Editorial

Gut Microbiota and Intestinal Decolonization of Pathogenic Microorganisms

De-Chang Chen

Department of Emergency and Critical Care Medicine, Shanghai Changzheng Hospital, Second Military Medical University, Shanghai 200003, China

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The occurrence of infections and mortality in critically ill patients in Intensive Care Units (ICUs) are as high as 53.9% and 30.0%, respectively.[1] In the United States, the number of patients who died from hospital-acquired infections exceeds 100,000 and the single-item cost is as high as 25 billion USD[2] per year. A study has demonstrated that colonization of conditional exogenous pathogens within the gut is an important pathologic basis of hospital-acquired infections in critically ill ICU patients.[3] The gut is inhabited by the largest variety of bacteria. On one hand, commensal bacteria can inhibit intestinal colonization of conditional exogenous pathogens, and on the other hand, intestinal bacterial flora plays important roles in immunity, metabolism and nourishment. Here, we address the composition of intestinal bacteria, the mechanism underlying decolonization of pathogens in the gut, change in the intestinal micro-ecological environment under pathologic conditions, and maintenance of the ability of the gut in decolonizing pathogenic microbiota.

Intestinal Microbiota

The gut is inhabited by 100 trillion (10^{14}) microbiota, including bacteria, fungi, viruses and protozoa. The European Union Project on Metagenomics of the Human Intestinal Tract collected 124 intestinal bacterial flora specimens from Europeans and conducted a deep sequencing on them by using the illumina-based metagenomic sequencing technique, which produced 576.7 gigabases of sequence. Based on this gene set, it is estimated that there are about 1000–1150 species of microbiota in the human gut.[4] According to their functions in the human body, they are classified into three types: (1) symbiotic bacteria or probiotics, which are intestinal-dominant bacteria mainly composed of strict anaerobic bacteria such as lactic acid bacteria, bifidobacteria, bacteroides, eubacteria, and nitrobacteria. They exist constantly and in large numbers in the host lifetime without causing harm to the host; rather they play physiological roles in nourishment, digestion, absorption, biologic antagonism and immunity,[4] as well as in maintaining health of the host. (2) Commensal bacteria, which are bi-directional, such as *Escherichia coli* (*E.coli*), enterococci and lactobacilli. On one hand, they have certain physiological functions, and on the other hand, they are pathogenic by increasing decaying and carcinogenic substances and toxins when reaching a certain quantity. (3) Passenger bacteria, most of which are pathogenic, such as proteobacteria and pseudomonas aeruginosa. They are not pathogenic when the amount is relatively small under a balanced micro-ecological environment, but may transform to pathogenic bacteria, in case the intestinal flora is imbalanced.

Mechanisms Underlying Intestinal Pathogen Decolonization

Intestinal symbiotic bacteria, also known as membrane flora, usually refer to those colonized on the mucosal surface of intestinal mucosal epithelial cells. They are important barriers to prevent pathogens from colonizing on the intestinal mucosa and occurrences of adhesion, invasion and gut-derived infections. The mechanisms of these effects

Address for correspondence: Prof. De-Chang Chen, Department of Emergency and Critical Care Medicine, Shanghai Changzheng Hospital, Second Military Medical University, Shanghai 200003, China
E-Mail: 18918520002@189.cn

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lie in their ability to express mucus-binding pili. These pili bind with mucus-binding protein expressed by intestinal mucosal epithelial cells, thus producing a mass effect by permanently attaching and colonizing on epithelial cells.[5] This effect can produce a powerful resistance to colonization of pathogenic microbiota. In addition, by competing with nutrients, they prevent pathogenic microbiota from growth and reproduction in large quantities by secreting antibacterial substances.[6,7] Furthermore, the mucus secreted by goblet cells in the intestinal mucosa forms a relatively thick mucous layer on the surface of intestinal mucosal epithelial cells, which is an important platform for interactions between symbiotic bacteria and intestinal mucosal epithelial cells, and also plays a role in preventing adhesion of pathogenic microbiota onto epithelial cells.[8] The pathologic foundation for enteric bacterial translocation and gut-derived infections no long exists if conditional exogenous bacteria cannot attach and colonize to intestinal mucosal epithelial cells, and therefore the decolonizing mechanism of the gut itself is very important in protecting the human body against the invasion of pathogenic bacteria.

Enteric decolonization of conditional exogenous bacteria can also be achieved by direct or indirect immune mediation. Symbiotic bacteria-derived molecules can provide the intestinal immune system with basic immune stimulation, thus enabling the system to be in a pre-excited state so that it can provide sufficiently strong immune response to decolonize pathogenic bacteria once they invade the gut and organs. For instance, NOD2-dependent responses to bacterial peptidoglycan fragments can promote Paneth cells to express antimicrobial cryptdin peptides.[9] Intestinal symbiotic bacteria can also induce Paneth cells and intestinal mucosal epithelial cells to express regenerating islet-derived protein IIIγ, which is a C-type lectin that binds to peptidoglycan to kill Gram-positive bacteria in the gut.[10]

Another important function of the intestinal immune system is to distinguish between enteric symbiotic bacteria and invading pathogenic microbiota, thus conferring immune tolerance on enteric symbiotic bacteria and immune response to exogenous pathogenic bacteria by decolonizing them. This function is usually achieved by pattern-recognition receptors (PRRs) on the surface of immune effector cells. They kill invading pathogenic microbiota by identifying pathogens of these pathogenic microbiota, completing signal transduction and inducing immune response.[11,12] Of the PRRs, toll-like receptors (TLRs) are mostly studied. Different subsets of TLRs can identify different types of pathogens. For instance, TLR2 mainly identifies lipoprotein and yeast; TLR4 mainly identifies lipopolysaccharide; and TLR5 can identify flagellin.[13] Activation of TLRs can induce dendritic cells to capture enteric pathogenic bacteria and process them to antigens and present them to lymphocytes to induce B-lymphocytes to secrete IgA to the surface of the intestinal mucosa, thus preventing pathogenic microbiota in the intestinal lumen from penetrating through the intestinal mucosal barrier.[14,15] $CD4^+$ T helper 17 (Th17) cells in the lamina propria can also prevent pathogenic microbiota in the intestinal cavity from attaching to intestinal epithelial cells.[16] The immune response specific to decolonization of pathogenic microbiota in the intestinal mucosa is regulated by immune regulatory T-cells to avoid injury to the intestinal mucosa due to excessive inflammatory response.[17]

Alteration in the Ability of Decolonization of Intestinal Microbiota Under Pathologic Conditions

The use of broad-spectrum antibiotics under pathologic conditions can kill large amounts of symbiotic bacteria. As a result, the membrane barrier is lost, the mass effect is weakened, and the ability of intestinal symbiotic bacteria against colonization of exogenous pathologic bacteria is lost. In a burn-endotoxin two-hit model,[18] the author fed the animals with the target bacterial strain *Klebsiella pneumoniae* (*K. pneumoniae*) carrying the drug-resistant gene SHV-18 and used antibiotics to intervene the animal model. The result showed that the original *E. coli* in the animal feces disappeared 2 days after antibiotic intervention, whereas the *K. pneumoniae* strain was still detectable even 4 weeks after discontinuation of the antibiotics. No *K. pneumoniae* strain was detected in the feces of the control group 2 days after administration of the target strain. In addition, the *E. coli* in the gut underwent drug resistance change 10 days after antibiotic intervention. The result of drug resistance gene detection showed that this gene came from the exogenous target strain *K. pneumonia*. This study indicated that the healthy gut has a decolonization function against exogenous pathologic microbiota, and that the use of antibiotics could induce or aggravate intestinal flora disturbance, thus enabling conditional exogenous pathogenic bacteria to colonize in the gut permanently and inducing drug resistance under the pressure of antibiotics, which causes drug-resistant genes to spread widely in the gut.

Other clinical pathologic factors can also cause intestinal flora disturbance and reduce the decolonization ability of the gut, such as parenteral nutrition and the use of parenteral proton-pump inhibitors (PPIs).[19]

Recovery of the Intestinal Colonization Ability

The use of broad-spectrum antibiotics in critically ill patients is the main reason for destruction of the intestinal membrane barrier and decreased ability of decolonization. A report presented at *Orlando Digestive Disease Week* in May 2013 pointed that short-term use of antibiotics could destruct membrane flora and markedly increase the load of pathogenic bacteria on the surface of the intestinal mucosa in mice.[20] Therefore, the principle of antibiotic therapy in critically ill patients should be appropriate initial treatment and de-escalation by using narrow spectrum of antibiotics whenever possible, and optimizing the antibiotic course of treatment for the sake of protecting the intestinal membrane barrier to the best.

Some recent studies showed that fecal microbiota transplantation (FMT) was effective for the treatment of intestinal infections due to intestinal flora alteration. There is a history of more than 1700 years in China about the use of diluted feces from healthy persons to treat intestinal infections.[21] In recent years, many countries use FMT to treat refractory or recurrent *Clostridium difficile* infection that does not respond to antibiotics with a curative rate of 90%.[3] The standard treatment of FMT can be implemented through upper digestive tract infusion or rectal perfusion. The purpose of FMT from healthy persons is to recover the healthy intestinal flora of the patient, enhance the ability of the gut in decolonizing exogenous pathogenic microbiota, and eliminate the pathologic foundation of enterogenic infections.

As there may also pathogenic bacteria existing in healthy persons, it is possible to import conditional exogenous microbiota to the gut of the patient through FMT. Some studies in recent years were also tried using symbiotic bacteria such as *Bifidobacterium* or *Lactobacillus* to treat intestinal flora alteration with fairly good outcomes. Some researchers isolated ten taxonomies of nonpathogenic symbiotic bacteria from healthy individuals and used them to treat refractory or recurrent *C. difficile* with the same good effect.[22]

Roy *et al*.[23] used *Bifidobacterium infantis*, *Lactobacillus*, and *Bifidobacterium lactis* to treat premature low-weight infants. The result showed that the infection rate in the study group was significantly lower than that in the placebo group. In addition, the length of hospital stay in the infants in the study group was significantly shorter and breast feeding was initiated significantly earlier as compared with the infants in the control group.[23]

Some studies have demonstrated that enteral nutrition has a great impact on intestinal microbiota. Animal experiments showed that starvation could decrease the amount of *Lactobacillus* and other membrane flora in the mouse gut. Another mouse model showed that parenteral nutrition for 6 days could alter the micro-ecological environment in the small intestine by increasing the amount of proteobacteria and bacteroidetes markedly, and decrease the amount of firmicutes dramatically.[24] Similar results were also obtained in other animal models. Firmicutes are symbiotic bacteria that have a powerful ability of decolonization on conditional exogenous microbiota. Parenteral nutrition or starvation can decrease the number of firmicutes in the gut, thus weakening its decolonization ability. Therefore, enteral nutrition should be initiated as early as possible in critically ill patients provided the anatomic conditions of the gut permit. Many studies have demonstrated that early enteral nutrition can decrease infective complications markedly.[20,25]

Gastric acid is a natural bactericide that can clear off bacteria enter the stomach from the oral cavity. Under pathological conditions, PPIs or H2 receptor antagonists (H2RAs) can increase the pH value in the stomach, thus weakening or depleting the antimicrobial ability of the gastric juice. As a result, bacteria reproduce in large quantities in the stomach and jejunum.[20] To ensure the antimicrobial ability of gastric fluid, PPIs or H2RAs are not clinically recommended unless there is stress ulceration with hemorrhage or the existence of high-risk factors that may induce stress ulceration.

Conclusions

An important pathologic basis of hospital-acquired infections in critically ill ICU patients is the colonization of conditional exogenous pathogens within the gut. The gut is inhabited by the largest variety of bacteria. Commensal bacteria can inhibit intestinal colonization of conditional exogenous pathogens, and intestinal bacterial flora plays important roles in immunity, metabolism, and nourishment.

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