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SUPPLEMENT ARTICLE

Sepsis and the Microbiome: A Vicious Cycle

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Sepsis has been characterized as a dysregulated host response to infection, and the role of the microbiome as a key influencer of this response is emerging. Disruption of the microbiome while treating sepsis with antibiotics can itself result in immune dysregulation. Alterations in the gut microbiome resulting from sepsis and its treatment have been implicated in organ dysfunction typical of sepsis across multiple tissues including the lung, kidney, and brain. Multiple microbiota-directed interventions are currently under investigation in the setting of sepsis, including fecal transplant, the administration of dietary fiber, and the use of antibiotic scavengers that attenuate the effects of antibiotics on the gut microbiota while allowing them to concentrate at the primary sites of infection. The emerging evidence shows that the gut microbiome interacts with various elements of the septic response, and provides yet another reason to consider the judicious use of antibiotics via antibiotic stewardship programs.

Keywords. sepsis; microbiome; immune system; antibiotics.

Sepsis is a leading cause of morbidity and mortality worldwide, and is among the most expensive health conditions in the United States [1]. Sepsis has recently been defined as a dysregulated host response to an ongoing or suspected infection. As a result, the majority of sepsis research has focused on the host immune system's response to understand the etiology of the dysregulation [2]. Although this immune-centered approach has led to an improved understanding of a host's response to the suspected infection, the role of the microbiome and the pathobiology of the inciting infectious agent remains poorly understood. In the last decade, microbiome research has begun to advance our understanding of how the microbiome at multiple colonization sites (lung, skin, gut) influences the immune dysregulation that develops during sepsis. The microbiome has been shown to play a key role in the development and regulation of the immune system, impacting both host susceptibility and response to infection. In this review we have limited our discussion to bacteria, as the sepsis literature pertaining to the bacterial microbiome is more extensive and mature than that of the virome or mycobiome.

HOW CAN THE MICROBIOME PREVENT INFECTION?

Infection underpins the primary insult and subsequent immune activation that defines the occurrence, course, and outcome of sepsis. The microbiome, in particular the intestinal microbiome, has emerged as an important component in this overall response. For example, maintaining a healthy intestinal microbiome establishes resistance to pathogen colonization within the intestinal tract, a major site of origin of sepsis-causing microbes. In addition, the intestinal microbiome can affect resistance against pathogens at sites remote from the intestines, such as the lung. A common perturbation to a healthy microbiota is exposure to antibiotics. Antibiotics, especially those with antianaerobic activity, dramatically alter the microbial ecology and result in the acquisition and domination by normally low-abundance but highly pathogenic species, such as *Enterococcus faecium* or *Klebsiella pneumoniae* [3, 4]. In one study, domination by *Enterococcus* increased the risk of subsequent Vancomycin-resistant Enterococci bacteremia by 9-fold whereas domination by *Proteobacteria* increased the risk of gram-negative bacteremia by 5-fold. Bacteremia occurred at a median of 7 days *after* intestinal domination by these pathogens [4]. The association between microbiota disruption and the risk of sepsis has been further supported by 2 large retrospective studies [5, 6]. In both studies, admission to the hospital for an infection-related complication and antibiotic exposure significantly increased the risk for subsequent sepsis-related hospitalization within 90 days of the index hospitalization. Taken together, these studies indicate that the normal microbiota are our first line of defense against pathogens, and that their disruption can increase the risk of serious life-threatening infection and sepsis.

HOW DOES THE INTESTINAL MICROBIOME INFLUENCE THE IMMUNE RESPONSE TO INFECTION?

As hosts and microbes coevolved to live symbiotically, they have developed a complex molecular dialog involving detection, responses, counter responses, and counter-counter responses, which results in continuous fine-tuning of the immune system

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and microbial ecology. The microbiota measurably influence the function of the immune system, including local barrier function at the site of colonization, hematopoiesis, T-cell differentiation and activation, cytokine production, antibody production, and phagocytosis [7–11]. Microbiota can influence the immune system via contact-dependent mechanisms involving surface structural elements or via secreted exoproducts (ie, metabolites). For instance, the most abundant phyla of the healthy gut, Bacteroides and Firmicutes, are major producers of short chain fatty acids (SCFA), which can regulate gene expression in regulatory T cells as well as alter the microbiocidal capabilities of macrophages [10, 11].

The significance of this microbiota-immune crosstalk in the setting of infection has now been well documented in animal models. Disruption of the microbiota with antibiotics prior to pathogen inoculation worsens the outcome in animal models of pneumococcal pneumonia [12], Escherichia *coli*, and *K. pneumoniae* [13], among others. These studies demonstrated that antibiotic treatment worsened the immune defect and overall outcome whereas restoration of the microbiota led to reestablishment of immune function and improved outcome. Among the various methods used to maintain or restore the lost microbiota in these studies included "prebiotic" carbohydrates, live strains of probiotics, and whole transfer of normal microbiota (fecal microbiota transplant, FMT) [12, 13]. Results from these studies demonstrate that maintenance of the gut microbiome over the course of the acute insult and after exposure to antibiotics can positively influence immune function and improve outcomes.

Indeed, there are now a small number of clinical case reports that describe patients with sepsis, multiorgan dysfunction, and shock that were associated with a major disruption in the microbiome, and in whom FMT and antibiotic cessation resulted in complete recovery. In these cases, antibiotic cessation and FMT was associated with reestablishment of the obligate anaerobic microbial populations that predominate in the colons of healthy individuals, and a decrease in proinflammatory cytokines and inflammatory markers [14]. Whether the findings of these provocative case reports may be applicable to a broader population of patients requires further studies of safety and efficacy, and well-designed controlled clinical trials.

WHAT ROLE DOES THE MICROBIOME PLAY IN ORGAN DYSFUNCTION DURING SEPSIS?

A central, defining feature of a poor outcome from sepsis is the development of organ dysfunction. Several of the most salient sepsis-associated organ dysfunctions, including lung injury, acute renal dysfunction, and encephalopathy, have been associated with microbiota disruption. For example, patients with acute respiratory distress syndrome harbor a pulmonary microbiome that is enriched with microbes that resemble those of the intestinal microbiome [15, 16]. In animal models of sepsis, lung injury can be prevented with antibiotic pretreatment or germ-free conditions [15, 17]. Animal studies also demonstrate that microbiota-directed therapies, such as FMT or SCFA administration, can prevent acute renal dysfunction [18, 19]. However, the relevance of these findings to the human condition remains to be established [20]. Finally, gut-origin polymicrobial dissemination to the brain has been detected in animal models as well as human patients dying of sepsis and correlates with the neuroinflammation that drives septic encephalopathy [21]. The contributions of microbiota to septic encephalopathy is further supported by the ability of members of the microbiota and their metabolites to alter the function of microglia, which appear to play a central role in sepsis-associated neuroinflammation [22, 23]. These studies and others suggest a role for the microbiome in organ dysfunction across multiple systems.

HOW DOES SEPSIS, ITS TREATMENT, AND CRITICAL ILLNESS ITSELF CHANGE THE MICROBIOME?

Work by our laboratory and others has demonstrated the existence of an ongoing bidirectional molecular dialogue between the host and its microbiota that can shift both host phenotype and microbial gene expression at both the species and community level [24, 25]. Following the onset of sepsis, there is frequently a loss of obligate anaerobes (Bacteroidetes and Firmicutes) that typically dominate in healthy individuals, and a "bloom" of normally low-abundant taxa such as Proteobacteria (which includes *E. coli* and *K. pneumoniae*) [3]. Although the mechanisms of this response remain to be elucidated, the selective pressures of physiologic stress (tissue injury, inflammation) and its treatment (antibiotics, artificial nutrition) all appear to drive the degree and duration of this disruption [4, 26, 27].

An unappreciated observation is that stress alone can alter intestinal microbiota composition as described above within minutes or hours of an insult [26]. One might speculate that this response is an attempt by the gut microbiome to "hibernate" as a way of suppressing any further antigenic challenge to the host upon whom its survival depends. However, as a result of modern care (eg, hospital confinement, antibiotics), the microbiome is frequently replaced by a "pathobiome" that has the potential to impair recovery and cause illness beyond that caused by the initial insult itself [25].

Among the various unavoidable methods to support septic patients is the use of artificial nutrition. This merits careful consideration, as dietary composition is among the most well studied factors that can shift intestinal microbiota composition, membership, and function [28]. For example, human volunteers fed an animal-protein based, high-fat, low-fiber diet have significant changes in their gut microbiota composition within a single day [28]. Compared to individuals fed a plant-based, high-fiber diet, they have lower concentrations of SCFAs and higher concentrations of secondary bile acids, which can inhibit

the growth of health-promoting Firmicutes and Bacteroidetes [28]. Nevertheless, patients hospitalized with sepsis are often subjected to casein-based, sterile, chemically defined diets delivered via an enteric tube that lack any dietary fiber [29, 30].

Finally, antibiotics, which are central to the treatment of infected patients, have a profound and lasting impact on the gut microbiome [31]. Antibiotics differentially impact the gut microbiome, with unintended consequences that go beyond the spectrum of activity of the antibiotic. For instance, vancomycin, which has selective gram-positive activity, can significantly reduce the intestinal population of gram-negative Bacteroidetes [3]. Metronidazole increases the risk of *Enterococcus* domination by up to 3-fold compared to vancomycin, fluoroquinolones, or β-lactams in human patients; fluoroquinolones may increase the risk of Proteobacteria domination [4]. Antibiotic-induced alterations in microbiota composition can trigger important functional changes, including reductions in the concentration of SCFAs, the emergence of antibiotic resistance, and the expression of previously latent virulence factors [25, 32, 33]. Recovery of the microbiota following antibiotic exposure generally requires weeks to months, as observed in healthy volunteers, and in some cases can take up to a year for full recovery [31].

Septic patients are subjected to a combination of these selective pressures, resulting in disruption of the microbiota and host immune function. Illustrating this principle, a recent study in mice demonstrated how these additive insults can result in sepsis driven by a gut pathobiome. In this study mice were subjected to a western-type high-fat, low-fiber diet, treated with 5 days of antibiotics, subjected to a 14-hour starvation period, and underwent a 30% hepatectomy. Whereas this is a recoverable surgery in mice fed a standard low-fat, high-fiber diet, the mice subjected to a western diet developed disseminated infection and died of lethal sepsis within 48 hours. The microbiota of these septic mice was depleted of Bacteroidetes and enriched in antibiotic-resistant Proteobacteria, which ultimately disseminated, causing lethal sepsis. Interestingly, these pathogens were present at ultralow abundance in the intestines of standard diet mice, but were presumably contained by the high abundance microbiota such as Firmicutes and Bacteroidetes. Importantly, none of the individual stresses *alone* was sufficient to produce disseminated infection or death, but rather, the combined effects of a western diet *and* antibiotics *and* surgery were required to produce the lethal phenotype [34]. This suggests that managing the pressures of antibiotics and diet can increase the resilience of the microbiota to major stress, and prevent the emergence of a pathobiome, pathogen dissemination, and sepsis.

The principle of negatively compounding additive pressures has been observed in cohorts of septic patients. When tube feeding practices were studied amongst critically ill septic patients, those in whom nutrition was interrupted were found to receive more antibiotics and a greater level of microbiota disruption (a loss of obligate anaerobes, lower SCFA, and more potential pathogens). In turn, these perturbations were associated with higher rates of bacteremia, organ failure, and death [27]. Another study revealed the emergence of an ultralow diversity, multidrug-resistant, virulent pathobiome in a cohort of patients with prolonged critical illness, which is associated with the additive pressures we have outlined. Many of these patients developed intestinal communities that were dominated by a single pathogen (*Enterococcus*, *Staphylococcus*, or *Enterobacteriaceae*). These low-diversity communities harbored significant antibiotic resistance profiles, which was associated with a significant increase in expression of virulence factors [25].

HOW CAN MICROBIOME SCIENCE INFORM THE CLINICAL CARE OF THE SEPTIC PATIENT?

As discussed, the microbiome plays a central role in sepsis—predisposing a host to infection, modulating the immune response, and contributing to the development of organ failure. Once sepsis is established, the combined effects of the physiologic stress state, artificial nutrition and sustained exposure to antibiotics can lead to a pathologic cycle of progressive ecological disruption of the gut microbiota resulting in a shift from health-promoting microbiota to disease-producing Pathobiota, as presented in Figure 1 [25, 34]. With this knowledge in mind, a new appreciation for the critical role of the health-promoting microbiota to drive a recovery-directed immune response during sepsis can inform novel approaches to care for the septic patient.

First and foremost in this approach may be to follow various society guidelines on antibiotic stewardship [35]. Antibiotics are necessary in suspected sepsis and the mortality benefit associated with timely antibiosis may increase the pressure to utilize and maintain therapy. We can infer from available data that conservative antibiotic use is less harmful to the microbiome. However, we are only beginning to define the tipping point at which this occurs. Some of these nuances include determining how specific agents impact the structure and function of the microbiota as well as what duration of antibiotics or level of antibiotics would be safe for maintaining the health of the microbiota. Another approach to mitigating the impact of antibiotics on the gut microbiota is the development of oral scavenger compounds that can absorb antibiotics, thus limiting microbiota disruption within the colon [36]. Multiple antibiotic scavengers are currently in development, and early results have demonstrated their ability to lower the concentration of antibiotics in the colon and thereby minimize the disruption of the intestinal microbiota while maintaining therapeutic levels of antibiotics systemically [36, 37]. However, this work remains investigational and further studies are needed to demonstrate clinical utility.

Beyond antibiotic stewardship, sepsis therapies derived from the growing body of microbiome science remain largely nascent and investigational. Administration of live commensals as probiotics

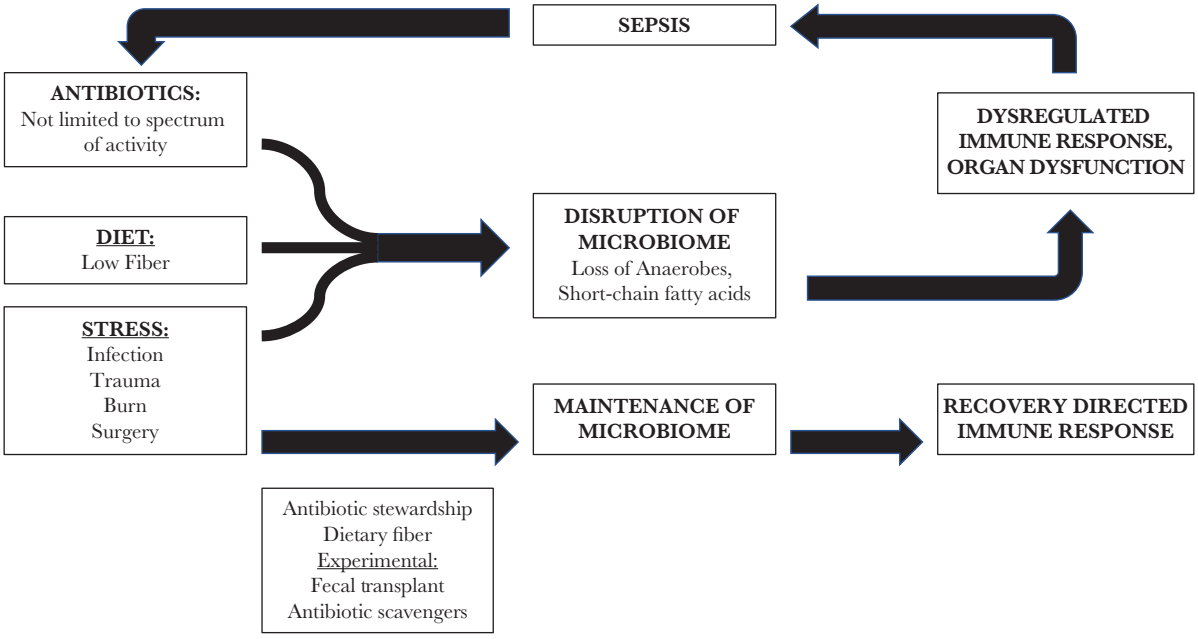

Figure 1. Microbiome and sepsis.

has been studied in a range of infections that may lead to sepsis. However, meta-analyses have found that probiotics were only beneficial in limited subgroups of patients and have an overall small effect size. As a result, probiotics are currently not recommended for prevention or treatment of any infection [35, 38]. Furthermore, recent evidence suggests that probiotic administration may actually delay the reconstitution of the native microbiota of healthy persons, raising potential concerns regarding these therapies [31].

Prebiotics are compounds in food that promote the growth of commensals, for instance fermentable fiber, which supports the growth of species that produce SCFAs. Currently, the Society of Critical Care Medicine and American Society for Enteral and Parenteral Nutrition recommends that fermentable fiber additives be considered in all hemodynamically stable critically ill patients receiving enteral nutrition [30]. In practice, this is not often done and clinical trials would be necessary to clarify the benefits in septic patients. Finally, FMT has been developed as a successful therapy for *Clostridioides difficile* infection and has been applied on an investigational basis in a small number of cases of sepsis; however, controlled studies will be necessary to clarify if these case reports are representative of a larger population of patients [14]. FMT as a potential therapy should be approached with extreme caution as a recent report demonstrated the transmission of a multidrug-resistant organism via FMT to 2 patients who subsequently developed lethal bacteremia [39].

CONCLUSION

There is compelling and convincing evidence that the microbiome plays a significant role in the occurrence, course, and outcome of sepsis via its effects on immune dysregulation leading to organ failure. In the future, microbiota-directed therapies may be used to drive a recovery-directed immune response as we learn how the microbiota favorably influence the immune response during critical illness. However, at the present time, therapies targeting the microbiome remain largely investigational, and judicious use of antibiotics and a rethinking of current nutritional formulations remain the only therapeutic interventions that can be recommended on the basis of current evidence.

Notes

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