly virulent pathogenic organisms (85).

Additionally, circulating opioids, both endogenously produced and exogenously administered, can alter the microbiome in critically ill patients. Dynorphin, an endogenous opioid released into the intestinal microenvironment during times of stress, has been shown to interact with *Pseudomonas aeruginosa* and promote a more virulent phenotype (86). Ex vivo studies corroborate these findings. Antibiotic treated *C. elegans* worms allowed to feed on culture media containing both common intestinal bacteria and synthetic opioids are susceptible to the increased killing capacity of *Klebsiella pneumoniae* as well as coagulase-negative *Staphylococcus* that is not present in the absence of opioids (87), indicating increased virulence of these pathogens in the setting of exposure to opioids.

### 3.4 Crosstalk between the immune system and the intestinal barrier

In the later stages of sepsis, patients develop widespread cellular apoptosis and upregulation of T cell co-inhibitory markers (88–90). Patients in this phase are at elevated risk due to inadequate control of primary infection and acquisition of secondary infections (91;92). The T cell co-inhibitory molecule PD-1 and its ligand PD-L1 have been extensively studied in preclinical models of murine sepsis with multiple lines of investigation demonstrating that blockade of either of these molecules results in restoration of immune functionality across several cell lines (89;93;94). Recent work has indicated that PD-L1 expression on intestinal epithelial cells may also play a role in maintaining intestinal integrity (95). PD-L1 is upregulated on intestinal epithelial cells in septic animals following CLP compared to sham animals and is also upregulated in septic patients. Furthermore, septic PD-L1−/− mice have decreased intestinal permeability compared to WT mice. In addition, septic PD-L1−/− mice have decreased tissue levels of IL-6, TNF and MCP-1. This suggests a direct relationship
between the immunological derangements seen in sepsis and subsequent intestinal hyperpermeability.

### 3.5 Nutrition in critical illness

Bacterial species that produce butyrate are significantly reduced in critical illness, which has been associated with epithelial cell loss, reduced mucosal tolerance, and bacterial translocation (25). In addition, due to a variety of reasons (including intestinal ischemia, ileus, malabsorption), many septic patients are unable to tolerate enteral feedings and are dependent on total parenteral nutrition (TPN) for caloric requirements. However, while TPN administration is able to deliver the same number of calories as enteral nutrition, they are not equivalent. TPN lacks the beneficial impact that enteral feeds have independent of caloric intake and importantly has been implicated in disruption of the intestinal barrier integrity, mucosal immune system and the composition of the microbiome.

Administration of TPN increases intestinal permeability, alters Paneth cell function and decreases the Firmicutes to Bacteroidetes ratio (96). Further, chronic TPN utilization in mice is associated with a transition from a microbiome environment predominantly containing Firmicutes to a pathobiome containing mostly gram-negative Proteobacteria (97). This dysbiosis is associated with an upregulation of multiple toll-like receptors on intestinal epithelial cells and a concomitant increase in inflammatory cytokines TNF and IFNγ. This elevated TNF expression has been associated with aberrant TNFR signaling, an important mechanism in cellular proliferation and cell survival. Decreased EGF levels in chronic TPN use as well as altered TNF receptor signaling thus promotes an altered state where the epithelial barrier is compromised due to increased cellular apoptosis.

### 3.6 Intestinal lymph

While initial studies of critical illness focused on harmful factors leaving the gut via the systemic circulation, there is significant evidence that mesenteric lymph can be an equally important carrier for toxic gut-derived factors (98). There are multiple lines of evidence supporting the "gut-lymph hypothesis." Gut-derived lymph flows from the mesenteric lymphatic duct directly into the pulmonary circulation. Importantly, ligation of the mesenteric lymph duct abrogates neutrophil driven lung injury and ARDS in multiple models of critical illness, including burns, trauma and shock in both small animal and large animal models (99). Further, lymph collected from animals subjected to trauma/hemorrhagic shock injury injected into naïve mice causes acute lung injury with the same toxic properties as seen in the original animal (100). Ligation of the mesenteric lymph duct in either trauma/hemorrhagic shock or burn injury also prevents decreases in cardiac contractility and cardiac output. Most importantly, ligation of the mesenteric lymph duct improves survival in multiple animal models of critical illness. The gut-derived factors carried in mesenteric lymph are still being identified but they typically do not contain intact bacteria, endotoxin or cytokines. Rather, lymph contains protein and lipid factors that signal through TLR4 dependent pathways in the lungs, suggesting that distant tissue injury occurs via pattern recognition receptor pathways stimulated by endogenous, inflammatory proteins released by the intestine.
Distinct from the gut-lymph hypothesis, intestine-specific deletion of Mttp, a protein required for chylomicron assembly, improves survival in mice subjected to *Pseudomonas aeruginosa* pneumonia (101). Knockout animals are protected against sepsis-induced increases in intestinal epithelial apoptosis and decreases in proliferation and villus length. Further, deletion of this protein prevents a sepsis-induced increase in IL-6 and is associated with an increase in serum high density lipoprotein (which is typically decreased in sepsis). In contrast, aged mice of the same genotype have worsened survival when subjected to pneumonia with the same microorganism (70). The mechanisms for this disparate age-dependent response are incompletely understood although aged mice with intestine-specific deletion of Mttp have increased intestinal epithelial apoptosis, with increases in both the Bax/Bcl-2 and Bax/Bcl-X<sub>L</sub> ratio as well as increased pulmonary myeloperoxidase.

### 3.7 Mucus

The mucus layer is compromised during critical illness, resulting in epithelial cell dysfunction. Although limited data is available in sepsis, ischemia/reperfusion models demonstrate a loss of hydrophobicity of the mucus layer, resulting in altered intestinal permeability (26). Further, rats subjected to trauma/hemorrhagic shock have decreased mucus, associated with villus height loss, increased epithelial apoptosis and increased intestinal permeability (50). Notably, enteral administration of a mucus surrogate prevents trauma/hemorrhagic shock-induced gut and lung injury (102).

### 4. Treatment targeting the microbiome

Based upon derangements to the microbiome in disease, multiple treatment modalities aimed at reconstituting the commensal environment have been proposed. Strategies have ranged from transplanting an entire exogenous microbiome from a healthy donor (fecal microbiota transplantation), to giving either healthy bacteria and/or stimulating the host to increase “good” bacteria (probiotics, prebiotics, synbiotics) or semi-selectively depleting pathogenic bacteria (selective decontamination of the digestive tract). Each of these approaches has shown promise in sepsis, yet each has limitations that prevent them from being standard of care in most environments.

Debatably the most impactful method of altering the microbiome is fecal microbiota transplantation. Due to the increased and often inappropriate use of antibiotics in critically ill patients, the emergence of antibiotic resistant pathogens has become more prevalent in the intensive care unit. With this, the incidence of *Clostridium difficile*, an increasingly morbid and potentially fatal infection has increased dramatically to upwards of 450,000 cases annually in the United States alone (103). While this microorganism is generally treatable with oral vancomycin or metronidazole, an increasing number of recurrent cases or treatment failures have been described. Unfortunately, conventional therapy for recurrent *Clostridium difficile* is generally ineffective. A landmark study recently demonstrated that fecal microbiota transplant increases the cure rate in recurrent *Clostridium difficile* infection from 30% to 93% (104). In order for a fecal microbiota transplant to be successful, however, patients cannot receive antibiotics, as continued antimicrobial therapy would be expected to immediately alter the transplanted microbiome. This has limited the usage of fecal...
microbiota transplant in the intensive care unit to a few case reports in patients with intractable diarrhea (105;106). Determining the role of fecal microbiota transplant in critically ill patients thus represents an experimental strategy in sepsis, which will require rigorous studies to determine what role this could play in future treatment.

In contrast, significant data exists on probiotic (and to a lesser degree prebiotic and synbiotic) use in the intensive care unit. Recent meta-analyses on over 2000 patients demonstrate that probiotics reduce the rate of ventilator associated pneumonia and infections in critically ill patients without differences in mortality, hospital length of stay or length of mechanical ventilation (107). However, conclusions are limited by low quality evidence, heterogeneity of study design and outcome measures. In 2016, a randomized, multi-center study examined the use of probiotics containing live *Bacillus subtilis* and *Enterococcus faecalis* via nasogastric tube in ventilated patients. Patients receiving probiotic therapy had a statistically significant reduction in the rate of ventilator associated pneumonia compared to those that did not (36.4 to 50.4%, p=0.031) (108). Ultimately, however, well designed trials are still required prior to wider adoption of probiotics in the intensive care unit.

The most controversial method of targeting the pathobiome is selective decontamination of the digestive tract. This strategy gives a combination of oral and systemic antibiotics with the goal of preventing or eradicating oropharyngeal and intestinal carriage of pathogenic microorganisms without adversely impacting the remaining microbiome of the patient or the intensive care unit at large. The data supporting selective decontamination of the digestive tract is compelling, with a meta-analysis of 29 higher quality trials demonstrating a decrease in mortality with an odds ratio of 0.73 (95% CI 0.64 to 0.84) (109). However, theoretical concerns about development of antimicrobial resistance with this strategy have limited its usage almost exclusively to a small number of countries with low basal resistance rates. While a debate on the pros and cons of this strategy are outside the scope of this review, ongoing large scale international trials should help clarify whether this approach to targeting the microbiome should be adopted on a broader scale.

5. **Concluding remarks**

All components of the intestinal microenvironment are vital to the maintenance of host homeostasis. In addition to its primary role of absorbing food, the intestine houses a complex microbial environment that is beneficial to host development and health. Unfortunately, this tightly woven web of connections breaks down in the setting of sepsis with resultant development of a pathobiome that can no longer be fully controlled by the immune system, allowing pathogens and their products to gain access to extraluminal spaces via alterations in intestinal permeability and apoptosis and via the mesenteric lymph. These problems are frequently exacerbated by common treatments not aimed at the intestine, but that unfortunately may impact the intestine in ways we are just beginning to understand (antibiotics, proton pump inhibitors, TPN).

Restoring the microbiome to health represents an exciting avenue for which clinical trials are already underway. Significant preclinical data also suggests that targeting intestinal epithelial integrity (apoptosis inhibition, growth factors, alteration of tight junction) and
immune competence (via checkpoint inhibitors) may also be attractive strategies. By targeting the multiple overlapping ways in which the intestinal microenvironment is altered in sepsis, the “motor” of organ failure may ultimately be diminished or turned off, preventing propagation of disease.

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Reference List


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HIGHLIGHTS

- The intestinal microenvironment is comprised of three elements—the epithelium, the local immune system, and intestinal microbiome. Each of these is interconnected and essential to maintaining host health.

- Sepsis mutates the symbiotic intestinal microenvironment to a dysbiotic environment that promotes epithelial cell hyperpermeability and apoptosis, hyperinflammation, and domination of pathogenic bacteria.

- Nutrition plays a large role in the metabolic and immunological environment within the gastrointestinal tract. Administration of TPN alters epithelial integrity, immunological function, and bacterial pathogenesis.

- Re-establishing a homeostatic intestinal microenvironment may be a useful treatment approach to sepsis.
Figure 1. The intestinal microenvironment in health and in critical illness
A) The intestinal microenvironment includes the intestinal epithelium, commensal bacteria, and the local immune system. These elements work in concert to promote a symbiotic relationship promoting host intestinal health. B) In sepsis, these elements become dysregulated resulting in a pathogenic environment where the epithelial barrier and host immune defense fail to control virulent bacteria.