The interplay between microbiota and inflammation: lessons from peritonitis and sepsis

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Mammals harbor a complex gut-associated microbiota, comprising bacteria that provide immunological, metabolic and neurological benefits to the host, and contribute to their well-being. However, dysregulation of the microbiota composition, known as dysbiosis, along with the associated mucosal immune response have a key role in the pathogenesis of many inflammatory diseases, including inflammatory bowel diseases (IBDs), type 1 and type 2 diabetes, asthma, multiple sclerosis, among others. In addition, outside the gut lumen, bacteria from microbiota are the causative agent of peritoneal inflammation, abdominal sepsis and systemic sepsis. Critical care interventions during sepsis by antibiotics induce dysbiosis and present acute and long-term poor prognosis. In this review, we discuss immunomodulatory effects of the microbial molecules and products, highlighting the role of Bacteroides fragilis, a human commensal with ambiguous interactions with the host. Moreover, we also address the impact of antibiotic treatment in sepsis outcome and discuss new insights for microbiota modulation.

Clinical & Translational Immunology (2016) 5, e90; doi:10.1038/cti.2016.32; published online 15 July 2016

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MAMMALIA AND INFLAMMATION: AN OVERVIEW OF MICROBE–HOST INTERACTION

The phrase ‘no man is an island’, exerted from a poem by John Donne, has acquired a whole new meaning in the past decades. The poet meant that no single man survives, prosper and thrive in isolation, without social contact. He was right in a most singular way, we indeed are never alone. In fact, we are always accompanied by an astonishing number of microorganisms that colonize our body surfaces and mucosa. The number of microorganisms living in this consortium is so vast that outnumbers our own cells. There is an unquestionable impact of this microbial community in our physiology, where these microorganisms and its genes contribute for important functions that are not coded in our own genome. The microorganisms inhabiting our body are collectively known as the human microbiota, and it affects how we eat, digest our food, fight diseases, it influences our metabolism and even how we relate to each other. Despite the anthropocentric point of view that we are highly evolved and self-sufficient organisms, research with germ-free animals tells a different story. Our microscopic counterparts are fundamental in every step of our journey through life, and without our microbiota we would not be able to survive in this world.

The impacts of the microbial communities that inhabit our bodies are more noticeable in the gastrointestinal tract (GIT), where the highest amount and diversity of bacteria are found. In the past decades, an increasing number of studies are finding connection between dysbiosis, that is, an imbalance in the composition of bacterial species, of gut microbiota and human diseases, such as inflammatory bowel diseases (IBDs), type 2 diabetes, obesity, allergies and colorectal cancer, but a cause and effect relation is still missing for most of these conditions. It has become evident that the microbiota composition also regulates immune response outside the gut. Alongside, we are beginning to define the composition of the gut microbiota in healthy individuals as well as its variability throughout different stages in life and among different genetic backgrounds. Evidence that environmental factors, particularly diet and antibiotic consumption, may affect the composition of the intestinal microbiota is becoming more prominent. Researchers have known for decades that the intestinal microbiota is primarily composed of anaerobic bacteria, but recent advances in DNA sequencing technologies have established that two major phyla represent 90% of the microbial community in the gut, the Bacteroidetes and Firmicutes. Others phyla found in the GIT are Actinobacteria, Proteobacteria, Verrucomicrobia, Deferrribacteres, TM7, Deinococcus and Fusobacteria.¹ The microbiota of the colon contains a staggering 10¹⁴ bacteria distributed among over 500 species in a single individual. Although the composition of species may vary among individuals, a healthy gut microbiota presents a functionally redundant diversity, meaning that more than one species may have the same metabolic function. This functional diversity confers resilience to our microbiota and helps the maintenance of homeostasis.

Dietary changes have been shown to have significant effects on the composition of the gut microbiota. In particular, an increased Firmicutes:Bacteroidetes ratio is associated with systemic and adipose tissue inflammation and development of metabolic syndromes, such as obesity, insulin resistance and type 2 diabetes.²,³ Studies in animal

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Received 16 February 2016; revised 13 April 2016; accepted 14 April 2016
models that link the gut microbiota with obesity showed that the absence of microbiota prevents the development of obesity in mice fed a high-fat diet, and the colonization of germ-free and antibiotic-treated mice with microbiota from genotypically obese mice induces higher adiposity in the recipient mice. In agreement, human twins discordant for obesity were used as donors to show that human fecal transplantation from obese individuals to germ-free mice also induced obesity. It has been shown that co-housing mice harboring an ‘obese microbiota’ with mice containing the ‘lean microbiota’ prevented the development of weight gain and obesity-associated metabolic syndromes development in obese cage mates. This was likely due to the sharing of the healthy gut microbiota. Indeed, the existence of an obesity-associated gut microbiome with higher capacity to harvest energy from diet and to induce insulin resistance is one of the therapeutic targets of fecal transplantation and probiotics prescription. The gut microbiota protects the host by outcompeting potential pathogens for nutrients and binding sites in the intestinal mucosa, it also induces maintenance of the mucus barrier and regulation of the immune response. These important roles of the gut microbiota are collectively known as ‘colonization resistance’. The microbiota also produces antimicrobial substances, such as bacteriocins, and stimulates Paneth cells in the intestinal epithelium to produce antimicrobial peptides. Studies performed in germ-free mice demonstrated that the intestinal microbiota is essential for the correct development and maturation of the mucosal and systemic immune system. One of the first demonstrations that individual bacteria of the microbiota contribute to immune development came from studies with the human commensal bacteria Bacteroides fragilis (B. fragilis). The monocolonization of germ-free mice with B. fragilis promotes lymphoid organogenesis and CD4+ T cells expansion, which is dependent on expression of a surface polysaccharide by the bacteria. The gut-associated lymphoid tissue (GALT) of germ-free mice does not become fully mature and the numbers of CD4+ T cells, epithelial T cells, dendritic cells (DCs) and IgA producing B cells are diminished. The balance among the population of T helper (Th) cells in these animals is highly skewed toward the Th2 profile, which is associated with the onset of allergic diseases. The healthy balance between effector Th cells is largely controlled by a subset of CD4+ T cells known as regulatory T cells (Treg) To maintain intestinal homeostasis, the mucosal immune system must selectively recognize and respond to pathogenic species while simultaneously maintaining tolerance to harmless and symbiotic members of the intestinal microbiota. Foxp3-expressing Treg represent an important mechanism in the prevention of autoimmune diseases, oral tolerance to dietary antigens, regulation of effector T cells and suppression of the immune response against the microbiota to limit intestinal inflammation. Interestingly, both the number of Treg as well as their ability to suppress inflammation are reduced in germ-free and antibiotic-treated mice, indicating the important role of microbiota to promote Treg development and function. The healthy balance among T helper cells may be restored by the colonization of these animals with commensal bacteria such as Bifidobacterium infantis or, more surprisingly, by the challenge with purified capsular polysaccharide from B. fragilis, which will be addressed in detail in the following section. The induction of colonic regulatory T cells by clusters IV and XIVa of the genus Clostridium has also been largely described. Oral inoculation of some strains of Clostridia from conventionally reared mice and healthy human fecal sample during the early life results in resistance to IBDs and allergies in adult mice. In addition to the active suppression of inflammation, Foxp3+ Treg regulate the diversity of IgA repertoire inside the germinal centers in the Peyer’s patches, influencing on the establishment of bacterial species responsible for immune homeostasis, which in turn facilitate the expansion of Treg. Therefore, the generation of colonic Foxp3+ Treg induced by microbiota is required for intestinal CD4+ T-cell homeostasis as reflected by the healthy balance between effector T-helper cells and Treg during symbiosis. In addition to Treg induced in the periphery, natural (or thymic) Treg can also mediate tolerance to intestinal commensals, and their antigen receptor repertoire is profoundly influenced by the composition of the microbiota, contradicting previous studies claiming that natural Treg recognizes only self-antigens. In the intestinal scenario, the major function of Treg is to dampen Th17 activity thereby facilitating bacterial intestinal colonization and avoiding unwanted inflammation. Although effector Th17 cells contribute to colonization resistance against enteric infections by inducing the recruitment of neutrophils and activation of epithelial cells, leading to clearance of extracellular pathogens, they are also involved in inflammatory diseases. Germ-free mice are presented with reduced numbers of Th17 cells in the small intestine and lamina propria, and emerging studies have pointed for a key role of the commensal microbiota, in particular segmented filamentous bacteria, in the development of intestinal Th17 cells. Apart from their protective role, the pathological role of Th17 cells during intestinal inflammation is well documented. The imbalance between Th17 proinflammatory and anti-inflammatory profile is correlated with higher incidence of IBDs, which includes ulcerative colitis and Crohn’s disease. Dysbiosis between symbionts (commensal bacteria with beneficial potential) and pathobionts (commensal bacteria with pathogenic potential), in combination with increased gut permeability that promotes the exposition to luminal content and antigens from microbiota, trigger a Th17-mediated immune response that fuels intestinal inflammation and development of IBDs. Antibiotics treatment ameliorates IBD symptoms in patients and experimental murine colitis. Furthermore, the microbial community of the gut is significantly altered in IBD patients. Individual members of the intestinal microbiota vary dramatically in their propensity to influence the development of IBDs, and it is difficult to identify potentially disease-driving members of the microbiota. Mice lacking components of the inflammasome, as the adapter protein ASC and NLRP6 receptor, are presented with reduced IL-18 production in the gut and harbor a colitogenic intestinal microbiota dominated by Prevotellaceae species, that can be transmitted to wild-type mice through cohousing, increasing the susceptibility to colitis. Recently, the same group has shown that intestinal bacteria selected on the basis of high coating with IgA conferred dramatic susceptibility to IBD in mice and humans, contributing significantly to the potential identification of colitogenic bacteria. Interestingly, colonization of germ-free mice with highly IgA-coated members of the intestinal microbiota from IBD patients promotes increased susceptibility to colitis. Among colitogenic microbiota induced in the inflammasome-driven colitis, the most abundant highly IgA-coated taxon was an unclassified genus from the Prevotellaceae family, corroborating with the data previously published. Therefore, the microbiota has an active role in the development and function of both pro- and anti-inflammatory T-cell responses, and the suppression of Th1 and Th17 cells by Treg prevents inflammation in experimental models of inflammatory diseases.

MICROBIOTA, SCFAS AND IMMUNOMODULATION

The metabolic activities of the intestinal microbiota have a profound effect on human nutrition and health. Over the past three decades,
SCFAs can modulate cellular functions either through the activation of G-protein-coupled receptors such as GPR41, GPR43 and GPR109A, or by inhibiting histone deacetylases (HDACs). In the last decade, numerous studies have demonstrated the immunomodulatory role of SCFAs, contributing with the regulatory arm of immune system to control intestinal and systemic inflammatory diseases. Butyrate and propionate promote Treg development in the colon by increasing histone acetylation of the Foxp3 locus, or through T-cell-intrinsic signaling via GPR43 receptor, leading to mitigation of colitis development and gut homeostasis. However, SCFAs also ameliorate inflammatory diseases such as colitis, arthritis and asthma.

Changes in microbiota composition and increase of circulating levels of SCFAs induced by high-fiber diet are also related to protection against respiratory airways inflammation. Dietary fiber drives bone marrow hematopoiesis altering the dendritic cell ability to promote Th2 response, and its metabolite propionate reduces allergic inflammation in the lung by signaling through GPR41 receptor. Acetate also has an important immunomodulatory role during experimental asthma, promoting acetylation at Foxp3 promoter through HDAC9 inhibition, leading to an increase of Treg suppression of allergic airways disease. This protection can be transferred to the offspring if the intake of high-fiber diet occurs during the pregnancy. In addition to a direct effect on Treg development and function, SCFAs can also shape macrophage and dendritic cell function. Butyrate reduces the proinflammatory phenotype of colonic macrophages and dendritic cells, through mechanisms mediated by histone acetylation and GPR109A, respectively, and renders them hyporesponsive to bacteria from microbiota and capacity to induce mucosal tolerance. Therefore, the production of immunomodulatory metabolites by microbiota is an important mechanism either for maintenance of intestinal homeostasis, contributing to the host-microbe mutualism, and for control of systemic inflammatory diseases.

In following sections, we will discuss the simultaneous role of gut microbiota in the maintenance of symbiosis and the establishment of extraintestinal infection. We will focus on B. fragilis, an important member of microbiota with many physiological (inside the gut) and pathological (outside the gut) functions during the microbiota–host interaction.

**B. FRAGILIS: THE LIGHT SIDE AND THE DARK SIDE OF THE FORCE**

### Polysaccharide A and its immunomodulatory potential

Both symbiotic and pathogenic bacteria express a redundant array of molecular patterns, collectively known as MAMPs (microbe-associated molecular patterns). The mechanisms by which our pattern recognition receptors, including Toll-like receptors (TLRs), distinguish between the commensal microbiota to maintain homeostasis, and enteric infections to trigger an effector response, is becoming clearer. In the last decade, many authors have described the production and expression of immunomodulatory molecules by the gut resident bacteria, which are important for the establishment of tolerance in symbiosis and protection against IBDs. *Bacteroides* species are among the earliest-colonizing and numerically prominent constituents of the gut microbiota in mammals. Although present in very small numbers, *B. fragilis* is a ubiquitous and important Gram-negative anaerobe that colonizes the mammalian lower gastrointestinal tract. *B. fragilis* expresses, among other molecules, a capsular polysaccharide complex (CPC) composed of a combination of polysaccharides (PS) coded by different biosynthetic regions in the bacterial genome. A single strain may code several CPC biosynthetic loci that are modulated by reversible phase variation in an ‘on’ and ‘off’ manner, allowing multiple combinations of different PS that improve evasion of the immune system and favors persistence of infection. The PS molecules have a peculiar characteristic; they harbor both positive and negative surface charges in the sugar repeating units conferring a zwitterionic nature that provides exceptional biological and immunomodulatory functions. Among polysaccharides of *B. fragilis*, polysaccharide A (PSA) is the most abundantly expressed and well-characterized molecule with immunomodulatory properties, contributing both to the establishment of gut homeostasis and the development of peritonitis and sepsis (Figure 1).

The first evidence of a symbiotic bacterial molecule that coordinates anti-inflammatory responses essential for the host health comes from *B. fragilis* studies. PSA-expressing bacteria protects from colitis induced by the pathobiont *Helicobacter hepaticus* through a functional requirement of IL-10-producing CD4+ T cells and suppression of IL-17 production by intestinal immune cells. Monocolonization of germ-free mice with *B. fragilis* induces Foxp3+ Treg development in the colon and increases their suppressive capacity through intrinsic Toll-like receptor 2 (TLR2) signaling by PSA. Accordingly, PSA is unable to protect TLR2-deficient mice from experimental colitis. The essential contribution of PSA is highlighted in studies using PSA-deficient *B. fragilis*, which results in defective colonic tissue colonization due to the inability to restrain Th17 responses. More recently, it was shown for the first time that PSA is involved in the...
induction of Foxp3⁺T<sub>reg</sub> expressing the ecto-ATPase CD39 in human CD4⁺ T cells.37

Acting together with CD73, CD39 prevents inflammation by converting proinflammatory extracellular ATP into anti-inflammatory adenosine. The high expression of CD39 is one of the mechanisms by which T<sub>reg</sub> play their suppression function.38 Treatment with B. fragilis abrogates encephalomyelitis autoimmune experimental development, a model of multiple sclerosis, and this effect depends on PSA expression.39 In line with this, purified PSA from B. fragilis prevents central nervous system (CNS) demyelination and inflammation by inducing T<sub>reg</sub> expressing CD39 in a TLR2-dependent manner, consistent with findings observed in colitis.40 This polysaccharide is also able to shape the migratory patterns of IL-10-producing CD39⁺ T<sub>reg</sub>, increasing their numbers in the CNS and attenuating the inflammatory response during encephalomyelitis autoimmune experimental. Absence of CD39 expression impairs accumulation of T<sub>reg</sub> and promotes elevated Th1/Th17 response in the CNS.41 In summary, these data indicate that PSA-expressing B. fragilis modulates both intestinal and extra-intestinal inflammation, which leads to autoimmunity.

In addition to the well-described anti-inflammatory functions of PSA, the uptake of this polysaccharide by antigen presenting cells (APCs) results in its processing and presentation via MHC class II molecules, leading to recognition by naive CD4⁺ T cells.42 This antigenic presentation is mediated by IFN-γ-producing Th1 cells in a TLR2-dependent proinflammatory manner, demonstrating a critical role of B. fragilis during peritonitis and intra-abdominal sepsis.43 The duality of proinflammatory versus anti-inflammatory effects of the PSA can be explained by differences in its localization (intestinal mucosa versus peritoneum) and the availability of molecules with adjuvant properties. Therefore, this unique polysaccharide possesses the capacity to elicit dichotomous T-cell responses through TLR2 activation (i) intrinsically on CD4⁺ T cells, leading to T<sub>reg</sub> development and suppressive function, and (ii) directly on APCs to promote IL-12 and IFN-γ production and to drive Th1 profile by increasing co-stimulatory molecules expression.36,43 This proinflammatory arm induced by PSA from B. fragilis will be discussed in the next section, where we intend to focus on pathogenic role of B. fragilis outside the gut, as the major causative agent of peritoneal infection.

**B. fragilis in the extraintestinal environment: lessons from peritonitis and intra-abdominal sepsis**

Despite its protective role, the bacteria that constitute our gut microbiota may be involved in serious pathogenic processes. In cases where the epithelial barrier of the gut is disturbed and breached, commensal bacteria escape the gut lumen, invade the peritoneal cavity and cause peritonitis. Peritonitis is the inflammation of the peritoneum, a membrane that lines the inner wall of the abdominal cavity. In an effort to stop bacterial spread, abscesses develop in the peritoneal cavity.35 Several diseases are involved in epithelial rupture, contributing to the development of peritonitis. The most commonly observed are abdominal infections, peptic ulcers, IBDs, appendicitis and diverticulitis. Post-surgical trauma or medical intervention, such as peritoneal dialysis and colonoscopy may also lead to intestinal perforation.45,46 Peritoneal infection is usually caused by a mixed infection containing two or more species of aerobic and anaerobic intestinal bacteria that act in synergy, although infections caused by a single agent were also observed.47 Studies using antimicrobial drugs specific for aerobic or anaerobic bacteria demonstrated that colonic anaerobic bacteria, such as B. fragilis, were responsible for abscess formation in animal infection.48 In fact, the species B. fragilis is recognized among the strict anaerobes as the most common cause of infections in humans.49 Infections caused by this pathogen include intra-abdominal sepsis and pelvic infections, intraperitoneal abscesses, hepatic bacteremia and endocarditis.49

Interestingly, B. fragilis is present in small amounts in the colonic microbiota and it is estimated that only 0.5% of the Bacteroides species in the gut are B. fragilis.50 Several virulence factors have been described in B. fragilis that account for its virulence and predominance in infections. The CPC bestows the bacteria with anti-phagocytic activity and is involved in adhesion to mesothelial cells of the peritoneum.51 In an elegant study, intra-peritoneal abscesses were induced by direct inoculation of gelatin shells containing capsulated strains of B. fragilis to the pelvic region of rats, whereas uncapsulated strains were unable to induce abscess formation unless these were accompanied by another abscess-inducing species such as Enterococcus spp.52 Administration of heat-killed capsulated strains of B. fragilis or even the purified capsule also induces abscess.52 These results clearly demonstrated the importance of B. fragilis capsule in abscess formation and paved the way for a series of studies uncovering the role of B. fragilis capsule in abscess formation. Despite this, the molecular and cellular mechanisms underlying abscess development, as well as host responses controlling the disease process, are not completely elucidated.

Studies using athymic or T-cell-depleted mice show that T lymphocytes are required for the initial induction of host responses leading to the intra-abdominal abscesses development.53 As described earlier, the zwitterionic polysaccharides that compose the CPC, such as PSA are processed into low molecular weight antigens in APCs and presented on class II MHC proteins for CD4⁺ T cells.54 The PSA is depolymerized into smaller carbohydrates fragments through the action of reactive nitrogen species generated by nitric oxide synthase.54 Further investigation concluded that the zwitterionic motif is not necessary for entry into APCs or processing, but it is essential for MHCII binding and subsequent CD4⁺ T cells activation.55 The CD28–CD86 co-stimulatory pathway was also shown to be required for proper T-cell activation and intra-abdominal abscesses development.56 In peritonitis, the recognition of PAMPs that are expressed by intestinal bacteria via pattern recognition receptors in presenting cells act in an adjuvant manner to induce higher expression of co-stimulatory molecules in APCs. An example of this is the recognition of PSA by TLR2 on dendritic cells.43 As a consequence, activation of T cells by PSA via dendritic cells stimulates the production of several proinflammatory cytokines, including IL-2, IL-12, IL-17, IFN-γ and TNF-α as well as chemokines involved in neutrophil recruitment, contributing to abscess formation.43,44

Purified CPC from B. fragilis was shown to confer protection against abscess formation and bacteremia in intra-abdominal sepsis caused by intraperitoneal injection of viable B. fragilis.57 Moreover, protection is transmitted to naive mice through transfer of splenic T cells, but not by serum antibodies specific for B. fragilis CPC, showing the importance of cellular response to PS antigens.58 Later, it was proven that the protection offered by PSA-experienced T cells is associated with a subtype of CD4⁺ T cells that produce IL-10 and present suppressive function, which we now recognized as Foxp3⁺ T<sub>reg</sub>.59 Thereby, colonization with B. fragilis, or treatment with purified PSA, in the absence of an adjuvant can result in exactly the opposite response in intra-abdominal sepsis by limiting or preventing inflammatory responses, implying a potential role of this immunomodulatory molecule in the treatment of many inflammatory disorders, such as IBDs.

Murine and human mononuclear leukocytes, including macrophages and dendritic cells, respond to B. fragilis or purified PSA
in vitro by producing proinflammatory cytokines, including TNF-α, IL-6, IL-1β, IL-8 in a TLR2-dependent manner. These events can contribute to the recruitment and accumulation of leukocytes in the infection site.43,58,59 Peritoneal macrophages stimulated by CPC produce TNF-α and IL-1β, which in turn induce the expression of the intercellular adhesion molecule-1 (ICAM-1) by mesothelial cells in the peritoneum. The upregulation of ICAM-1 induces the mesothelial cells to become an appropriate platform for adhesion of polymorphonuclear leukocytes, an important step in abscess formation.60 In addition, mesothelial cells produce inflammatory cytokines and chemokines in response to B. fragilis, which is reduced in the absence of TLR2 expression.61 As observed for PSA, the recognition of LPS from B. fragilis, an atypical lipopolysaccharide when compared with other Gram-negative bacteria, is absolutely dependent on TLR2.61 In line with this, TLR2−/− mice exhibit impaired intra-abdominal abscess development in response to challenge with B. fragilis.43 The widely used model of murine peritonitis includes intra-peritoneal inoculum of B. fragilis (or purified PSA) in combination with a potentializing agent with adjuvant properties, usually sterile cecal content (SCC), which elicits a strong inflammatory response that fuels intra-abdominal sepsis and abscess development.62,63 The use of SCC mimics the extravasation of colonic contents from the intestine into the peritoneal cavity, but the exact contribution of the potentializing agent remains to be understood. Unpublished results from our group have shown that the presence of fiber in the SCC is essential for NLRP3 inflammasome activation and IL-1β production by macrophages and dendritic cells. In addition, NLRP3−/− and IL-1R−/−, but not IL-1R−/− mice were protected from abscess development after challenge with B. fragilis in combination with SCC, and that the same phenotype was observed when the challenge included SCC obtained from mice fed on zero-fiber diet (Pandini et al., manuscript in preparation). These results are in agreement with previous studies showing the contribution of bran, in place of SCC, as potentializing agent in the abscess induction by B. fragilis.63 Collectively, these data suggest that SCC including dietary fiber provide inflammatory signals, which function as a danger signal outside the gut fueling inflammation during peritonitis.

**MICROBIOTA BEHAVIOR AND SYSTEMIC SEPSIS**

Sepsis is defined as a systemic inflammatory response to infection and has been 1 of the top 10 causes of death worldwide, with an overall mortality rate of 20–30% in USA.64 In developing countries like Brazil, the mortality rate is still higher at 40–60%.65 The term sepsis refers to sepsis complicated by acute organ dysfunction, and septic shock is defined when sepsis is further complicated either by hypotension refractory to fluid resuscitation or by hyperlactatemia.66,67 Despite the fact that nowadays the scientific community has reasonable knowledge about the physiopathology of sepsis, the treatment still consist of antibiotics, fluids resuscitation/vasoconstrictors and supportive care.68 The several attempts of treatment with drugs or antibodies directed to inflammatory molecules or to bacterial components failed in the clinical trials. Moreover, the lack of a successful treatment for sepsis so far aggravates its long-term complications. Even though the mortality rate has decreased in USA, the incidence of sepsis has increased, which leads to a great number of survivors suffering from potential long-term effects. Even more worrying is that several works have demonstrated that 80% of septic survivors die within 5 years from lung infections, cancer and cardiovascular diseases,69,70 or otherwise these patients have poor quality of life and long-standing physical and intellectual wounds.64,65,67 These morbidity and mortality trends continue for at least 15 years after discharge.72

There is no doubt that antibiotic therapy is critical to rescue patients from death, but it is also fundamental to have in mind that the long-term complications of sepsis treatment has poor prognosis. This section aims to bring forward discussion on the impact of antibiotic intervention, dysbiosis and immunosuppression following severe sepsis. There are few data in the literature about this subject and lots of questions about the microbiota impact on acute sepsis and on its complications.

**MICROBIOTA AND ANTIBIOTIC THERAPY DURING SEPSIS**

It is expected that critical illness, such as sepsis, and its interventions of intensive care (as enteral feeding, proton-pump inhibitors, systemic catecholamines and antibiotics) significantly alter the microbiota. The modifications in the microbiota during sepsis may induce susceptibility to several diseases, and also can be the cause of immunosuppression following sepsis.73 However, few studies address this issue so far. The dysbiosis following sepsis aggravates the multiple organ failure and increases the risk of death. The diversity and the balance among the bacterial phyla are critical for a healthy microbiota. Under physiological conditions, the community composition is regulated by three intrinsic factors: the immigration of bacteria into the community via the oropharynx, elimination of the bacteria by feces, and the proliferation rate of the community’s members. All these three processes are altered in septic patients to result in a gut ecology that has a lower diversity community and an overgrowth of a small number of species. It promotes an accumulation of bacteria in the stomach and gut such as E. coli, Pseudomonas aeruginosa, Enterococcus spp., Staphylococcus aureus and Klebsiella spp., which in turn may drive bacteria translocation, extra-abdominal infection and multi-organ failure. Therefore, due to sepsis and its clinical interventions, the intestinal bacteria condition is significantly altered, leading to dysbiosis which directly impact on the immune response balance73,74 (Figure 2).

In an interesting study that supports the statements above, the authors demonstrated that perinatal antibiotic treatment increases the risk of sepsis in neonatal mice. The mechanism seems to be an increase in neutrophils in the blood shortly after birth induced by microbiota.75 This study suggested that the epithelial cells in the gut lumen recognize the LPS from gut bacteria through TLR4 and the activation of this receptor triggers the release of IL-17 by lymphoid-like cells in the lamina propria. This cytokine induces the release of granulocyte colony-stimulating factor, which in turn triggers granulopoiesis in the bone marrow and the release of neutrophils in the blood. When neonatal mice were treated with clinically relevant antibiotics, bacterial diversity was reduced and hindered the agranulocytosis. These mice were more susceptible to sepsis, as the number of neutrophils to eradicate the infection was severely reduced. Therefore, the microbiota is critical in host defense as it primes neutrophils to protect the host against infections and late-onset sepsis.75

**COMMENSAL LIFESTYLE VERSUS PATHOGENIC LIFESTYLE**

An important point is when and how the microbiota turns to be hostile—some commensal bacteria seem to shift their behavior to virulent pathogens after an episode of sepsis. In this situation, the gut is exposed to several factors as antibiotic, physiological stress and opioids, leading to low-diversity microbiota, as we stated before. As a consequence, bacteria that had a commensal lifestyle when grouped together, in the low-diversity scenario they shift to pathogenic lifestyle, characterizing dysbiosis.74,76 In addition, it was also postulated that the local environmental condition drives the lifestyle of the bacteria.
Bacteria sense the amount of resources in the gut and also produce energy products for epithelial cells that line the colon. Butyrate from bacteria metabolism in the gut induces T\textsubscript{regs} development and function, which maintains the mucosal tolerance, as mentioned earlier. On the other hand, butyrate is also critical for the function/metabolism of the epithelial cells. During critical illness the butyrate-producing bacteria are almost undetected, which causes epithelial cells death, lost of mucosal tolerance, bacteria translocation, and consequently abdominal and extra-abdominal infection.\textsuperscript{73}

Another important substrate for the bacterial community is phosphate. Bacteria can sense the abundance or absence of phosphate-containing energy sources. During sepsis, it is possible to observe a low level of phosphate in the low-diversity pathogenic lifestyle community. Thus, it has been postulated that the abundance of phosphate, an important substrate for bacterial growth and proliferation, can revert bacteria virulence and impair the shift to pathogenic lifestyle of certain bacteria. This effect is observed even in the presence of endogenous stress mediators (such as products of ischemia, inflammatory mediators, and so on). Therefore, phosphate-containing compounds could be a novel strategy for prevention of critical illness such as sepsis and its long-term complications.\textsuperscript{75}

Taken into account all of the data described above, it is unequivocal that hospitalization, antibiotic therapy, opioids and all source of intervention for critical illness contribute to dysbiosis. Dysbiosis in turn provides a series of circumstances that promote a low-diversity community in the gut, a shift to pathogenic lifestyle of this community, nutrient deprivation and an imbalance of the immune response marked by a shift for inflammatory T cells (as Th17) instead of T\textsubscript{regs} \textsuperscript{74} (Figure 2). All of these events are already evident during sepsis, mainly during severe sepsis and septic shock.

**CORRELATION BETWEEN IMMUNOSUPPRESSION FOLLOWING SEVERE SEPSIS AND MICROBIOTA ALTERATION**

The long-term complications following severe sepsis are becoming apparent in the last decade, although it is still poorly understood. Recently, with the growing knowledge about the role of the microbiota on homeostasis and on several diseases, clinical studies on sepsis are examining possible explanations for the immunosuppression based on severe sepsis-induced dysbiosis. There are some data in the literature supporting this idea and our group has strong evidence of cellular reprogramming after severe sepsis and antibiotic therapy. Mice subjected to severe sepsis by cecal ligation and puncture surgery, and treated with antibiotic are more susceptible to Aspergillus fumigatus challenge, resulting in ~80% mortality.\textsuperscript{78} Our group and others have shown that the immune cells, such as dendritic cells, T\textsubscript{regs} and neutrophils, from antibiotic-treated septic mice were presented with altered activation, leading to an increase of host susceptibility for secondary infection and multi-organ dysfunction.\textsuperscript{78–80} Our observations suggest that sepsis targets such as lungs, kidneys and peritoneal cavity, were also posed with an altered inflammatory/immune response profile, which is deleterious to a secondary insult, and this fact may be contributed by the dysbiosis following sepsis.

In conclusion, dysbiosis induced by antibiotic treatment during sepsis could be one of the causes of the long-term complications. Although the antibiotic therapy is critical to rescue the septic patient from the first insult, the late complications in the microbiota composition/behavior were never taken into account. We believe that the normal microbiota restoration will be a promising intervention for septic patients, preventing the morbidity and the high mortality still observed in these patients that survive the acute episode of sepsis. It is still too early to state, but the fecal microbial transplantation treatment has been demonstrating success to reconstitute the gut microbe...
community of patient with recurrent *C. difficile* infections and against severe sepsis (case reports).\(^1\),\(^2\) The fecal microbial transplantation may function as a co-adjuvant treatment, and in combination with a restricted antibiotic therapy for sepsis may present as a novel intervention to reduce the long-term complications observed after sepsis and antibiotic prescriptions.

**CONCLUDING REMARKS**

In this review, we intended to discuss how T-cell responses in the mucosa driven by the microbiota communicate with the peripheral immune system. We also discussed the inflammatory response against the microbiota when it leaves its natural habitat of the gut lumen and become opportunistic pathogens (for example, periodontitis and sepsis), as well as the microbiota behavior during the systemic infection.

Due to obvious technical difficulties, most of the studies elaborated to evaluate the effect of bacteria in the inflammatory response are based on infection models using a single, or a few, etiologic agents. In reality, our immune system is constantly exposed to a myriad of bacterial species, along with their products and metabolites, and these relationships have been selected through millions of years of natural selection. Our gut immune system has evolved not only to allow the colonization of an enormous amount of microorganisms, but has become inherently dependent on this colonization for proper maturation and development. As mentioned above, imbalances in this environment is a source of serious chronic diseases. The manner of initial colonization upon delivery (vaginal delivery when compared with a cesarean section), exposure to probiotics, antibiotic consumption during life and adopted diet patterns have direct implications in susceptibility to many inflammatory diseases, including asthma, IBDs, autoimmunity, metabolic syndromes among others.

Next-generation sequencing technologies have allowed us to probe into the composition of the microbiota as never before, although most of these species remain uncultured. Moreover, the functional redundancy in the species that compose the microbiota create great diversity and the result is that a microbiota common to every human being has not been described, and some researchers believe that it will never be. However, as is enumerating bacterial species in the gut, the identification of how these bacterial species crosstalk with our immune system in a healthy manner and contribute to homeostasis is of utmost significance.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**ACKNOWLEDGEMENTS**

We thank Tyler Lieberthal for the help with figures.

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