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Impact of bacterial DNA in severity of sepsis

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Impact of bacterial DNA in severity of sepsis

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ผลกระทบของดีเอ็นเอของแบคทีเรียต่อความรุนแรงของภาวะติดเชื้อในกระแสเลือด

น.ส.วรีรัตน์ แก้วดวงเดือน

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การพบดีเอ็นเอที่ปราศจากแบคทีเรียในเลือดระหว่างภาวะติดเชื้อ อาจเกิดได้ทั้งจากการตายของแบคทีเรียในเลือดและการเคลื่อนย้ายของดีเอ็นเอสายสั้นนอกตัวเชื้อแบคทีเรียผ่านกำแพงลำไส้ นอกจากนี้ การมีอยู่ของดีเอ็นเอของแบคทีเรีย ร่วมกับโมเลกุลของลิโปโพลีแซคคาไรด์ (LPS) จากผนังเซลล์ของเชื้อแบคทีเรียแกรมลบ ยังอาจนำไปสู่การอักเสบที่มากเกินควรในระหว่างการติดเชื้อ ดังนั้น คณะผู้วิจัยจึงทำการทดลองในสัตว์ทดลองและเซลล์มาโครฟาจเพื่อสำรวจอิทธิพลของดีเอ็นเอที่ปราศจากแบคทีเรียในสภาวะการติดเชื้อในกระแสเลือด ผลการทดลองพบว่าสาสารถตรวจพบดีเอ็นเอที่ปราศจากแบคทีเรีย (bacterial free DNA) ในเลือดของหนูทดลองในโมเดลภาวะการติดเชื้อโดยการผูกแล้วเจาะลำไส้ (cecal ligation and puncture) และ การฉีดดีเอ็นเอที่สกัดจากแบคทีเรียในหนูทดลองร่วมกับการฉีด LPS พบว่าเกิดการอักเสบรุนแรงกว่าการฉีด LPS เพียงอย่างเดียวโดยพิจารณาจากระดับไซโตไคน์ TNF-α, IL-6 และ IL-10 ในกระแสเลือด นอกจากนั้นการกระตุ้นเซลล์มาโครฟาจด้วย ดีเอ็นเอที่ปราศจากแบคทีเรีย ร่วมกับ LPS พบว่าเกิดการตอบสนองมากกว่าการกระตุ้นด้วย LPS เพียงอย่างเดียว โดยพิจารณาจากการเพิ่มขึ้นของไซโตไคน์ TNF-α, IL-6 และ IL-10 ในน้ำเลี้ยงเซลล์รวมถึงการแสดงออกของยีนที่ก่อให้เกิดการอักเสบที่เพิ่มขึ้น (*iNOS* และ *IL-1*β) ในเซลล์มาโครฟาจ ผลการทดลองสนับสนุนว่ามีการกระตุ้นร่วมกันระหว่างดีเอ็นเอที่ปราศจากแบคทีเรียและ LPS อย่างน้อยผ่านทางการกระตุ้นเซลล์มาโครฟาจ ในระหว่าการติดเชื้อในกระแสเลือดซึ่งอาจจะนำไปสู่การอักเสบที่รุนแรงมากยิ่งขึ้น กล่าวโดยสรุปดีเอ็นเอที่ปราศจากแบคทีเรียในหนูที่เกิดภาวะติดเชื้อในกระแสเลือดนั้นเกิดขึ้นได้จากการตายของแบคทีเรียในเลือดและการเคลื่อนย้ายของดีเอ็นเออิสระจากลำไส้ ซึ่งทำให้เกิดการอักเสบที่รุนแรงขึ้น ข้อมูลเหล่านี้จึงสนับสนุนความสำคัญของดีเอ็นเออิสระในเลือดระหว่างภาวะติดเชื้อในกระแสโลหิตที่อาจจะนำไปสู่การวิจัยเพิ่มเติมต่อไป

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Bacterial sepsis can be caused by intestinal damage, leading to the passage of viable bacteria and their components into the circulatory system. Bacterial-free DNA in the blood during sepsis can be derived from bacterial death and intestinal translocation of free DNA in the gut contents. The presence of bacterial DNA in combination with lipopolysaccharide (LPS) can lead to more serious outcomes of sepsis. To explore the impact of bacterial-free DNA in sepsis, animal and macrophage experiments were performed. Firstly, the appearance of bacterial-free DNA and bacteriome in the blood was presented in mice with cecal ligation and puncture sepsis. Secondly, injection of LPS with bacterial-free DNA in mice was more severe than LPS injection alone. Finally, the increased TNF-α, IL-6, and IL-10 cytokine, and several pro-inflammatory genes (*iNOS* and *IL-1*β) in macrophages support the synergy between bacterial DNA and LPS. In conclusion, the presence of bacterial-free DNA in sepsis was partly due to the degradation of viable bacteria in blood and the translocation of free DNA from the gut contents resulted in the profound inflammation. These data support the importance of free DNA in the blood during sepsis.

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Warerat Kaewduangduen

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CHAPTER I

INTRODUCTION

Sepsis is considered one of the global priorities established by the World Health Council and the World Health Organization (1). As more than 30 million people worldwide have a high rate of 6 million deaths annually from sepsis, with the continuously increased incidence due to the increased average age of the population (2). In addition, septic patients often have long-term effects such as impaired immune systems, neuropsychiatry, and cardiovascular dysfunctions that result in lower quality of life and increased mortality (3). Despite the high mortality rate, there is still no specific treatment or medication for sepsis at present (4). Polymicrobial sepsis that initiated from gastrointestinal (GI) tract infection is one of the most common causes of sepsis and one of the important factors of sepsis severity is bacteremia (5). Bacteremia in GI sepsis could be originated from the local intestinal infection or an impact of gut permeability defect (gut leakage) that is induced by intestinal ischemia during sepsis due to microvascular vasoconstriction or hypotension (6). Interestingly, gut permeability defect (gut leakage) allows the translocation of several components (e.g., LPS) or viable organisms from gut pathogens (mostly Gram-negative bacteria) into blood circulation (5, 7).

Among several pathogen molecules in gut, bacterial DNA is also interesting and the impact of DNA from gut translocation or from bacteremia, might affect sepsis

severity. Up to 50% of patients with severe sepsis or sepsis shock were found to have a possibility of non-infectious cause (negative culture) but caused by a severe systemic inflammatory response to other factors (8). In addition, cell-free DNA has also been detected in septic patients and patients with inflammatory bowel disease presumed to come from inactivated bacteria or dead organisms of invasive microorganisms or translocation of microorganisms or bacterial DNA in the intestine (9, 10). In general, bacterial DNA undergoes spontaneous breakdown through various reactions that produce similar-sized DNA fragments (65 kDa) (11-14) of lipopolysaccharides (LPSs) (50-100 kDa) (15), a gram-negative bacterial molecule used as the current indirect biomarker of leaky gut (16, 17). The bacterial-free DNA fragments might be small enough to move through intestinal barriers into blood circulation during sepsis, but the indicative models of bacterial-free DNA translocation remain limited.

In addition, the effect of bacterial DNA on virulence during sepsis is inconsistent (18-20). Previous reports showed worsening severity of infection, consistent with the bacterial-free DNA found in dead bacteria (21-23), while; improved infectious outcomes were consistent with reductions in bacterial-free DNA by external blood purification (24, 25). Nevertheless, the clinical importance of bacterial-free serum DNA in septic patients is mentioned as an insignificant impact (26, 27). Physiologically, DNA is released after bacterial decay and enters the

circulation, that could stimulate a wide range of systemic immune responses (21). Bacterial DNA can stimulate immune cells such as B cells, macrophages, and lymphocytes to produce pro-inflammatory cytokines such as tumor necrosis factor-**α** (TNF-**α**), interleukin 6 (IL-6), interleukin 12 (IL-12), and interferon-**γ** (IFN-**γ**) that may represent biomarkers for the severity of the infection (21). Macrophages are one of the key cells in hyper-inflammation sepsis (28) and are found in various organs (29), therefore they are used in many studies (30-33). Administered-CpG DNA in mice induces a slight inflammatory response, in part, via TLR-9 of macrophage activation. Whereas simultaneous injection of CpG DNA and LPS in mice strongly induces an inflammatory response through TLR-4 and TLR-9 of macrophages activation (34-36). However, concomitant administration of bacterial-free DNA and LPS might be a superior model due to the difference between bacterial-free DNA and CpG DNA (37), as well as the model of sepsis in similar patients.

Therefore, bacterial DNA together with other molecules during sepsis may lead to more serious conditions, such as septic shock and eventually lead to death, than sepsis without free bacteria DNA in the blood. This was the starting point for our research to study the role of bacterial DNA in combination with LPS in both in vitro and in vivo to determine whether DNAemia actually leads to infection severity. Simultaneously, we further explored the existence of bacteria-free DNA in the cecal ligation and puncture (CLP) model, likely resulting from bacterial degradation in the

blood and intestinal translocation. Our research may be another part of the knowledge in the developing future diagnosis, therapy, and treatment of human infectious and inflammatory diseases.

CHAPTER II

HYPOTHESIS AND OBJECTIVE

Research question

Does the synergy between bacterial DNA and lipopolysaccharide enhance sepsis severity?

Hypothesis

The synergy between bacterial DNA and lipopolysaccharide enhance sepsis severity.

Objective

To study the synergy between bacterial DNA and lipopolysaccharide that could be enhanced sepsis severity.

CHAPTER III

LITERATURE REVIEW

Gut microbiota

The entire population of microorganisms that colonize the gastrointestinal tract or the intestines collectively is called the human gut microbiota (38). Usually, microbial or commensal microorganisms in the intestines of healthy people are predominantly composed of bacteria, collectively known as the bacteriome, consisting of Firmicutes are the most relative abundance, followed by *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria*, respectively. Also, in the digestive tract, a community of viruses is found collectively known as a virome. They mainly consist of single-stranded and double-stranded DNA bacteriophages belonging to Siphoviridae, Podoviridae, Myoviridae, and Microviridae family. In addition to bacteriome and virome, gut mycome and eukaryome are found in the digestive tract that communities of fungi and parasites, respectively (39, 40). The gut microbiota throughout human life plays an important role in nutrient metabolism for use as energy, immune modulation, and host defense to maintain the host's gut in homeostasis (41). On the other hand, dysbiosis or variance in the gut microbiota that is decreased commensal microorganisms or increased pathogens can lead to immune system dysfunction and translocate of the microbiota, causing gut-derived infection or sepsis (39, 40). The variability of this gut microbiome depends on a

number of factors such as genetics, diet, age, medication, and other external factors (42). However, the main factor contributing to dysbiosis is microbial barrier disruption as the gut microbiota competes for restricted nutrients and modulates the host's immunity as the first barrier against pathogen invasion. That a large number of pathogens invasions can cause dysbiosis (43). In addition, the metabolites produced by the metabolism convert nutrient components into energy by gut microbiota also have both positive and negative effects on the human body. For example, short-chain fatty acids and butyrate affect the intestinal barrier function that promotes modulating the proliferation of epithelial cells and builds strong immunity (42), amino acid metabolism of microorganisms promotes signaling or antimicrobial peptide (44), and bile salts modify also improves mucus production and the integrity of the intestinal mucosa as well as inhibiting other microorganisms (45). On the other hand, the metabolic process-derived phenol is considered a toxic microbial metabolic disorder and destroys intestinal epithelial cells and tight junction, causing increased permeability (40). Likewise, the metabolism of certain drugs such as xenobiotics can affect various diseases or symptoms such as diarrhea, inflammation, and even anorexia (46). In addition, antibiotic exposure in patients, particularly in septic patients, who received antibiotics for a long time resulting in some strains of bacteria resistant to antibiotics and leads to the movement of microorganisms and dysbiosis due to loss of commensal microorganism or colonization resistance (47). Not only the balance of gut microbiota protects against disease or symptoms from

dysbiosis but also the intestinal immune system. Both the innate immune system and the adaptive immune system play an important role in preventing invasion of the gut microbiota (48).

Gut microbiota and innate immune system

The innate immune system plays an important role in maintaining a balance between tolerance to commensal microorganisms and immunity to opportunistic infections. The epithelial cell layer serves as the first barrier in the digestive tract consists of goblet cells and paint cells that live in this layer (49). Both of these cells can produce antimicrobial molecules that a key component of innate immunity such as mucin, defensin, and lysozyme. These antimicrobial molecules have direct sterilization properties, produce an inner mucous layer to maintain the mucous barrier and stabilize the gut microbiota (40). As long as the microbiota translocation through the epithelial cell layer, innate immune cells such as neutrophil cells, macrophage cells, and dendritic cells are also available to eliminate the infection. These innate immune cells can recognize pathogen-associated molecular patterns (PAMPs) or microbe-associated molecular patterns (MAMPs) via pattern recognition receptors (PRRs) such as Toll-like receptor (TLR), NOD-like receptors (NLRs), RIG-I-like receptors (RLRs), and C-type lectin receptors (Dectin-1) (45, 48). The activated cells carry various signals into the cell which has two signals, including myeloid differentiation factor 88 (MyD88) and TIR-domain-containing adapter-inducing

interferon-β (TRIF). MyD88 is an activator that most of the signals induce via the NF-kB pathway and trigger genes involved in inflammation such as tumor necrosis factor (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6). In part of TRIF is an adapter protein, which sends a signal through the IRF (interferon regulatory factor), causing the activation and production of type 1 interferon (IFN-α and IFN-β) to kill the virus (42, 50). However, previous research has been shown that innate immune dysregulation and microbiota dysbiosis can lead to uncontrollable inflammatory responses and septic complications. In patients infected with *Clostridium difficile* that caused diarrhea was found that altered fecal microbiota, loss of the protective microbial barrier, as well as production of pro-inflammatory cytokines and chemokines that affect host inflammatory damages, epithelial cell death, and increased mucosal permeability (51).

Gut microbiota and adaptive immune system

Antigens from the microorganism that enters the body are presented to T lymphocytes (CD4+ T cell and CD8+ T cell) via MHC molecule class I or class II by antigen-presenting cells, such as dendritic cells and macrophage cells. The activated antigen-specific T lymphocytes undergo clonal expansion, differentiation and stimulate B lymphocytes to convert to plasma cells to produce antibodies, mostly IgA. These antibodies are drawn to the site of infection or are transported across the epithelial cells into the lumen to neutralize and prevent the binding of microbiota to

the epithelium (42, 45). In the part of the activated T lymphocytes such as T helper cells are release cytokines such as interferon gramma (IFN-**γ**), interleukin-4 (IL-4), and interleukin-17 (IL 17). This interleukin-17 also induces mucin production and improves barrier function (42). In addition, **γδ**T cells are the most common subset of T cells compared to other tissues. These **γδ**T cells can recognize both protein and lipid antigens without getting by MHC molecules (40). Indeed, in both infectious patient and animal infection models, studies of the gut microbiota are involved in building and maintaining an adaptive immune system in the intestinal mucosa. The diversity of these adaptive immune cells also builds a barrier that is like a complete wall to the intestines to prevent the translocation of gut microorganisms. Meanwhile, the gut microbiota also plays an important role in modulating the production of secretory IgA. However, alteration in the intestinal microflora and specific adaptive immune response associated with the gut microorganism translocation remains unclear (39, 45).

Gut leakage

In normal conditions, the gastrointestinal tract consists of intestinal epithelial cells that act as a barrier in the intestinal wall to prevent microbial invasion, toxins and isolate the external environment from the host cells (52). The mucosal lining in the single layer of epithelium also contains proteins, including tight junctions and desmosomes, among others that regulate intestinal permeability by connecting between cells and absorbing nutrients. Tight junctions are protein complexes primarily composed of junctional adhesion molecules (JAMs), occluding, and claudins in the paracellular space and have a dynamic barrier structure. (53). Increased paracellular permeability of the epithelium or impaired tight junctions between intestinal epithelial cells causes the translocation of microorganisms, including their products, from the lumen into the lamina propria, the bloodstream, and other organs that called this incident that leaky gut (54). The development of the leaky gut induces an immune response due to the lamina propria site in the gut contains innate immune cells and adaptive immune cells (52) such as macrophages, T cells, B-cells, and plasma cells that help maintain intestinal homeostasis (Figure 1).

In addition to leaky gut due to disruption of tight junctions and actin cytoskeleton of intestinal epithelial cells leading to sepsis (55), leaky gut has also been reported to lead to chronic conditions and other diseases in both patients and animal models such as Inflammatory Bowel Disease (IBD), type 1 diabetes, multiple

sclerosis (MS), systemic lupus erythematosus (SLE), and more (52, 56). Furthermore, the high permeability of the intestinal barrier can also affect the blood-brain barrier due to hormone secretion or lead to nervous system degeneration (57).

The cause of leaky gut syndrome depends on many factors such as eating processed foods, drinking alcohol, stress, certain medications, age, chemical therapy, and radiotherapy (52). Besides, certain foods can lead to leaky gut flora, such as gluten, a type of glycoprotein found in the endosperm of plants. Both in the intestinal of healthy people exposed to the commensal bacteria and the intestinal of celiac disease patients exposed to gluten are stimulate the secretion of zonulin, a type of protein that destroying the tight junction and stimulating the innate immune system to inhibit the colonization of the gut microbiota (58). Additionally, iron overload in thalassemia mice has been reported to induce disruption of intestinal barrier permeability and translocation of LPS and (1 ⟶ 3)-β-D-glucan (BG) into the blood circulation. This affects the severity of sepsis increases in thalassemia mice (7). These external factors also influence alter in the gut microbiota known as dysbiosis. On the other hand, gut microbiota can contribute to the leaky gut syndrome (40, 52). Therefore, probiotics and prebiotics are used to reduce the permeability disruption and increase the balance of commensal microbiota in the intestinal (54, 59). The gut microbiota of patients infected with *Clostridium difficile* is rebalanced by fecal

microbiota transplant (FMT), providing a novel therapeutic strategy for achieving healthy conditions (60).

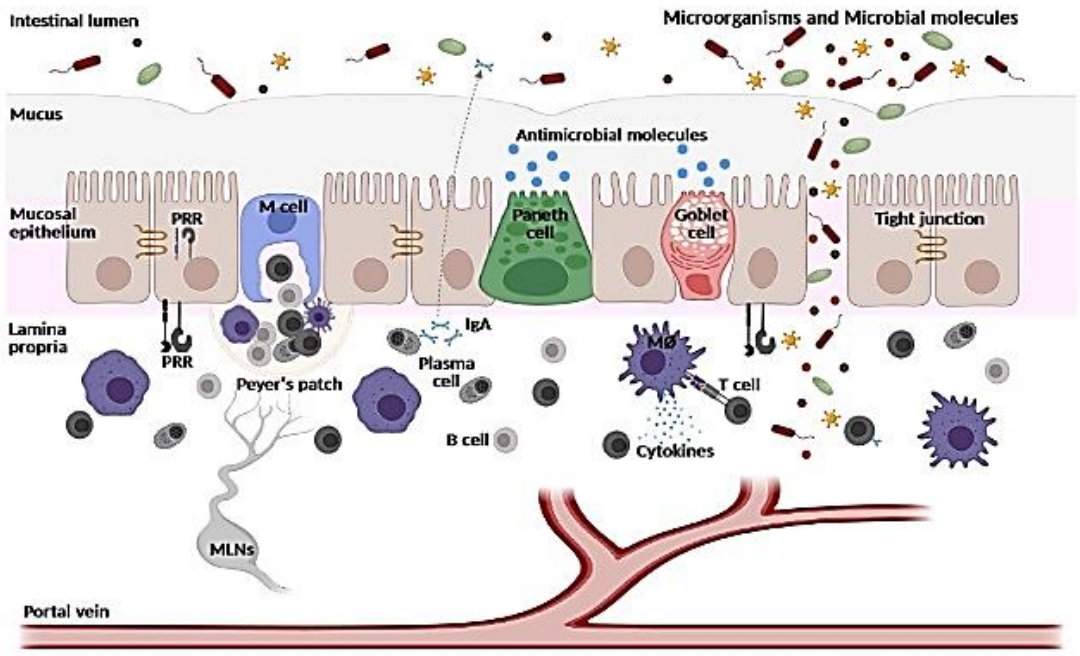


Figure 1 Immune response to prevent gut microbiota translocation due to causing gut leakage.

Figure created using Biorender (https://biorender.com/) is modified from reference number 62; A. Khan, et al., 2021 (61).

Sepsis

The term sepsis has been in use for decades and the evolution of the definition has changed over time. As recently as 2016, sepsis was redefined by the European Society of Intensive Care Medicine and the Critical Care Medicine Society as a dysregulated host infection response that results in life-threatening organ dysfunction (62). Sepsis can be broadly divided into three levels of severity. First, sepsis-1 was defined as an inflammatory response to infection and must have a suspected or confirmed source of infection in conjunction with the Systemic Inflammatory Response Syndrome (SIRS) and/or Sequential Organ Failure Assessment scores (SOFA) or Quick Sequential Organ Failure Assessment (qSOFA) score greater than or equal to two points (63). Severe and persistent host inflammatory processes in sepsis leading to organ dysfunction with a Sequential Organ Failure Assessment (SOFA) score greater than or equal to 2 points were indicated as severe sepsis (64). Finally, septic shock is a condition of sepsis in which poor tissue perfusion, causing the patient to have blood circulation and cell metabolism disorders leading to a higher mortality rate of 40% compared to only 10% of sepsis deaths (65). The criterion for clinical diagnosis of septic shock is those septic patients unresponsive to fluid resuscitation and necessary to receive a vasopressor to raise mean arterial pressure (MAP) to the range of 65-70 mmHg, as well as the presence of lactic acid levels in the blood of more than 2 mmol/L after resuscitation (62, 63) (Figure 2).

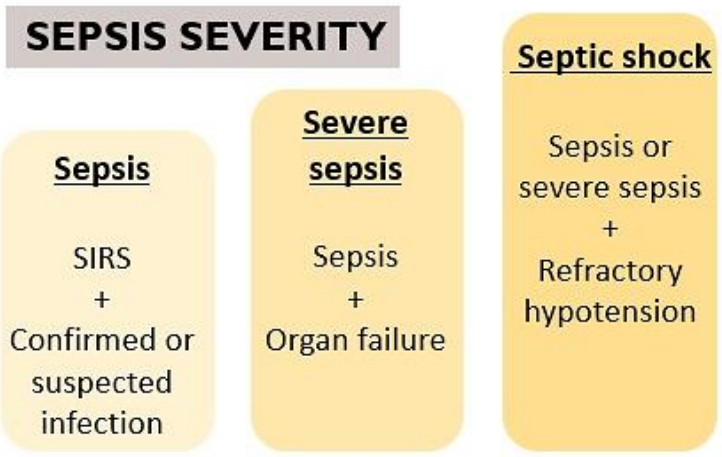


Figure 2 Sepsis is broadly divided into three levels of severity.

Figure created using Biorender (https://biorender.com/)

70th World Health Assembly In 2017, the World Health Organization (WHO) made the management and prevention of sepsis a global health priority (66). Currently, there are approximately 1.7 million adult sepsis cases in the United States annually with an increasing trend every year and approximately 270,000 deaths. Mortality in ICU patients was found to be associated with approximately one in three sepsis (67). In Thailand, according to data from the Ministry of Public Health and the National Health Security Office, the incidence of sepsis is approximately 175,000 per year, and these patients die approximately 45,000 per year, or about one-third (68). The Extended Prevalence of Infection in Intensive Care (EPIC II) study found that the most common source of infection for septic patients admitted to ICU in 75 countries were the lungs (64%), abdomen (20%) bloodstream (15%), and urinary tract (14%).

The most common sepsis-associated pathogens from culture sites were gram-negative bacteria (62%), gram-positive bacteria (47%), and fungi (19%), respectively. The most common gram-negative strains were *Pseudomonas spp.* (20%) and *Escherichia coli* (16%), the most common gram-positive strains were *Staphylococcus aureus* (20%), and the most common fungi were *Candida spp*. (63). Meanwhile, common microorganisms isolated from the prevalence study of pediatric sepsis (SPROUT) revealed gram-negative bacteria (28%), gram-positive bacteria (27%), fungi (13%), and viruses (21%) which the most common infection sites were the lungs (40%), bloodstream (19%), and abdomen (8%), respectively (69).

The mortality or response to sepsis in septic patients may be related to a number of factors, such as the amount and severity of the pathogens, site of infection, emergency surgery, trauma, disease (e.g., congestive heart failure, cirrhosis, cancer), immunosuppression, genetic factors, age, sex, and others (Figure 3). These factors may affect the pathophysiology of patients in different septic states. Some patients with sepsis have balanced and effective immunity to fight infection, while others have insufficiently effective immunity or extending to dysregulated conditions. This is a complexity for which the exact cause is unknown. However, the sepsis pathophysiology is due to molecular, cellular, and immune changes causing circulatory and metabolic disturbances leading to multiple organ malfunctions (63, 70).

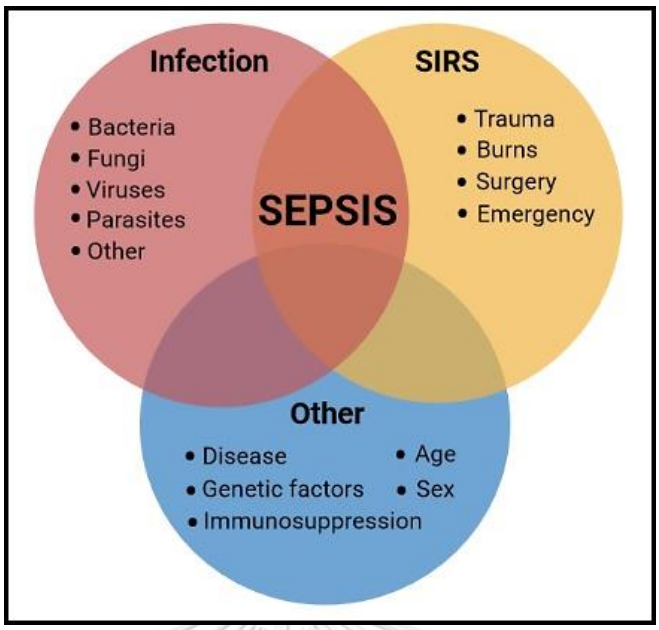


Figure 3 Factors related to mortality or response to sepsis.

Figure created using Biorender (https://biorender.com/)

Dysregulated host response in sepsis

Pathogens invade the body through epithelial barriers and interact with innate immune cells in the bloodstream and tissues. This causes an immune response to destroy pathogens and cause inflammation. The innate immune cells, including macrophages, monocytes, neutrophils, and natural killer cells, are responsible for phagocytosis and elimination of both microorganisms and damaged host cells by recognizing part of the pathogen known as pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) through pattern recognition receptor (PRR) on the cell surface. The resulting signals are sent into the cell by stimulation via the NF-κB pathway and cause cytokine storms and excessive

other mediators such as TNF-α, IL-1, IL-6, E-selection, and co-stimulation molecular (71). The resulting different cytokines work together that affect the liver by stimulating the production of acute-phase protein, such as increased C-reactive protein and vascular permeability leads to the blood supply to the site of attachment infection or increased inflammation (70). In addition, there was an increased accumulation of cells involved in eliminating pathogens, complement activation, hypotension, and hypothalamus stimulation resulting in fever. Pathogen-associated molecular patterns (PAMPs) also affect endothelial cells by inducing nitric oxide release leading to hypoperfusion and resulting in damage to the endothelium lead to hyperpermeability and edema (72). These include activation of circulating clotting factors, coagulation pathways, aggregation and adhesion of platelets leading to thrombosis in the blood vessels (70) (Figure 4).

Clinical manifestations of sepsis

The immune system in septic patients is unable to control the various inflammatory mediators in the proper amount. It can cause various sepsis conditions and lead to multiple organ system failures. The heart can be directly affected and result in myocardial depression or septic cardiomyopathy at different levels. The increased vascular permeability in the early stages of sepsis results in tissue edema and can be so severe that it causes hypovolemia. As well as the blood flow to the organs of the body is reduced and leads to myocardial depression and organ

hypoperfusion (73). In addition, the most common primary hematological manifestations in sepsis including leukocytosis, neutropenia, thrombocytopenia, and disseminated intravascular coagulation (DIC). Disruption of blood coagulation and fibrinolysis results in disseminated intravascular coagulation, resulting in tissue depletion of blood, nutrients, and oxygen (63). Subsequently, metabolic acidosis occurs in the body as cells receive less oxygen, causing the cells to rely on anaerobic metabolism and leading to hyperlactatemia (74). The development of hypoxia and metabolic acidosis results in hyperventilation or tachypnea and leading to acute respiratory distress syndrome (ARDS) in patients with sepsis (75). As well as liver dysfunction, previously reported liver failure in septic patients has a pronounced effect on morbidity and mortality (76). Renal parenchyma, hemodynamic disorder, and endothelial dysfunction cause acute kidney injury (AKI) leading to sepsis-related morbidity and death (63). In addition, the production of hormones during sepsis in the body is clearly affected and effected many systems such as the cardiovascular system, immune system, and metabolism. It was found that catecholamine levels were significantly elevated over a long period of time and eventually died (63) (Figure 5).

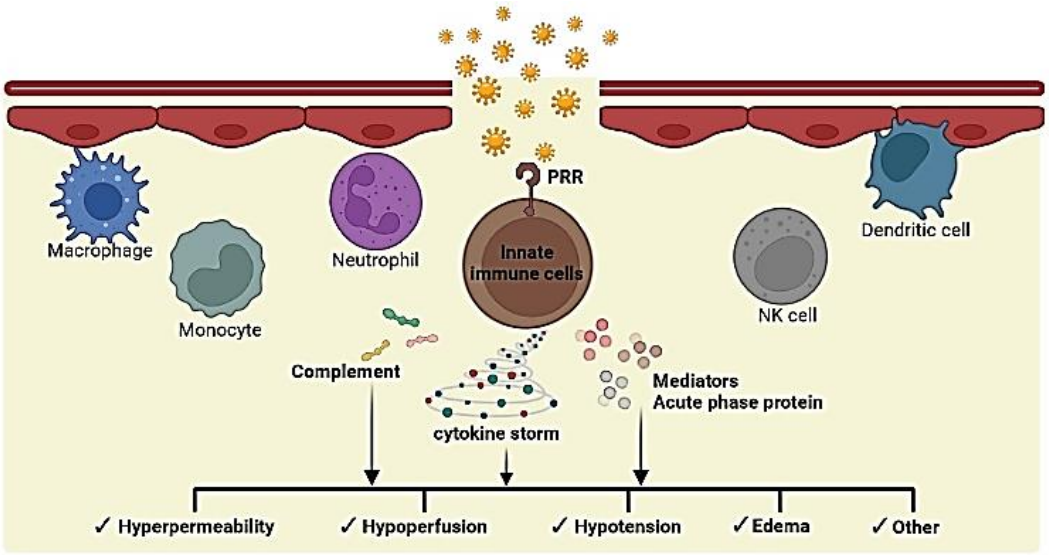


Figure 4 Dysregulated host response occurs in sepsis.

Figure created using Biorender (https://biorender.com/) is modified from reference number 74; J. Chen and H. Wei, 2021 (77).

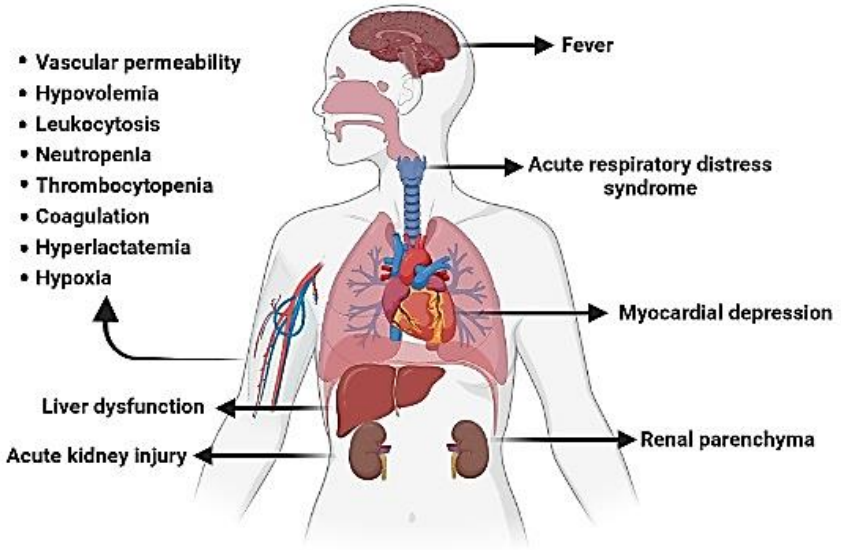


Figure 5 Clinical manifestations arise from sepsis.

Figure created using Biorender (https://biorender.com/) is modified from reference number 79; K. M. Tourelle, et al., 2021 (78).

Treatment and management of sepsis

In 2017, the 70th World Health Assembly revised guidelines for the prevention, diagnosis, and clinical management of sepsis. Morbidity and mortality from sepsis can be prevented with early diagnosis, timely and appropriate treatment, and effective infection prevention and control (66). The current treatment that can reduce mortality and complications in this group of patients is early goal-directed therapy (EGDT) (63). Initially, broad-spectrum antibiotics (meropenem, ciprofloxacin, and vancomycin) should be considered to cover probable pathogens and promptly administered appropriate antimicrobials after specimens have been cultured (79). At the same time, the septic patient should receive fluid resuscitation quickly and sufficiently to adjust circulatory dynamics with the goal of providing adequate oxygenation of the body's organs and increasing blood pressure. In fluid resuscitation, intravascular volume is assessed by physical examinations such as jugular venous pressure (JVP), central venous pressure (CVP), and pulmonary capillary wedge pressure (PCWP) (63). In septic patients who are resuscitated with adequate intravascular volume but not sufficient to reduce hypotension, therefore, vasopressors, steroids (hydrocortisone), or adrenaline should be considered as appropriate. Dopamine and norepinephrine are the first-line vasopressors that increase cardiac contractility and increase blood pressure (80). Additionally, organ function supportive treatments such as oxygen supplement therapy, mechanical

ventilator, metabolic support, and vitamin supplementation (ascorbic acid) are being considered (63) (Figure 6).

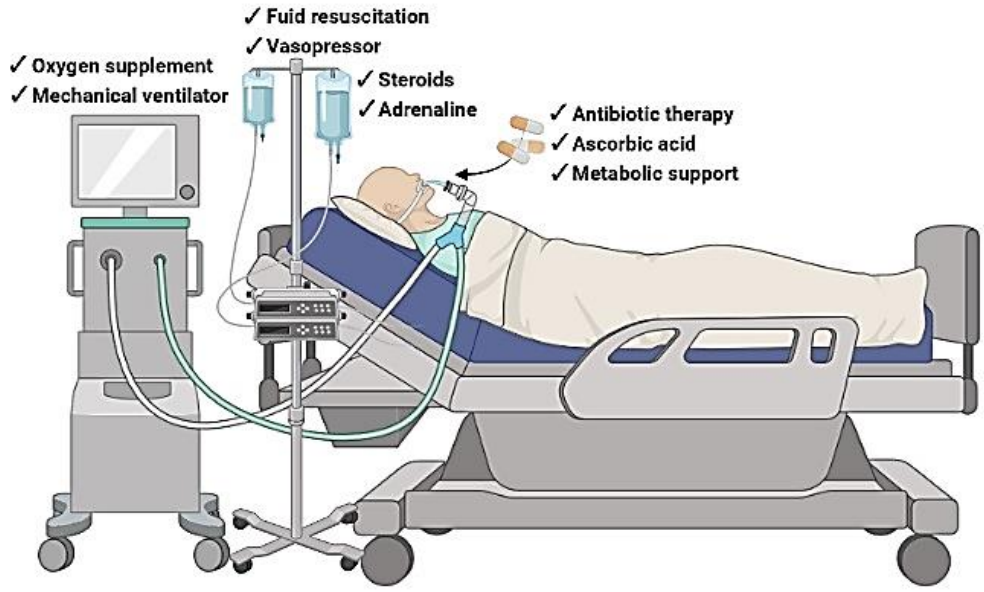


Figure 6 Treatment and management of septic patients.

Figure created using Biorender (https://biorender.com/) is modified from reference number 70; J. E. Gotts and M. A. Matthay, 2016 (69).

DNAemia

Most of the space inside a healthy person's body is a sterile microenvironment such as blood. The circulatory system appears microorganisms only intermittently, such as during sepsis (22). During an infection, the bacteria break down and release their DNA into the host circulation (21). Indeed, blood levels of circulating free DNA (cf-DNA) have been reported to increase in a time-dependent manner in sepsis mouse models. The sources cf-DNA can be caused by bacteremia and cell death, either necrotic tissue or apoptotic cells at the site of infection, especially endothelial cells. Therefore cf-DNA may be useful as a biomarker for predicting the conditions for septic conditions at the initial stage (23). Additionally, cf-DNA has also been reported in sepsis patients presumably due to dead organisms or inactivated bacteria decay that may be one of the causative agents (non-infectious cause) of severe sepsis or sepsis shock with up to 50% (8-10). Previous studies have reported that bacterial DNA is found not only in the blood of infected patients but also in the blood of healthy people. The characteristics of the bacteria that continuously spreads into the bloodstream but do not cause sepsis. It may be an indicator of bacterial DNA detected in healthy individuals known as DNAemia (22). Asymptomatic bacteremia sometimes arises from non-surgical manipulation procedures, such as dental procedures and bacterial translocation from the gastrointestinal tract (81, 82). The most frequent bacteria isolated by blood culture of patients after orthodontic procedures are *Streptococcus viridans* group and

Staphylococcus species (82). However, previous research on the use of next-generation sequencing (NGS) in bacterial DNA assays has shown that the quantitative taxonomic composition of DNA in healthy and sepsis individuals differs. In the blood of healthy people dominated by anaerobic bacteria (76.2%), mainly in anaerobic *Bifidobacteriales* order about 73%. While septic patients found decreased *Bifidobacteria* DNA and increased *Proteobacteria* DNA, which could be associated with intestinal dysfunction and lead to intestinal bacterial translocation (22, 83). *Bifidobacteriale* order is one of the bacteria that colonize the human gut and has immune-modulating properties that have the possibility to prevent infection (84). Recent research reports that bacterial DNA has been found in the blood of nearly all seriously ill COVID-19 pneumonia patients. This is thought to be caused by abnormalities in the intestinal barrier and causing gut bacterial translocation. Since the virus can be detected in the feces of COVID-19 patients and enterocytes in the intestine expressing angiotensin-converting enzyme 2 (ACE2) receptors, indicating that the gastrointestinal tract is another site associated with COVID-19 infection. (85). Bacterial DNA was also found in the blood of older adults (average age 79 years) and associated with serum levels of zonulin, a marker of intestinal permeability. Bacterial DNA tends to affect many aspects of host physiology, such as inflammatory disease, type 2 diabetes, and cardiovascular disease (86).

Bacterial DNA activate the immunity system

Deoxyribonucleic acid (DNA)

Deoxyribonucleic acid, also known as DNA, is a nucleic acid that stores the genetic information of both eukaryotic and prokaryotic organisms. Most of the DNA is found in the nucleus of cells known as nuclear DNA, while DNA is also found in mitochondria which are called mitochondrial DNA (87). DNA has two important functions i) DNA replication to create identical DNA in all respects for new cells and ii) transcription to RNA, the resulting RNA is responsible for the arrangement of amino acids in protein synthesis, and proteins are used as structural components of various components within the cell and enzymes in living organisms. DNA is made up of a subunit called a nucleotide, which is a combination of deoxyribose sugar, phosphoric acid, and nitrogenous base. Nitrogenous bases are also divided into two groups: purine bases, namely thymine (T) and cytosine (C), and pyrimidine bases, namely adenine (A) and guanine (G). There are four types of nucleotides, including adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytosine triphosphate; CTP), and thymidine triphosphate (thymidine triphosphate; TTP). (88) The ordering of nucleotides of the four nucleotides affects the diversity and specificity of each organism. The structure of DNA is the connection of multiple nucleotides by phosphodiester bonds. Two polynucleotide chains are arranged parallel in opposite directions by pairing with hydrogen bonds and entangled in a right-hand helix like a

spiral staircase, and also have sugars and phosphate groups at the outer core of the molecule called the double helix (89, 90) (Figure 7).

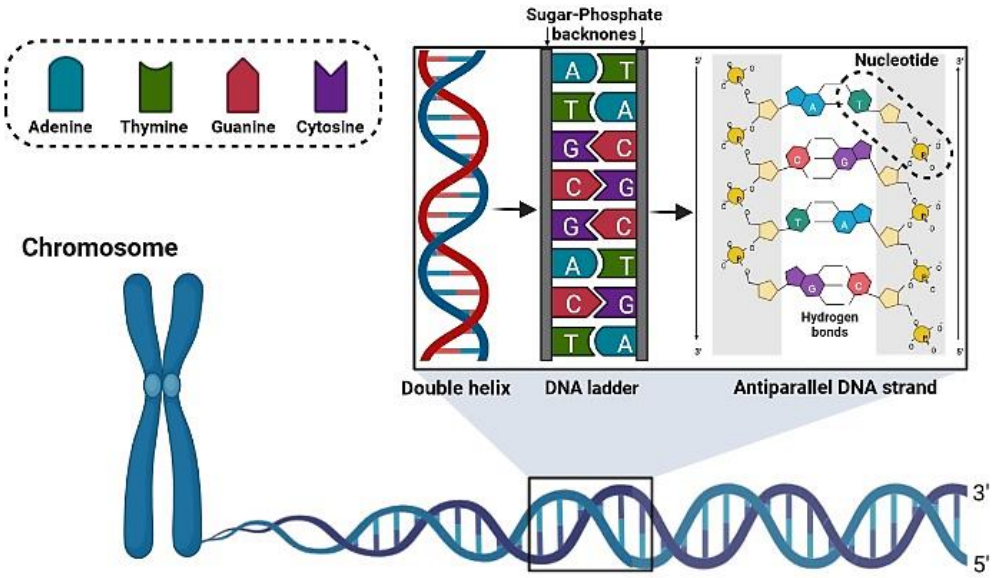


Figure 7 The chemical structure of deoxyribonucleic acid (DNA).

Figure created using Biorender (https://biorender.com/) is modified from reference number 94; G. Salieb-Beugelaar, 2022 (91).

DNA of eukaryotic cells (human, animal, fungi, or yeast) and prokaryotic cells (bacteria or archaea) are similar in some ways and different in others. In terms of similarities, both eukaryotic and prokaryotic DNA serves to store information for the functioning and reproduction of different organisms. Moreover, both are replicated by DNA polymerase. As for differences, eukaryotic DNA is present in the cell nucleus, but prokaryotic DNA is found in the cytoplasm. Next, eukaryotic DNA has a double-

stranded and linear shape, whereas prokaryotic DNA has a double helix and a circular shape. Finally, human genome, classified as eukaryotic cells, is approximately 2.9 billion base pairs.; on the other hand, the size of prokaryotic DNA is approximately 160,000 to 12.2 million base pairs, depending on the species (92, 93).

The mechanism by which DNA enters the cells

The DNA released by the broken bacteria during infection and enters the circulation is quickly cleared by the spleen and liver, having a half-life of about 4 minutes. A large number of bacterial DNA continually entering the circulation can induce a severe immune response. The circulating Bacterial DNA is absorbed by the spleen and triggers the white blood cells to release cytokines (21). In addition, bacterial DNA plays a role in priming the immune system and leads to widespread immune cell death in the following activation through the hyper-immune responses. However, the role of free DNA in sepsis is still unclear (86) and most reports of the free DNA are mainly on cancer and viral infections (21). In bacterial DNA, unmethylated CpG DNA motifs activate immune cells such as B cells, natural killer cells, lymphocytes, macrophages, and neutrophils (37, 94, 95). While eukaryotic DNA, CpG motifs are high methylation, thus allowing the host to differentiate between self-DNA and bacterial DNA. The host cells or mammalian cells exhibit a variety of DNA sensors as one of the defense lines in infection (96). DNA sensors are distributed

in cell compartments and play a key role in detecting microbial DNA entering cells through various mechanisms.

TLR9-MyD88 pathway

Most bacteria undergo decay and their DNA is transported into cells via endocytosis or phagocytosis to activate toll-like receptor 9 (TLR9) (21). TLR9 is the main receptor that recognizes bacterial DNA (pathogen-associated molecular patterns) and signals in host cells (damage-associated molecular patterns) (97). TLR9 is expressed in various tissues such as the spleen is most abundantly expressed and immune cells, including macrophages, neutrophils, lymphocytes, and natural killer cells (21). Innate immune cells transport extracellular bacteria into the cell to endosomes and phagosomes, where the N-terminal domain of TLR9 recognizes unmethylated CpG-DNA motifs of bacteria (97). CpG-DNA motifs activated TLR9 induce various signals generation through myeloid differentiation primary response gene 88 (MyD88) and induce nuclear factor **κ**B (NF-**κ**B) pathways that cause the eventual secretion of proinflammatory mediators and antimicrobial molecules (98). However, different cell types may trigger different downstream pathways. Bacterial DNA activates lymphocytes to produce interleukin 6, interleukin 12, and IFN-**γ** similar to activates macrophages to produce interleukin 6, Interleukin 12, and tumor necrosis factor **α** (TNF-**α**) (99). There are also other DNA sensors such

as DHX9, which sense CpG-B DNA using the DUF domain and activate the signal via the MyD88-NF-κB pathway to increase the expression of TNF-α and interleukin 6 (21) (Figure 8).

cGAS-STING pathway

Specific bacteria can enter host cells and release DNA directly to the cytosol in response to cytosolic DNA sensors. Cyclic GMP-AMP synthase (cGAS), a cytosolic DNA sensor, senses cytosolic double-stranded DNA (dsDNA) of bacteria can and cannot distinguish microbial DNA from host DNA. The bacterial DNA-binding cGAS forms dimers and synthesizes cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) to activate the STING pathway on the endoplasmic reticulum surface. The stimulated STING is signaled through TANK Binding Kinase 1 (TBK1) and IFN regulatory factor 3 (IRF3) to stimulate the secretion of type I IFN (100). This signaling also activates the NF-κB pathway to increase the expression of inflammatory mediators and antimicrobial molecules, including TNF-α and interleukin 6 (86). Additionally, cGAS-STING activates cell death pathways leading to the death of T lymphocytes, B lymphocytes, neutrophils, macrophages, hepatocytes, and cardiomyocytes (21, 101). Therefore, signaling through the STING pathway is an important response to both bacterial and viral DNA in cells that mediate innate immunity (21) (Figure 8).

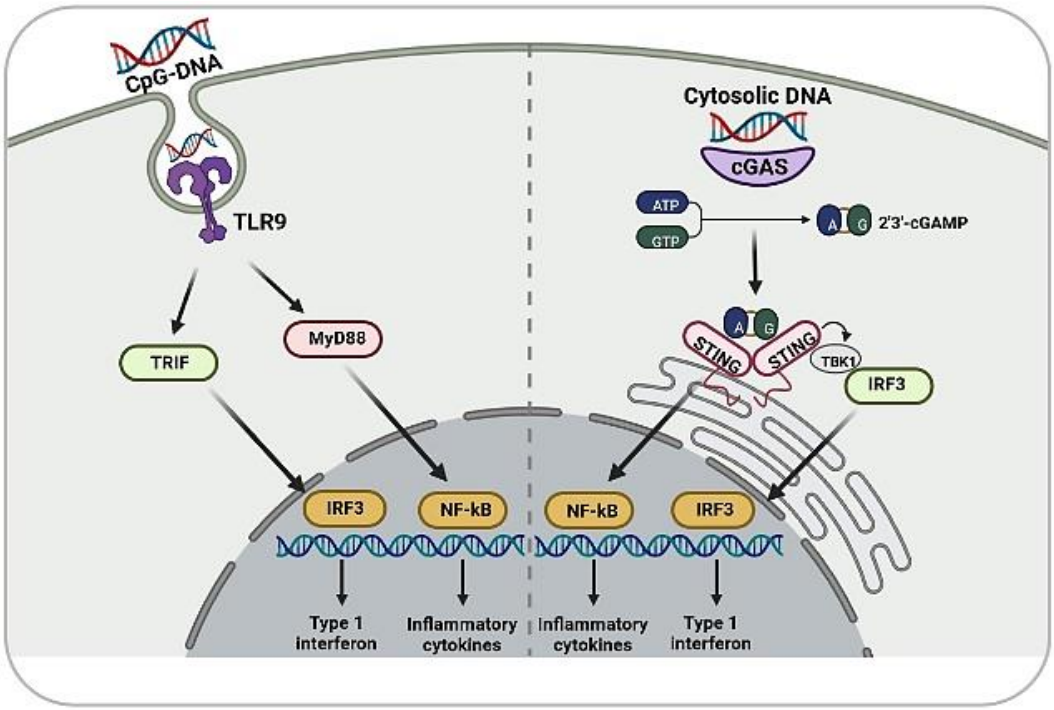


Figure 8 Mechanism of bacterial DNA activation.

Figure created using Biorender (https://biorender.com/) is modified from reference number 103 and 104; A. Decout, 2021 and G. Montamat, 2021 (102, 103).

Effects of Bacterial DNA

Macrophage cells respond to the stimulation of bacterial DNA via TLR9 by secreting various inflammatory cytokine (104) and macrophage responses to CpG-containing phosphodiester oligonucleotides (PO-ODN) are also dependent on DNA length (37). Short CpG-ODN (< 44 nucleotides) was found to be less immunostimulating than *Escherichia coli* DNA and probably not involved in ligand-mediated TLR9 cross-linking. Additionally, bacterial DNA was found to stimulate neutrophils, including IL-8 secretion, CD11b upregulation, and neutrophil migration

via the TLR9-independent and MyD88-dependent pathways (105) and cause a respiratory burst, cell death, or NETs formation that is a novel program for cell death (101). Bacterial CpG-DNA has been reported to be able to stimulate an immune response through DNA sensors, but not enough to cause cell or animal death (34). CpG-DNA and LPS work synergistically to stimulate the production of inflammatory cytokines such as TNF-**α**, IL-6, IL-10, and IFN-**γ** through activation of the NF-**κ**B pathway, as well as increase the binding of DNA at the cell surface in monocytes and macrophages, nitric oxide production, B cell proliferation, and Ig secretion (36). Generally, bacterial DNA provokes a non-serious immune response. Indeed, it has been reported that bacterial DNA may induce septic shock in mouse models due to acute liver failure caused by the apoptotic cells through the induction of high levels of TNF-**α** (35). However, there are no definitive clinical reports yet. On the other hand, CpG- oligonucleotides or synthetic oligonucleotides have been tried for vaccines to modulate inflammation and enhance immunity, as immune responses can increase the host's resistance to different pathogenic pathogens (106).

Macrophages

The macrophage and monocyte are among the leukocytes originating from the hematopoietic stem cells in the bone marrow that are considered the same type. The monocytes float in the blood, while the macrophage traps pathogens and foreign bodies in tissues. Each organ has a different name for macrophages, such as mesangial cells in the kidney, Kupffer cells in the liver, histocytes in the spleen and connective tissue, microglial cells in the central nervous system, or osteoclast in bone. It is often among the first to recognize pathogens and foreign matter as they are located in many areas and have various functions (107, 108).

First, macrophages are able to ingest pathogens and foreign matter through a variety of cell surface receptors known as the phagocytosis process. Macrophage has receptors that make them easier to ingest, known as opsonic receptors, such as the Fc receptor, a receptor for the Fc fraction of immunoglobulin, or the complement receptor, a receptor for complement proteins attached to the microbial surface. There are also pattern recognition receptors (PRRs) such as the toll-like receptor, the lectin receptor, and the scavenger receptor that enable macrophages to recognize pathogens. Macrophages also kill microorganisms through other mechanisms such as lysozyme release, reactive oxygen species (ROS), and nitric oxide (NO). Concurrently, it releases certain enzymes that play a role in wound healing, such as collagenase and elastase (Figure 9). Secondly, macrophages are another important cell

responsible for the secretion of chemokines and cytokines, both pro-inflammatory cytokines and anti-inflammatory cytokines (109, 110). Some cytokines, such as Interleukins, can also stimulate the expression of adhesion molecules, selectins, integrins, immunoglobulin, and mucin-like glycoproteins, which help attract macrophages and other cells to the site of inflammation or injury. Finally, macrophages are one of the antigen-presenting cells that act as an antigen presenter to the adaptive immune system, such as T cells through major histocompatibility complex (MHC) class I or II and co-stimulatory molecules, such as CD40, CD80, and CD86. This causes the activation of an adaptive immune response to continue functioning (107, 108) (Figure 10).

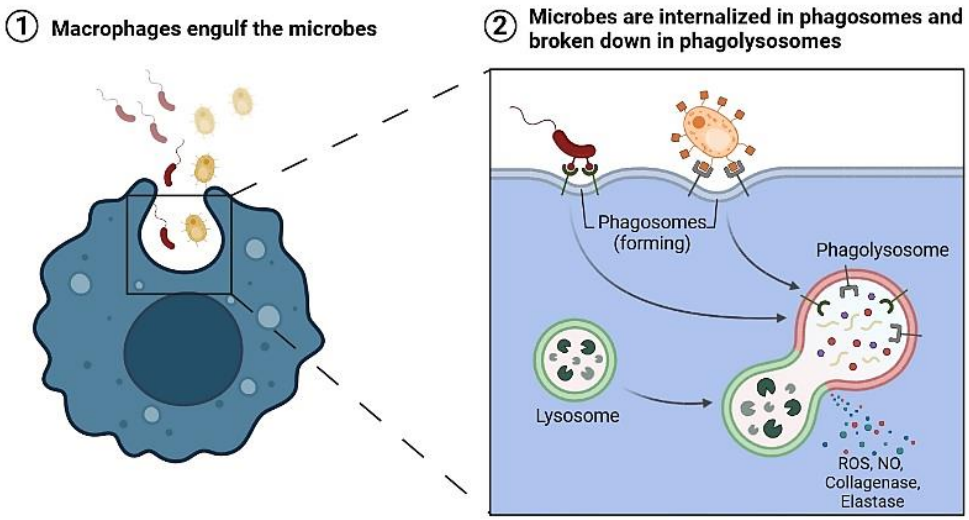


Figure 9 Phagocytosis process of macrophage cell.

Figure created using Biorender (https://biorender.com/) is modified from reference number 112; K. Lindell, 2022 (111).

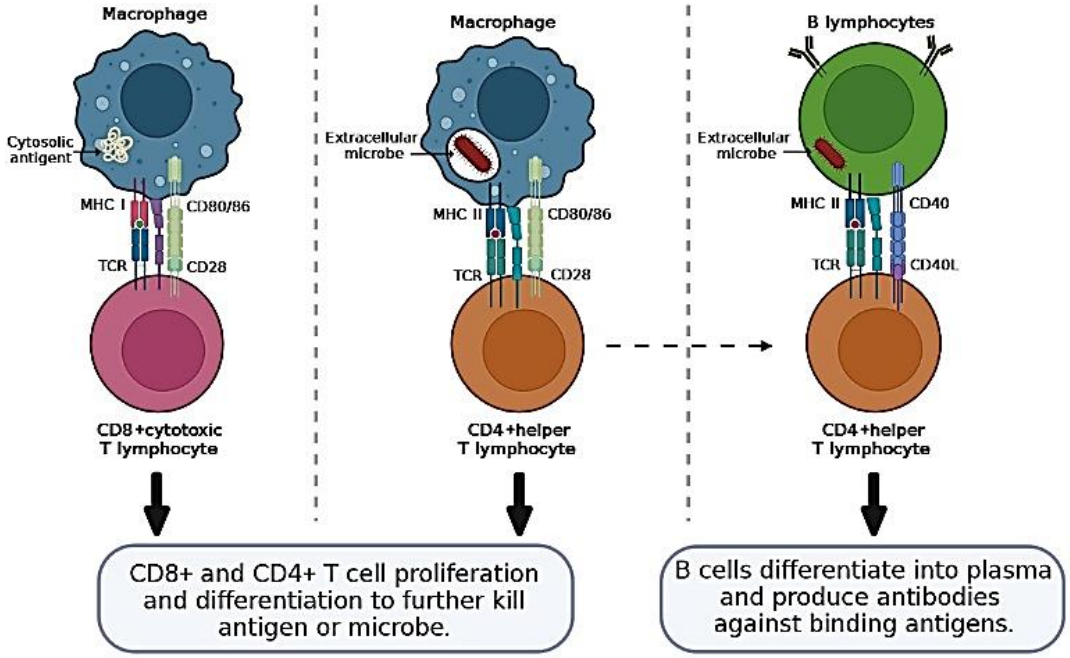


Figure 10 The antigen-presenting function of the macrophage.

Figure created using Biorender (https://biorender.com/) is modified from reference number 113-115; C. Nicchitta, 2020; C. Pifferi, et al., 2017 and A. Lin and K. Loré, 2017 (112-114).

Otherwise, macrophages are considered to be dominant plastic cells capable of transitioning from one phenotype to another, a process called macrophage polarization. Macrophage polarization consists of classically activated macrophages (M1) and alternately activated macrophages (M2) based on response to stimuli or cytokine signals. M1 macrophages produce proinflammatory cytokines such as TNF-**α**, IL-1, IL-6, cyclooxygenase-2(COX-2), nitric oxide (NO), and reactive oxygen species (ROS), as well as expressions of CXCL9, CXCL10, and CXCL11, are polarized by

stimulation of pathogen-associated molecular patterns (PAMPs) and Th1 cytokines such as IFN-γ and TNF-α. The inflammatory responses are regulated in the normal state by anti-inflammatory cytokine production such as IL-10 and TGF-β of the Th2 cytokines IL-4 and IL-13-induced M2 macrophage. Additionally, M2 macrophages are expressed by arginase-1 (Arg-1), found in inflammatory zone (FIZZ1), CCL17, CCL18, CCL22, and CCL24 (108, 115, 116) (Figure 11).

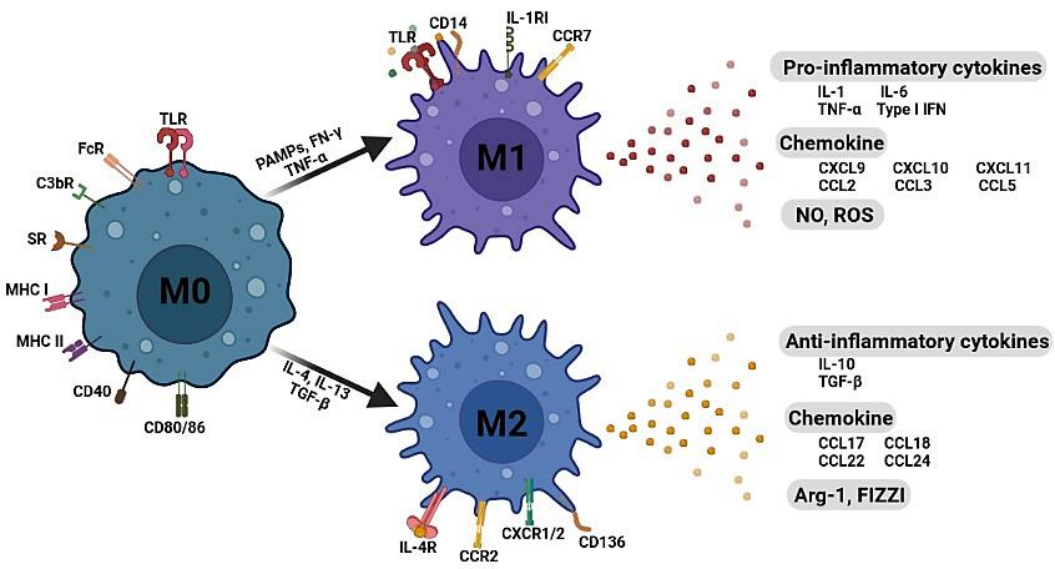


Figure 11 Macrophage polarization and specific function of M1 and M2 macrophage.

Figure created using Biorender (https://biorender.com/) is modified from reference number 118 and 119; M. Benoit, et al., 2008 and D. Lu, et al., 2021 (117, 118).

Lipopolysaccharide (LPS) activate the immunity system

Lipopolysaccharides (LPS) are important constituents present in the outer membrane of the gram-negative bacterial cell wall and have properties as endotoxins that induce immune responses during sepsis (119). LPS is a thermostable molecule with a molecular weight of more than 100,000 Daltons (120). The structure of LPS consists of Lipid A and polysaccharides. Lipid A is composed of phosphorylated N-acetyl glucosamine dimer and 6 or 7 saturated fatty acids attached to it and forming a hydrophobic region that is attached to the membrane. It is also a toxic component with immune-inducing properties. The length and position of the esterified acyl chain, as well as the phosphate groups in the lipid A structure, have different levels of activation to the immune response. The polysaccharide portion is divided into core polysaccharides (R antigen) and O polysaccharides (O antigen). Core polysaccharide consists of sugars short chain attached to one NAG in the sixth position and 2-keto-3-deoxy octanoic acid (KDO), which is always the polysaccharide found in LPS. While O polysaccharide is composed of at least 20 sugars and attached to the core polysaccharide. The sugar content of O polysaccharides varies with the bacterial species and determines the smooth type and the rough type of bacteria (121-123) (Figure 12).

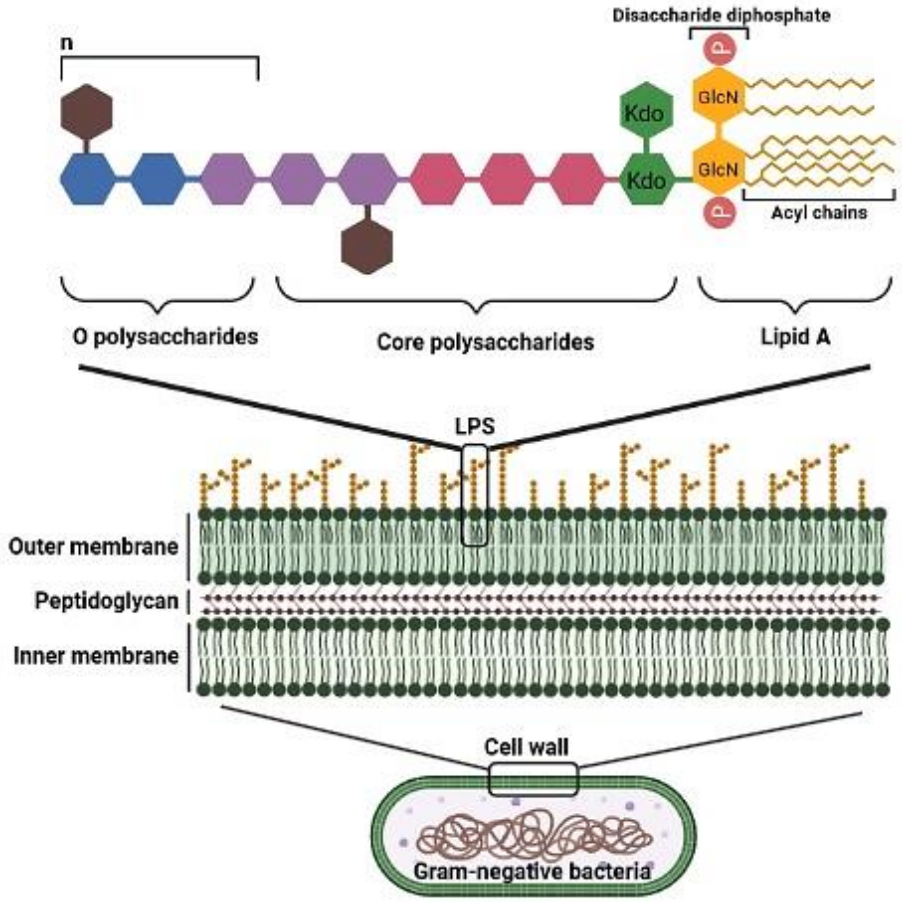


Figure 12 Cell wall and lipopolysaccharide (LPS) structures of Gram-negative bacteria.

Figure created using Biorender (https://biorender.com/) is modified from reference number 125; L. Mazgaeen and P. Gurung, 2020 (124).

Innate immune cells such as macrophages, neutrophils, and dendritic cells recognize LPS, one of the pathogen-associated molecular patterns (PAMPs), via toll-like receptor 4 (TLR4), a pattern recognition receptor (PRR) present on the cell surface and lead to stimulating subsequent inflammation. The immune response is

initiated by serum protein binding the monomer of LPS from gram-negative bacteria and transferring it to the soluble CD14 molecule (sCD14). CD14 then transferred LPS to the TLR4/MD-2 receptor complex, leading to homodimerization of TLR4 and dimerization of the cytoplasmic TIR-domain (Toll-interleukin-1 receptor), respectively. The resulting signal is delivered to myeloid differentiation primary response gene 88 (MyD88) adapter proteins and TIR domain-containing adapter proteins (TIRAP), leading to the induction of proinflammation cytokines via transcription factor NF-**K**B and mitogen-activated protein kinase (MAPK) signaling. Meanwhile, LPS recognition of the TLR4/MD-2 complex within the endosome is signaled via the TIR domain-containing adapter (TRIF), leading to activation of interferon regulatory factor 3 (IRF3) and type-1 interferons expression (125, 126). All these inductions lead to the overproduction of inflammatory cytokines and broadly pathophysiological effects such as fever, alteration in white blood cell count, disseminated intravascular coagulation, hypotension, shock, and severe to the point of death (121, 127) (Figure 13).

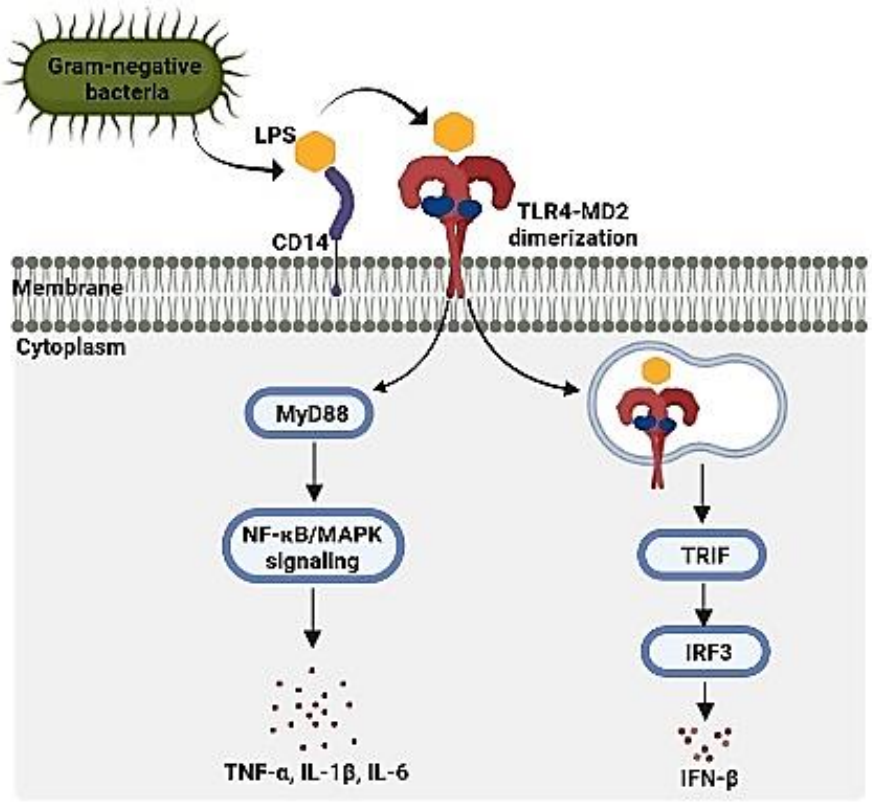


Figure 13 Mechanism of lipopolysaccharide (LPS) activation.

Figure created using Biorender (https://biorender.com/) is modified from reference number 129; Z. Nová, et al., 2019 (128).

CHAPTER IV

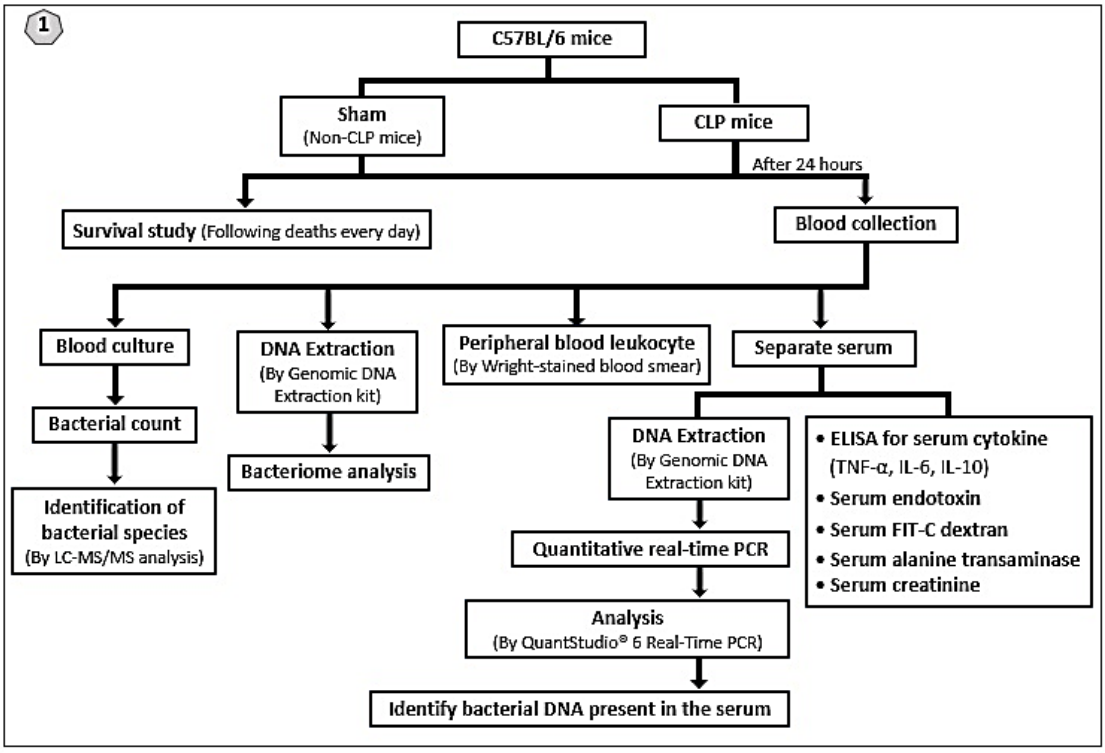
METHODOLOGY

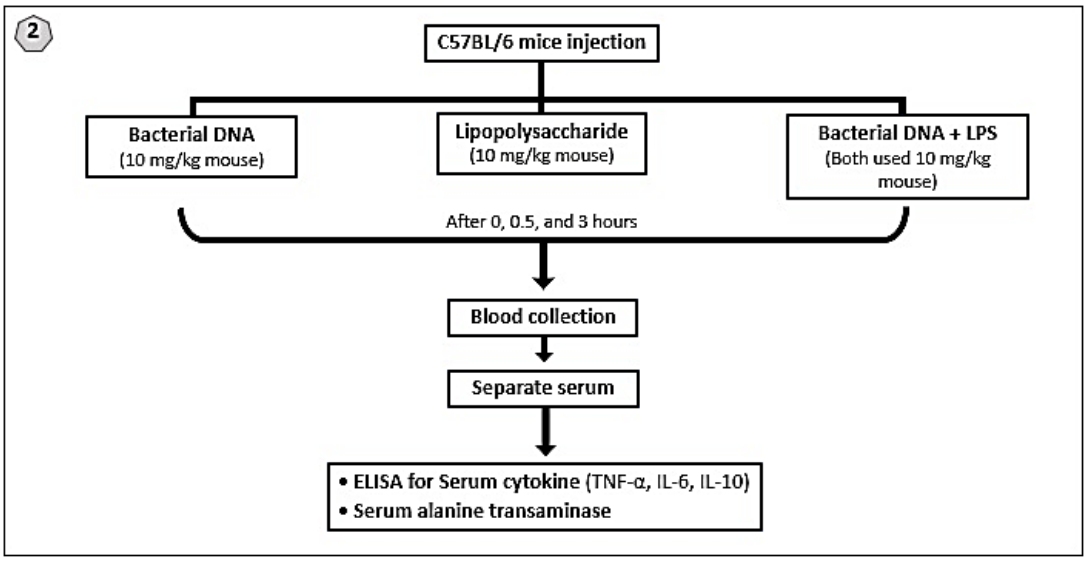
Conceptual framework

Bacteria-free DNA with or without LPS induces more inflammation in macrophage cells and the mouse model.

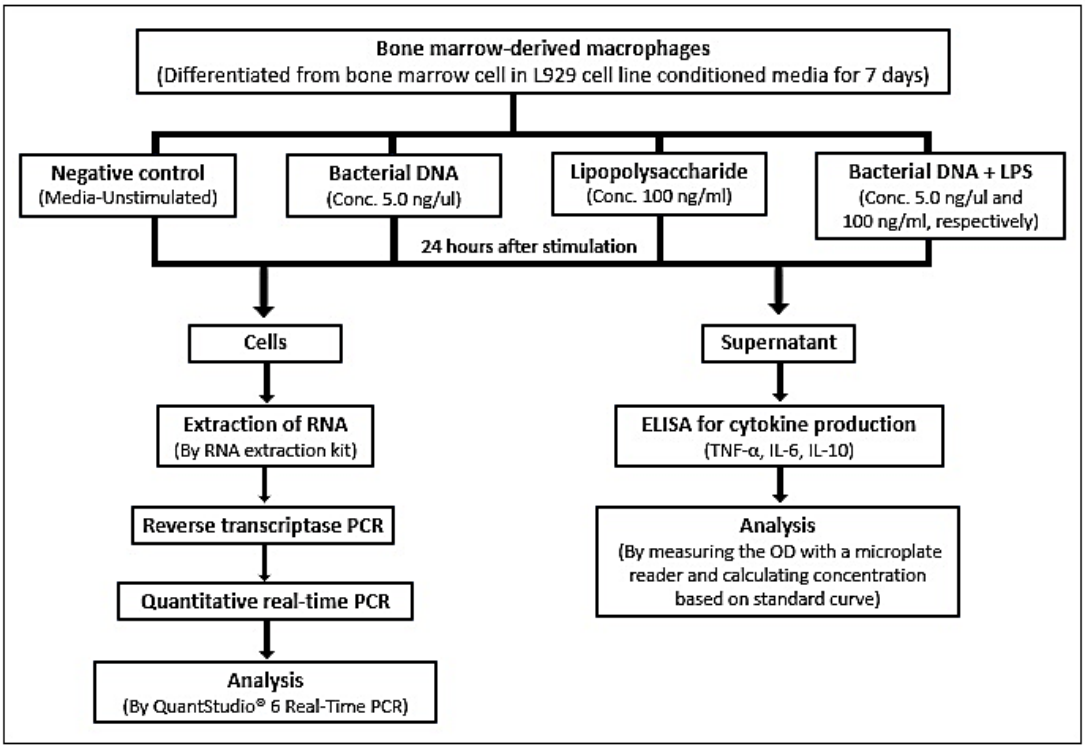
Experiment design

In vivo




2
C57BL/6 mice injection
Bacterial DNA
(10 mg/kg mouse)
Lipopolysaccharide
(10 mg/kg mouse)
Bacterial DNA + LPS
(Both used 10 mg/kg mouse)
After 0, 0.5, and 3 hours
Blood collection
Separate serum
• ELISA for Serum cytokine (TNF-α, IL-6, IL-10)
• Serum alanine transaminase

In vitro


Bone marrow-derived macrophages
(Differentiated from bone marrow cell in L929 cell line conditioned media for 7 days)
Negative control
(Media-Unstimulated)
Bacterial DNA
(Conc. 5.0 ng/ul)
Lipopolysaccharide
(Conc. 100 ng/ml)
Bacterial DNA + LPS
(Conc. 5.0 ng/ul and 100 ng/ml, respectively)
24 hours after stimulation
Cells
Supernatant
Extraction of RNA
(By RNA extraction kit)
ELISA for cytokine production
(TNF-α, IL-6, IL-10)
Reverse transcriptase PCR
Quantitative real-time PCR
Analysis
(By QuantStudio® 6 Real-Time PCR)
Analysis
(By measuring the OD with a microplate reader and calculating concentration based on standard curve)

CHAHTER V

MATERIALS AND METHODS

Animal and Animal Models

C57BL/6 mice at 8-week-old weighing approximately 20–22 g were used in all experiments and purchased from Nomura Siam (Pathumwan, Bangkok, Thailand). Mice were used as sepsis models using cecal ligation and puncture or sham surgery (7). Briefly, the cecum was ligated at 10 mm below the ileocecal valve and then punctured twice middle between the ligation and the cecal tip with a 21-gauge needle under isoflurane anesthesia. A small amount of feces was extruded after the needle was removed. While the sham mice were anesthetized and mid-abdominal incision without puncture nor ligation. CLP and sham mice were sacrificed at 24 hours by cardiac puncture under isoflurane anesthesia with sample collection. On the other hand, mice were intravenously (IV) injected with extracted DNA of *Escherichia coli* (ATCC 25922) (ATCC, Manassas, VA, USA) and intraperitoneally (IP) injected with lipopolysaccharide (LPS) (Escherichia coli 026: B6; Sigma-Aldrich) at the same concentration of 10 mg/kg mouse. Blood samples were collected before LPS injection (0 h) and 0.5 h post-injection. Then, mice were sacrificed at 3 h post-injection by cardiac puncture under isoflurane anesthesia with blood collection.

Identification of organisms in the blood

CLP and sham mice were collected blood 24 hours into the EDTA tube, then 100 µl of blood was spread plate on the blood agar (Oxoid, Hampshire, UK). Bacterial colonies found on blood agar were counted and enumerated after 24 hours of incubation at 37 °C, and the bacterial species were then examined using mass spectrophotometry (LC-MS/MS analysis) (Vitek MS; bioMérieux SA, Marcy-l'Etoile, France), according to the routine hospital protocol.

Additionally, blood (200 µl) from each mouse was collected for blood microbiota analysis performed as previously described (129). Shortly, the metagenomic DNA was extracted from the prepared samples using DNA Mini Kit (Qiagen, Redwood City, CA, USA). The quality of extracted DNA was performed using a Nanodrop ND-100 (Thermo Scientific, Waltham, MA, USA). 16S rRNA gene V4 library was constructed using universal prokaryotic primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') with appended 50 Illumina adapters and 30 Golay barcode sequences. The bioinformatic analyses were performed according to Mothur's Standard Operating Procedures (SOP) (130).

DNA extraction of *Escherichia coli* (*E. coli*)

Escherichia coli (ATCC 25922) (ATCC, Manassas, VA, USA) from a freshly streaked trypticase soy agar plate (TSA) (Oxoid, Hampshire, UK) were incubated in 5 mL of tryptic soy broth (TSB) at 37 °C for approximately 16 hours while shaking at 250 rpm. *E. coli* suspension was extracted with FavorPrep™ Tissue Genomic DNA Extraction Mini Kit (Favorgen Biotech, Wembley, WA, Australia) and quantified by a Nanodrop ND-100 (Thermo Scientific, Waltham, MA, USA). DNA was stored at -80 °C until use (131).

Macrophage cells activation and analysis

Macrophages cells were derived from bone marrow of mice as previously described (132). Briefly, bone marrow from femurs and tibias were collected by centrifugation at 6,000 rpm for 4 °C and cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) with sodium pyruvate in a humidified 5% CO_2 incubator at 37 °C for 7 days. Conditioned media of the L929 cell line, containing macrophage-colony stimulating factor, at 20% weight by volume (w/v) were used to induce macrophages from the pluripotent stem cells. Bone marrow (BM)-derived macrophages at 1×10^6 cells/well in 6-well plates in a total of 1 ml of completed DMEM were stimulated with the extracted DNA (mentioned previously) of *E. coli* at 5 ng/μL or stimulated in

combination with 100 ng/ml lipopolysaccharides (LPS) (Escherichia coli 026: B6; Sigma-Aldrich). After 24 hours of incubation, the supernatant was examined for TNF-**α**, IL-6, and IL-10 cytokine production by the enzyme-linked immunosorbent assay (ELISA) (Invitrogen). The cell pellets were extracted with RNA using the FavorPrep^{TM} Tissue Total RNA Purification Mini Kit (Favorgen, Taiwan) and quantified by a Nanodrop 100 Spectrophotometer (Thermo Fisher Scientific). The RNA samples were converted to cDNA with High-Capacity cDNA Reverse Transcription (Thermo Scientific) and examined gene expression with the SYBR Green PCR Master Mix (Applied biosystem, Foster City, CA, USA) for quantitative real-time polymerase chain reaction (RT-PCR) (Thermo Scientific), respectively. The results were demonstrated in relative quantification of the comparative threshold method (2-**ΔΔ**Ct) and normalized by housekeeping gene (***β-actin***). Primers for pro-inflammatory signals (*iNOS* and *IL-1**β***), and anti-inflammatory signals (*Arginase-1*, *TGF-**β***, and *Fizz-1*) were used (Table 1).

Table 1 List of primers used in the experiment of macrophage cells activation

Primer name	Forward primer	Reverse primer
Inducible nitric oxide synthase (iNOS)	5'-ACCCACATCTGGCAGAATGAG-3'	5'-AGCCATGACCTTTCGCATTAG-3'
Interleukin-1β (IL-1β)	5'-GAAATGCCACCTTTTGACAGTG-3'	5'-TGGATGCTCTCATCAGGACAG-3'
Arginase-1 (Arg-1)	5'-CTTGGCTTGCTTCGGAACTC-3'	5'-GGAGAAGGCGTTTGCTTAGTTC-3'
Transforming Growth Factor-β (TGF-β)	5'-CAGAGCTGCGCTTGCAGAG-3'	5'-GTCAGCAGCCGGTTACCAAG-3'
Resistin-like molecule-α (FIZZ-1)	5'-GCCAGGTCCTGGAACCTTTC-3'	5'-GGAGCAGGGAGATGCAGATGA-3'
β-actin	5'-CGGTTCCGATGCCCTGAGGCTCTT-3'	5'-CGTCACACTTCATGATGGAATTGA-3'

Blood and Serum Analysis

The parameters of bacterial DNA quantity, peripheral blood leukocytes, serum endotoxin (LPS), intestinal permeability defect, systemic inflammation, liver injury, and kidney dysfunction were evaluated from blood samples. The bacterial-free DNA present in the blood was extracted with FavorPrep™ Tissue Genomic DNA Extraction Mini Kit and examined using the 16s rRNA 5'-GATGAACGCTGGCGGCGTGC-3' (forward primer), 5'-CAATCATTTGTCCCACCT TC-3' (reverse primer) by quantitative RT-PCR with QuantStudio 6 Flex Real-time PCR System (Thermo Scientific), respectively. For peripheral blood leukocytes, blood was mixed with 3% v/v of acetic acid in a ratio of blood and acetic acid at 1:20 by volume for red blood cells lysis prior to

hemocytometer counts. After that, the percentages of neutrophils and lymphocytes were examined using a Wright-stained blood smear.

Blood collected in sterile tubes was centrifuged at 8,000 rpm for 10 minutes at 4 ℃ to separate the serum. Serum endotoxin (LPS) was determined using the Limulus Amebocyte lysate test (Associates of Cape Cod, East Falmouth, MA, USA), and values of LPS < 0.01 EU/mL were recorded as 0 due to the limitation of the standard curve. Intestinal permeability defect was examined by oral administration with FITC-dextran, a 4.4 kDa intestinal nonabsorbable molecule (Sigma-Aldrich, St. Louis, MO, USA), at 12.5 mg per 25 g mouse. After 3 h administration, FITC-dextran was in serum detected using a fluorospectrometer (NanoDrop 3300; ThermoFisher Scientific, Wilmington, DE, USA).

Serum inflammation cytokines, including TNF-a, IL-6, and IL-10 representing systemic inflammation, were measured using enzyme-linked immunosorbent assay (ELISA) (Invitrogen, Waltham, MA, USA). The degree of liver and kidney injury were determined by measuring plasma alanine transaminase (ALT) and creatinine using EnzyChrom ALT Assay (EALT-100, BioAssay, Hayward, CA, USA) and QuantiChrom Creatinine-Assay Kits (DICT-500) (BioAssay), respectively.

Data analysis

Statistical differences were examined using an unpaired student's t-test or one-way analysis of variance (ANOVA) with Tukey's to analyze the two or multiple group trials, respectively, and presented as mean ± standard error (SE), with $p < 0.05$ considered significant. All in vivo experiments were repeated three times before being statistically analyzed using GraphPad Prism 7 (GraphPad Software, Inc., San Diego, CA, USA).

CHAPTER VI

RESULT

The presence of bacterial-free DNA in the blood of mice with the cecal ligation and puncture-induced sepsis

The cecal ligation and puncture (CLP) model, a mouse model similar to the septic patient (133), was performed to explore bacterial-free DNA in the blood in sepsis. The severity of the CLP mice was identified by survival analysis, blood bacterial burdens, serum bacterial-free DNA, serum endotoxin, serum FITC-dextran (gut permeability defect) (Figure 14A–E), serum cytokines (TNF-α, IL-6, and IL-10), serum creatinine (kidney injury), serum alanine transaminase (liver damage), and peripheral blood leukocyte (neutropenia and lymphopenia) (Figure 14G–I). The bacterial characteristics observed in the CLP group indicated a combination of different colony morphology in the blood culture and mass-spectrometry analysis, it was possible to identify the presence of both Gram-negative and Gram-positive bacteria. Gram-negative bacteria were found, including *Escherichia coli*, *Enterobacter spp.*, and *Pseudomonas spp.* Meanwhile, Gram-positive bacteria were found, including *Streptococcus spp.*, *Staphylococcus spp.*, and *Enterococcus spp.* (Figure 14F).

In parallel, the bacterial microbiome analysis was investigated in the blood of CLP mice (Figures 15A–C). Blood microbiome analysis of CLP mice at the genus level showed that *Escherichia coli* and *Streptococcal spp.* were more abundant, while *Enterobacteriaceae* were lower without the differences compared to sham (phylum level analyses) (Figures 15A, C). In blood bacteriome analysis, the greater abundance of *Escherichia coli* and *Streptococcal spp.* were also related to the species separated by the morphological colony from blood culture (Figure 14F).

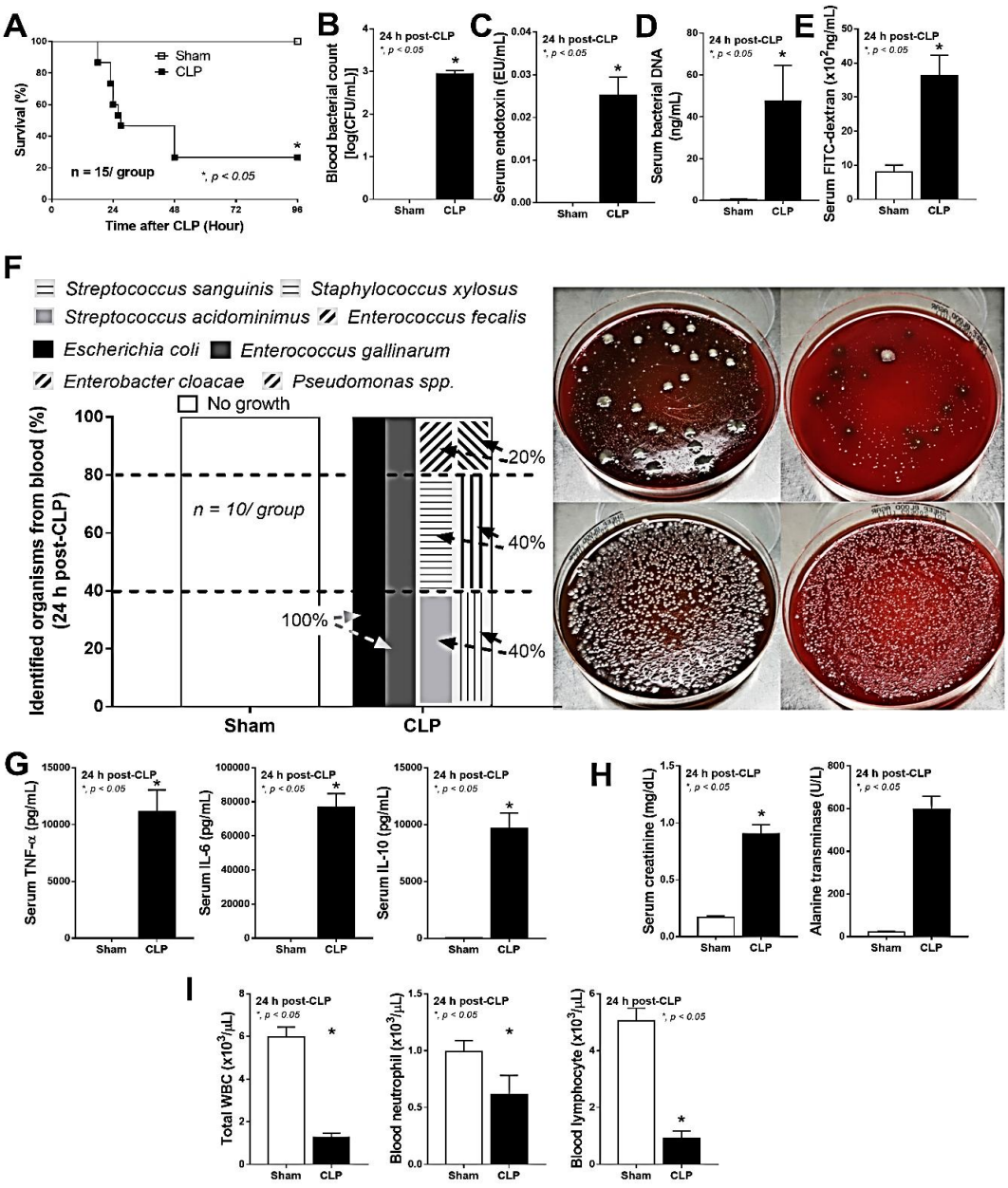


Figure 14 The cecal ligation and puncture (CLP) model represented bacteremia and the affectation of both intestinal infection and gut permeability defect.

Blood characteristics of both mice were identified by analysis of sepsis survival analysis, bacterial burdens, bacterial-free DNA and bacterial colony characteristics presences, endotoxin, gut leakage, an inflammatory cytokine, kidney injury, liver damage, and peripheral blood leukocyte at 24 hours after the experiment (n = 15/group for A and n = 9–10/group for B–I).

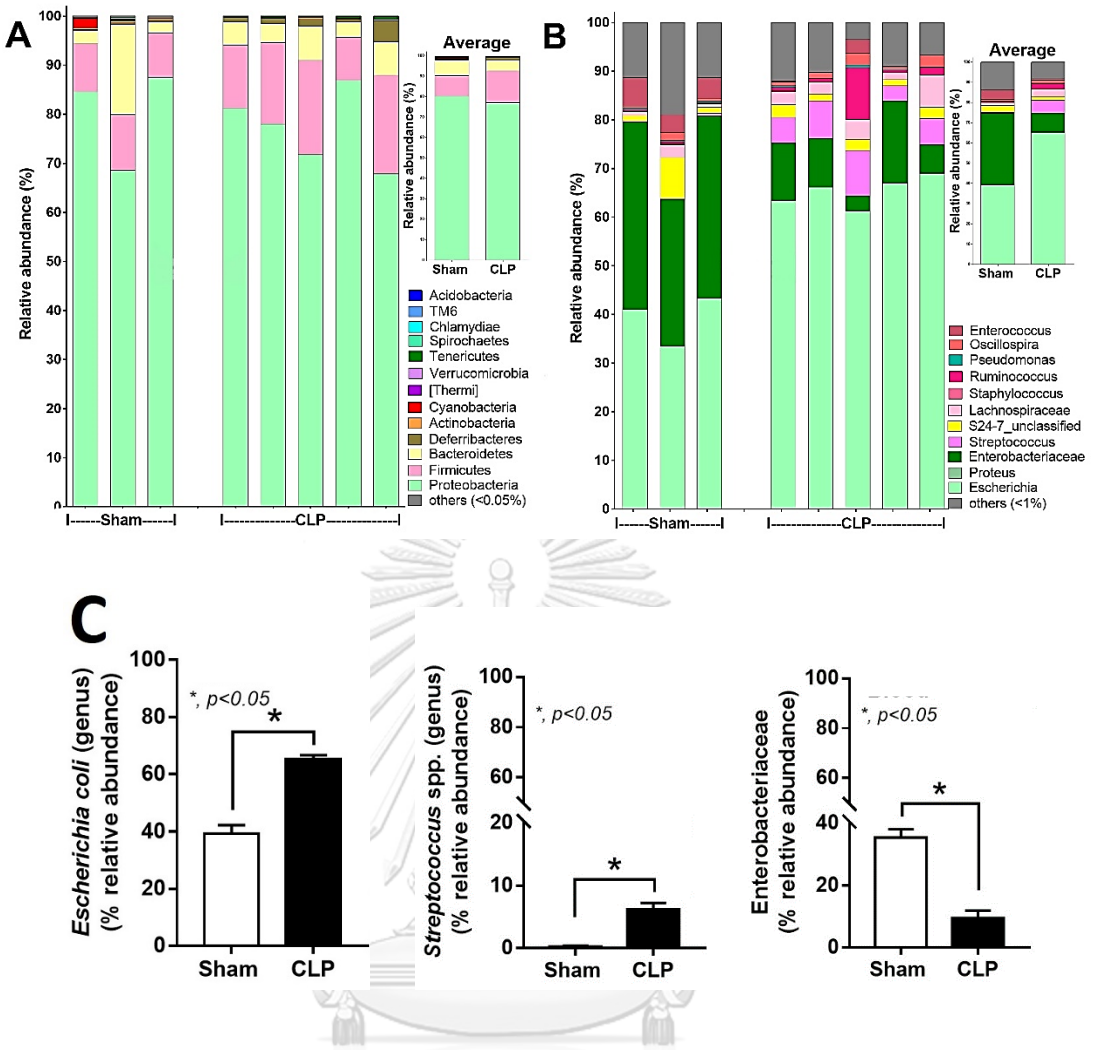


Figure 15 Blood bacteriome analyses in cecal ligation and puncture (CLP) mice and sham at 24 hours after surgery, represented the abundance of bacteria in both the phylum and species.

The increased inflammatory effect during sepsis might be due to the synergy between bacterial-free DNA and LPS in the mouse model

With the breakdown of bacteria in blood, bacterial DNA and endotoxin (LPS) might trigger a combined immune response (25, 134). Then, bacterial-free DNA and LPS were co-administered in mice. The mouse model with LPS injection alone showed inflammation throughout the body (serum TNF-**α**, IL-6, and IL-10) and liver damage (serum alanine transaminase) (Figures 16A–D). At 0.5 and 3 hours after injection, mice with bacterial DNA alone caused less systemic inflammation (serum TNF-**α**, IL-6, and IL-10) and liver injury (serum alanine transaminase) than other groups (Figures 16A–D). Compared with LPS injection alone, bacterial DNA plus LPS induced a more serum cytokine, especially IL-6 and IL-10, and serum alanine transaminase (Figures 16A–D). Consequently, it is possible the presence of bacterial-free DNA would increase severity during sepsis, in part due to the combination of endotoxemia.

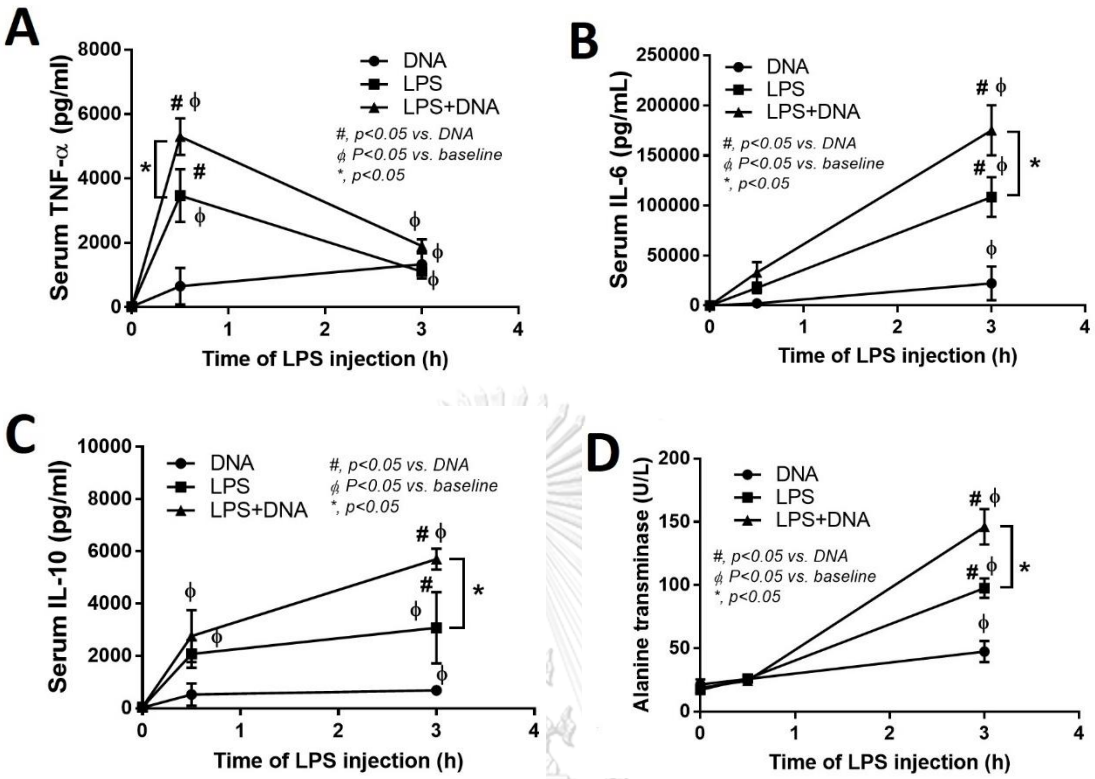


Figure 16 The influence of co-injection with bacterial-free DNA (DNA) and lipopolysaccharide (LPS) on the circulatory system.

Characteristics of mice after injection at 0.5 and 3 hours with bacteria-free DNA (DNA) alone or lipopolysaccharide (LPS) alone and DNA and LPS combination (DNA + LPS), as indicated by serum cytokines and serum alanine transaminase (n = 7–9/time-point).

Additive inflammatory effect in macrophages due to the synergy of bacterial-free DNA and LPS

In vitro experiments with bone marrow-derived macrophages demonstrated the importance of macrophages in recognizing molecular patterns associated with pathogens, both bacterial DNA and LPS (135). Stimulation of bone marrow-derived macrophages with bacterial DNA in combination with LPS showed a more pronounced inflammation (supernatant TNF-**α**, IL-6, and IL-10) than activation with bacterial DNA alone and LPS alone (Figures 17A–C). Furthermore, the gene expression of pro-inflammatory signals, including *iNOS* and *IL -1**β***, and anti-inflammatory signals, including *Arg-1* and *Fizz-1* of the macrophages activated with bacterial DNA in combination with LPS were the highest among the others (Figures 17D–H). For the activation of bone marrow-derived macrophages with bacterial DNA alone, inflammation (supernatant IL-10, but not TNF-**α** and IL-6) was higher than in the control media but lower than LPS stimulation alone and bacterial DNA stimulation in combination with LPS (Figures 17A–C). These data support a synergy of bacterial-free and LPS (34-36).

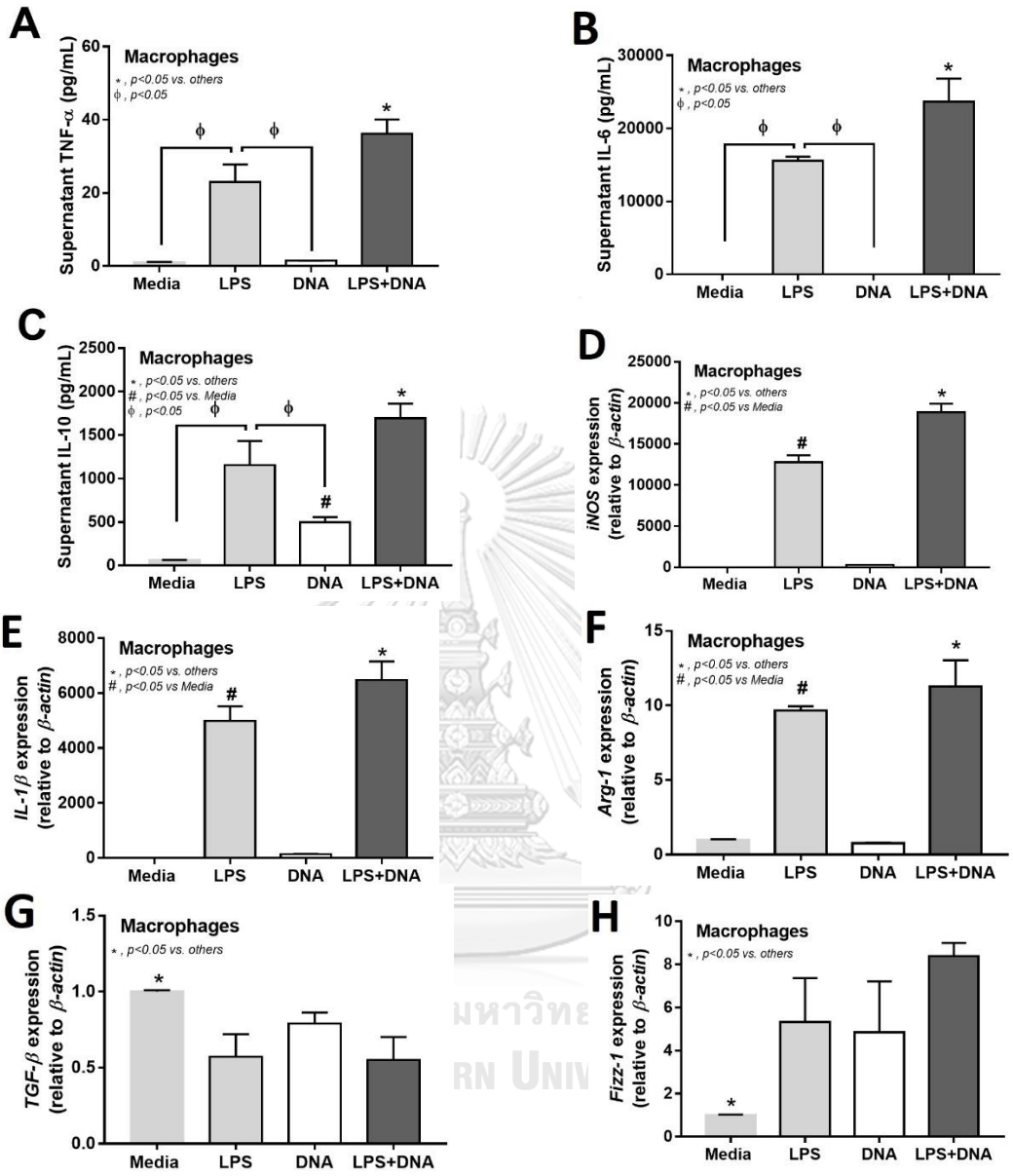


Figure 17 Effects of combined stimulation of bacterial free DNA (DNA) and lipopolysaccharide (LPS) on macrophage responses.

Characteristics of bone marrow (BM)-derived macrophages after stimulation at 0.5 and 3 hours with media control, bacteria-free DNA (DNA) or lipopolysaccharide (LPS) alone, and DNA and LPS combination (DNA + LPS), as indicated by inflammatory cytokines and genes expression of pro-inflammatory signals and anti-inflammatory signals.

CHAPTER VII

DISCUSSION

Translocation of bacterial DNA from the intestine into the blood circulation

Although most sources of bacterial-free DNA in the blood during sepsis may result from the breakdown of bacteria, intestinal defects that occur during sepsis could also cause the transfers of bacterial-free DNA into the circulatory system (16, 17). In general, the intact bacterial DNA has a relatively large molecular size, ranging between 100 and 15,000 kbp or approximately $6.5 \times 10^4 - 9.8 \times 10^6$ kDa that cannot pass through the intestinal barrier (136, 137). However, bacterial-free DNA is rapidly naturally degraded by spontaneous physical and biochemical attacks such as depurination and deamination, resulting in a DNA size of less than 100 bp (65 kDa) (11-14) and has the opportunity to transfer through the intestinal barrier (138, 139). In CLP (the most resemble model to the septic patients) (133), we demonstrated the presence of bacterial-free DNA in the serum that possibly resulted from bacterial death in blood and intestinal translocation. Blood culture of CLP mice demonstrated i) diverse bacterial colony morphologies in blood agar, ii) sepsis-induced gut barrier defect (FITC-dextran test), and iii) bacterial-free DNA in blood as indicated by real-time PCR and blood bacteriome analysis.

Blood bacteriome analysis of sham and CLP mice at the phylum level demonstrated a high abundance of Proteobacteria, a group of pathogenic bacteria.

While blood bacteriome analysis at the genus level found an abundance of *E. coli*, one of the Proteobacteria and a common cause of gut-derived infection (16), in CLP mice was higher than in sham. In addition, the presence of Proteobacteria in blood of sham group suggested a possibility of the transient intestinal translocation in healthy mice despite the absence of gut barrier defect by the FITC-dextran assay. This might be due to the induction of pathogenic bacteria, especially Proteobacteria (140, 141). Based on the data obtained from the CLP surgery, we suggested that the bacterial-free DNA in serum during sepsis may not only come from bacterial decay but also be transferred from the intestines into the circulatory system.

Bacterial DNA enhances LPS-induced pro-inflammatory responses in mice and macrophage cells

Bacterial-free DNA is one of the pathogen-associated molecular patterns with inflammation-stimulating properties, its role in immune responses is still uncertain (18-20). Although the benefits of the reduction in blood bacterial-free DNA during sepsis is still in debate (25), the presence of bacterial-free DNA in septic patients was reported to be a pro-inflammatory molecule (34). Despite previous reports on differences in immune responses between bacterial-free DNA and CpG DNA (37), as well as the influence of co-administration between CpG DNA and LPS in mice (34), data on the influence of crude DNA remains limited. For this reason, a model of bacterial-free DNA and LPS injection was performed to investigate the effects of

crude bacterial-free DNA (but not synthetic CpG DNA) on LPS responses. Here, mice injected with bacterial-free DNA in combination with LPS induced more severe systemic inflammation (serum cytokines) and liver injury (liver enzymes) than LPS injection alone, possibly due to the immune responses against bacterial DNA.

Based on these data, the function of bacterial-free DNA in combination with LPS may induce systemic inflammation via macrophage M1 polarization (*iNOS* and *IL-1β* expression) (34, 36). Indeed, the immune responses against a combination between bacterial-free DNA and LPS was further highlighted in bone marrow-derived macrophages as DNA-stimulated macrophages alone induced low pro-inflammation compared with LPS stimulation as previously mentioned (131). However, the combination of bacterial-free DNA and LPS enhanced inflammatory responses (supernatant cytokines and inflammatory signals) when compared with LPS alone.

In conclusion, the profound inflammatory response from co-activation between bacterial DNA (from bacterial breakdown) and LPS (from intestinal translocation) demonstrated the influence of serum bacterial-free DNA on effective immune responses. In figure 18, the possible synergy of bacterial DNA and LPS from several published literature demonstrates the possible simultaneous immune activation of TLR-4 and TLR-9 from LPS and bacterial DNA, respectively. Therefore, inhibition of DNA activation or depletion of bacterial-free DNA in the blood could be

a promising strategy or adjunctive intervention for some sepsis cases. More studies should be further tested.

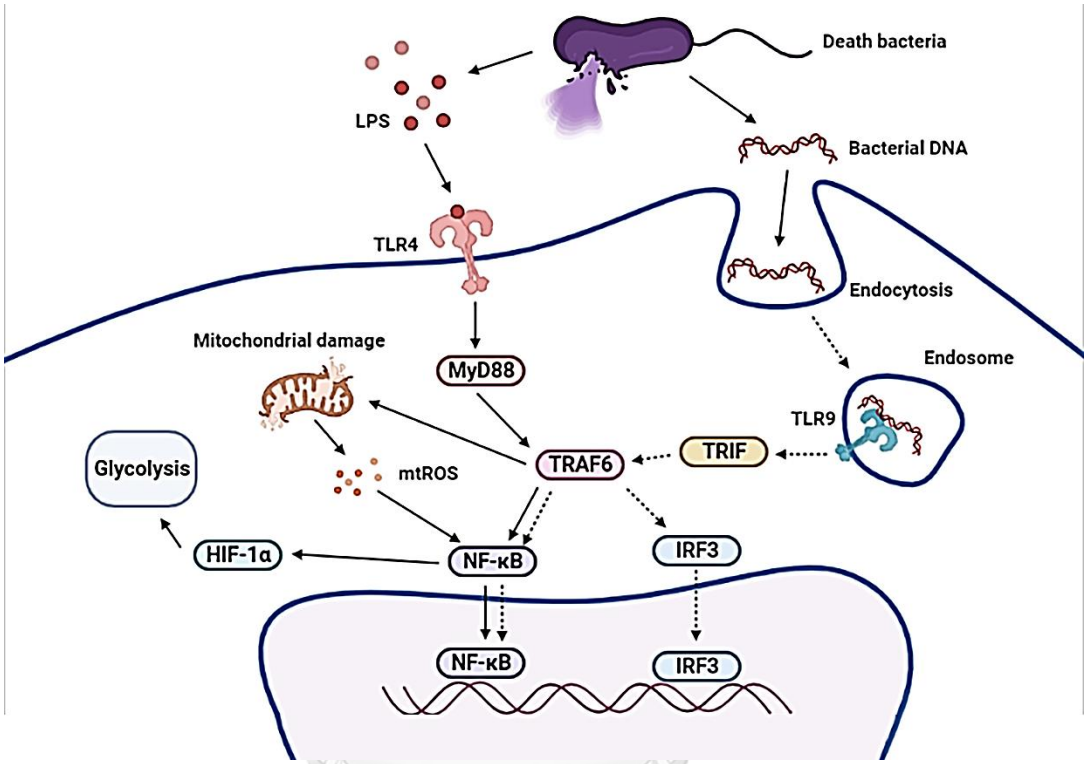


Figure 18 The functional hypothesis suggests simultaneous activation of the endosomal TLR-9 and surface TLR-4 by bacterial DNA and LPS, respectively.

LPS induce an inflammatory response via MyD88, while DNA induces TRIF (30, 142). The potent inflammatory stimulation requires high cellular energy through glycolysis (143) and mitochondria (that might cause mitochondrial damage) (131, 144). Figure created using Biorender (https://biorender.com/, accessed on 29 January 2022).

APPENDIX A

MATERIALS AND EQULPMENT

1. 6 wells plate (Cell Culture plate)	USA
2. 96 wells flat bottom plate	USA
3. 96 wells PCR microplate	USA
4. Biosafety Cabinet Class II NU-4oo-600E	USA
5. Centrifuge 5415R	USA
6. Centrifuge U-32R	Germany
7. CO_2 incubator NU-5500E	USA
8. Decimal balance XT-220A	Switzerland
9. Filter pipet tip 10, 200, 1000 µL	USA
10. Incubator shaker N-biotek	Korea
11. Mass spectrophotometry	France
12. Microbiological incubator memmert	Germany
13. Micropipette 1, 10, 200, 1000 µL	Germany
14. NanoDrop 1000	USA
15. Pipet tip 10, 200, 1000 µL	USA
16. ProFlex PCR system	USA
17. QuantStudio® 6 Real-Time PCR system	USA
18. Serological pipette 5, 25, 50 mL	USA
19. T75 Flasks	USA
20. Varioskan Flash Multimode ELISA reader	USA
21. Vortex Genie 2	USA
22. Water bath memmert	Germany

APPENDIX B

BIOLOGICAL/CHEMICAL AGENTS AND REAGENYS

1. Absolute ethanol	USA
2. Blood agar	UK
3. Dulbecco's Modified Eagle Medium (DMEM)	USA
4. EnzyChrom ALT Assay	USA
5. Enzyme-linked immunosorbent assay (ELASA) kit	USA
6. Fetal Bovine Serum (FBS)	USA
7. Fluorescein isothiocyanate (FITC)-labeled CD206 antibody	USA
8. Genomic DNA Extraction Mini Kit	USA
9. Genomic DNA Extraction Mini Kit	Australia
10. HEPES	USA
11. High-Capacity cDNA Reverse Transcription Kit	USA
12. Horse Serum	USA
13. Lipopolysaccharide (LPS; Escherichia coli 026: B6)	USA
14. Multiscribe reverse transcriptase	USA
15. Normal saline	Thailand
16. Penicillin-Streptomycin	USA
17. Phosphate Buffer Saline (PBS)	Thailand
18. PowerUpTM SYBRTM Green Master Mix	USA
19. Primer	USA
20. QuantiChrom Creatinine-Assay (DICT-500)	USA
21. RNase/DNase free H_2O	Germany
22. Sodium pyruvate	USA
23. Tissue-Tek OCT compound	UK

24. Total RNA Purification Mini Kit	Taiwan
25. Trypticase soy agar plate (TSA)	UK
26. Tryptic soy broth (TSB)	UK

APPENDIX C

REAGENTS PREPARATION

1. Complete DMEM (Dulbecco's Modified Eagle Medium (DMEM) with high glucose)

DMEM	33.375 mL
10% FBS	3 mL
1% Sodium pyruvate	375 µL
1% HERES	375 µL
1.3% Pen-strep	375 µL
Horse serum	2.5 mL
L929 supernatant	10 mL

2. 1X DNase I Buffer

10 mM Tris-HCl	78.8 mg
2.5 mM $MgCl_2$	11.9 mg
0.5 mM $CaCl_2$	2.8 mg
ddH_20	50 mL

3. 70% ethanol

100% ethanol	70 mL
Sterile water	30 mL

4. 1X Phosphate Buffer Saline (PBS)

Stock solution (10X PBS)	100 mL
ddH_20	900 mL

5. Lipopolysaccharide

Stock solution (10 µg)

Lipopolysaccharide (1 mg)	5 µL

ddH_20	495 µL
Working solution (100 ng)	
Stock solution (10 µg)	60 µL
CDMEM	5.940 mL

6. Primer

Working solution (10 mM)	
Stock solution (100 mM)	20 µL
ddH_20	180 µL

7. Quantitative Real-Time Polymerase Chain Reaction (qPCR) master mix

10X RT Buffer	2.0 µL
25X dNTP Mix (100 mM)	0.8 µL
10X RT Random Primers	1.0 µL
MultiScribe™ Reverse Transcriptase	1.0 µL
Nuclease-free H_2O	4.2 µL

8. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) master mix

PowerUp™ SYBR™ Green Master Mix (2X)	5.0 µL
Forward primer	0.2 µL
Reverse primer	0.2 µL
Nuclease-free H_2O	2.6 µL
complementary DNA (cDNA)	2.0 µL

9. Stop solution ELISA

2N H_2SO_4	2.805 mL
ddH_20	47.195 mL

10. 50X TAE buffer

Tris base	242 g
Glacial acetic acid	57.1 mL
0.5M EDTA (pH 8.0)	100 mL
ddH_20 (Total volume)	1000 mL

11. Wash Buffer

1X PBS	1000 mL
0.05% Tween 20	500 µL

REFERENCES

(1) I. T. Schrijver, C. Théroude and T. Roger. Myeloid-Derived Suppressor Cells in Sepsis. Journal 10,Issue (2019): 2019327.

(2) C. Cao, M. Yu and Y. Chai. Pathological alteration and therapeutic implications of sepsis-induced immune cell apoptosis. Journal 10,Issue (2019): 2019782.

(3) E. C. van der Slikke, A. Y. An, R. E. W. Hancock and H. R. Bouma. Exploring the pathophysiology of post-sepsis syndrome to identify therapeutic opportunities. Journal 61,Issue (2020): 2020103044.

(4) M. W. Adelman, M. H. Woodworth, C. Langelier, L. M. Busch, J. A. Kempker, C. S. Kraft, et al. The gut microbiome's role in the development, maintenance, and outcomes of sepsis. Journal 24,Issue (2020): 2020278.

(5) M. G. Mureșan, I. A. Balmoș, I. Badea and A. Santini. Abdominal Sepsis: An Update. Journal 4,Issue (2018): 2018120-125.

(6) F. Haussner, S. Chakraborty, R. Halbgebauer and M. Huber-Lang. Challenge to the Intestinal Mucosa During Sepsis. Journal 10,Issue (2019): 2019891.

(7) K. Sae-Khow, A. Charoensappakit, P. Visitchanakun, W. Saisorn, S. Svasti, S. Fucharoen, et al. Pathogen-Associated Molecules from Gut Translocation Enhance Severity of Cecal Ligation and Puncture Sepsis in Iron-Overload β-Thalassemia Mice. Journal 13,Issue (2020): 2020719-735.

(8) N. de Prost, K. Razazi and C. Brun-Buisson. Unrevealing culture-negative severe sepsis. Journal 17,Issue (2013): 20131001.

(9) Y. Long, Y. Zhang, Y. Gong, R. Sun, L. Su, X. Lin, et al. Diagnosis of Sepsis with Cell-free DNA by Next-Generation Sequencing Technology in ICU Patients. Journal 47,Issue (2016): 2016365-371.

(10) J. Yang, Z. Li, Y. Liu and S. Guo. Tracking bacterial DNA patterns in septic progression using 16s rRNA gene amplicon sequencing analysis. Journal 14,Issue (2021): 2021753-767.

(11) T. Lindahl. Instability and decay of the primary structure of DNA. Journal 362,Issue (1993): 1993709-715.

(12) K. M. Nielsen, P. J. Johnsen, D. Bensasson and D. Daffonchio. Release and persistence of extracellular DNA in the environment. Journal 6,Issue (2007): 200737-53.

(13) S. Overballe-Petersen, K. Harms, L. A. Orlando, J. V. Mayar, S. Rasmussen, T. W. Dahl, et al. Bacterial natural transformation by highly fragmented and damaged DNA. Journal 110,Issue (2013): 201319860-5.

(14) E. Willerslev, A. J. Hansen, R. Rønn, T. B. Brand, I. Barnes, C. Wiuf, et al. Long-term persistence of bacterial DNA. Journal 14,Issue (2004): 2004R9-10.

(15) B. Jann, K. Reske and K. Jann. Heterogeneity of lipopolysaccharides. Analysis of polysaccharide chain lengths by sodium dodecylsulfate-polyacrylamide gel electrophoresis. Journal 60,Issue (1975): 1975239-46.

(16) P. Amornphimoltham, P. S. T. Yuen, R. A. Star and A. Leelahavanichkul. Gut Leakage of Fungal-Derived Inflammatory Mediators: Part of a Gut-Liver-Kidney Axis in Bacterial Sepsis. Journal 64,Issue (2019): 20192416-2428.

(17) A. Leelahavanichkul, N. Worasilchai, S. Wannalerdsakun, K. Jutivorakool, P. Somparn, J. Issara-Amphorn, et al. Gastrointestinal Leakage Detected by Serum (1⟶3)-β-D-Glucan in Mouse Models and a Pilot Study in Patients with Sepsis. Journal 46,Issue (2016): 2016506-518.

(18) J. Schrenzel. PCR for the diagnosis of sepsis: hope or hype? Journal 15,Issue (2011): 2011111.

(19) M. J. Struelens. Detection of microbial DNAemia: does it matter for sepsis management? Journal 36,Issue (2010): 2010193-5.

(20) I. Ziegler, S. Cajander, G. Rasmussen, T. Ennefors, P. Mölling and K. Strålin. High nuc DNA load in whole blood is associated with sepsis, mortality and immune dysregulation in Staphylococcus aureus bacteraemia. Journal 51,Issue (2019): 2019216-226.

(21) Z. Cheng, S. T. Abrams, J. Austin, J. Toh, S. S. Wang, Z. Wang, et al. The Central Role and Possible Mechanisms of Bacterial DNAs in Sepsis Development. Journal 2020,Issue (2020): 20207418342.

(22) T. Gosiewski, A. H. Ludwig-Galezowska, K. Huminska, A. Sroka-Oleksiak, P. Radkowski, D. Salamon, et al. Comprehensive detection and identification of bacterial

DNA in the blood of patients with sepsis and healthy volunteers using next-generation sequencing method - the observation of DNAemia. Journal 36,Issue (2017): 2017329-336.

(23) S. Hamaguchi, Y. Akeda, N. Yamamoto, M. Seki, K. Yamamoto, K. Oishi, et al. Origin of Circulating Free DNA in Sepsis: Analysis of the CLP Mouse Model. Journal 2015,Issue (2015): 2015614518.

(24) A. Jörres. Blood purification in sepsis. Journal 22,Issue (2018): 2018357.

(25) D. Lilleri, G. Gerna, M. Furione, M. E. Bernardo, G. Giorgiani, S. Telli, et al. Use of a DNAemia cut-off for monitoring human cytomegalovirus infection reduces the number of preemptively treated children and young adults receiving hematopoietic stem-cell transplantation compared with qualitative pp65 antigenemia. Journal 110,Issue (2007): 20072757-60.

(26) P. Chen, S. Li, W. Li, J. Ren, F. Sun, R. Liu, et al. Rapid diagnosis and comprehensive bacteria profiling of sepsis based on cell-free DNA. Journal 18,Issue (2020): 20205.

(27) W. Liao, X. Zuo, G. Lin, Y. Zhou, Y. Fu, S. Cai, et al. Microbial cell-free DNA in plasma of patients with sepsis: a potential diagnostic methodology. Journal 29,Issue (2020): 2020129-137.

(28) D. M. Mosser, K. Hamidzadeh and R. Goncalves. Macrophages and the maintenance of homeostasis. Journal 18,Issue (2021): 2021579-587.

(29) M. E. Kotas and M. A. Matthay. Mesenchymal stromal cells and macrophages in sepsis: new insights. Journal 51,Issue (2018): 2018

(30) T. Celhar, S. Pereira-Lopes, S. I. Thornhill, H. Y. Lee, M. K. Dhillon, M. Poidinger, et al. TLR7 and TLR9 ligands regulate antigen presentation by macrophages. Journal 28,Issue (2016): 2016223-32.

(31) O. M. El-Sayed, N. A. Dewyer, C. E. Luke, M. Elfline, A. Laser, C. Hogaboam, et al. Intact Toll-like receptor 9 signaling in neutrophils modulates normal thrombogenesis in mice. Journal 64,Issue (2016): 20161450-1458.e1.

(32) J. M. Roda, R. Parihar and W. E. Carson, 3rd. CpG-containing oligodeoxynucleotides act through TLR9 to enhance the NK cell cytokine response to antibody-coated tumor cells. Journal 175,Issue (2005): 20051619-27.

(33) L. Sacramento, S. C. Trevelin, M. S. Nascimento, D. S. Lima-Jùnior, D. L. Costa, R. P. Almeida, et al. Toll-like receptor 9 signaling in dendritic cells regulates neutrophil recruitment to inflammatory foci following Leishmania infantum infection. Journal 83,Issue (2015): 20154604-16.

(34) S. Cornélie, E. Wiel, N. Lund, G. Lebuffe, C. Vendeville, G. Riveau, et al. Cytosine-phosphate-guanine (CpG) motifs are sensitizing agents for lipopolysaccharide in toxic shock model. Journal 28,Issue (2002): 20021340-7.

(35) T. Sparwasser, T. Miethke, G. Lipford, K. Borschert, H. Häcker, K. Heeg, et al. Bacterial DNA causes septic shock. Journal 386,Issue (1997): 1997336-7.

(36) A. K. Yi, J. G. Yoon, S. C. Hong, T. W. Redford and A. M. Krieg. Lipopolysaccharide and CpG DNA synergize for tumor necrosis factor-alpha production through activation of NF-kappaB. Journal 13,Issue (2001): 20011391-404.

(37) T. L. Roberts, J. A. Dunn, T. D. Terry, M. P. Jennings, D. A. Hume, M. J. Sweet, et al. Differences in macrophage activation by bacterial DNA and CpG-containing oligonucleotides. Journal 175,Issue (2005): 20053569-76.

(38) E. Dekaboruah, M. V. Suryavanshi, D. Chettri and A. K. Verma. Human microbiome: an academic update on human body site specific surveillance and its possible role. Journal 202,Issue (2020): 20202147-2167.

(39) S. Mukherjee, N. Joardar, S. Sengupta and S. P. Sinha Babu. Gut microbes as future therapeutics in treating inflammatory and infectious diseases: Lessons from recent findings. Journal 61,Issue (2018): 2018111-128.

(40) C. Wang, Q. Li and J. Ren. Microbiota-Immune Interaction in the Pathogenesis of Gut-Derived Infection. Journal 10,Issue (2019): 20191873.

(41) T. L. Lin, C. C. Lu, W. F. Lai, T. S. Wu, J. J. Lu, Y. M. Chen, et al. Role of gut microbiota in identification of novel TCM-derived active metabolites. Journal (2020): 2020

(42) J. Y. Yoo, M. Groer, S. V. O. Dutra, A. Sarkar and D. I. McSkimming. Gut Microbiota and Immune System Interactions. Journal 8,Issue (2020): 2020

(43) F. C. Pereira and D. Berry. Microbial nutrient niches in the gut. Journal 19,Issue (2017): 20171366-1378.

(44) E. P. Neis, C. H. Dejong and S. S. Rensen. The role of microbial amino acid

metabolism in host metabolism. Journal 7,Issue (2015): 20152930-46.

(45) B. W. Haak, H. C. Prescott and W. J. Wiersinga. Therapeutic Potential of the Gut Microbiota in the Prevention and Treatment of Sepsis. Journal 9,Issue (2018): 20182042.

(46) N. Koppel, V. Maini Rekdal and E. P. Balskus. Chemical transformation of xenobiotics by the human gut microbiota. Journal 356,Issue (2017): 2017

(47) I. Wuethrich, W. P. B, Y. Khodamoradi and M. Vehreschild. The role of the human gut microbiota in colonization and infection with multidrug-resistant bacteria. Journal 13,Issue (2021): 20211-13.

(48) D. Zheng, T. Liwinski and E. Elinav. Interaction between microbiota and immunity in health and disease. Journal 30,Issue (2020): 2020492-506.

(49) S. M. Jandhyala, R. Talukdar, C. Subramanyam, H. Vuyyuru, M. Sasikala and D. Nageshwar Reddy. Role of the normal gut microbiota. Journal 21,Issue (2015): 20158787-803.

(50) T. Herta, A. Bhattacharyya, C. Bollensdorf, C. Kabus, P. García, N. Suttorp, et al. DNA-release by Streptococcus pneumoniae autolysin LytA induced Krueppel-like factor 4 expression in macrophages. Journal 8,Issue (2018): 20185723.

(51) E. R. Littmann, J.-J. Lee, J. E. Denny, Z. Alam, J. R. Maslanka, I. Zarin, et al. Host immunity modulates the efficacy of microbiota transplantation for treatment of Clostridioides difficile infection. Journal 12,Issue (2021): 2021755.

(52) B. A. Paray, M. F. Albeshr, A. T. Jan and I. A. Rather. Leaky Gut and Autoimmunity: An Intricate Balance in Individuals Health and the Diseased State. Journal 21,Issue (2020): 2020

(53) C. Chelakkot, J. Ghim and S. H. Ryu. Mechanisms regulating intestinal barrier integrity and its pathological implications. Journal 50,Issue (2018): 20181-9.

(54) Q. Mu, J. Kirby, C. M. Reilly and X. M. Luo. Leaky Gut As a Danger Signal for Autoimmune Diseases. Journal 8,Issue (2017): 2017598.

(55) W. Panpetch, W. Chancharoenthana, K. Bootdee, S. Nilgate, M. Finkelman, S. Tumwasorn, et al. Lactobacillus rhamnosus L34 Attenuates Gut Translocation-Induced Bacterial Sepsis in Murine Models of Leaky Gut. Journal 86,Issue (2018): 2018

(56) W. Panpetch, V. Sawaswong, P. Chanchaem, T. Ondee, C. P. Dang, S. Payungporn,

et al. Candida Administration Worsens Cecal Ligation and Puncture-Induced Sepsis in Obese Mice Through Gut Dysbiosis Enhanced Systemic Inflammation, Impact of Pathogen-Associated Molecules From Gut Translocation and Saturated Fatty Acid. Journal 11,Issue (2020): 2020561652.

(57) M. E. M. Obrenovich. Leaky Gut, Leaky Brain? Journal 6,Issue (2018): 2018

(58) G. Caio, L. Lungaro, N. Segata, M. Guarino, G. Zoli, U. Volta, et al. Effect of Gluten-Free Diet on Gut Microbiota Composition in Patients with Celiac Disease and Non-Celiac Gluten/Wheat Sensitivity. Journal 12,Issue (2020): 2020

(59) V. T. Pham, N. Seifert, N. Richard, D. Raederstorff, R. E. Steinert, K. Prudence, et al. The effects of fermentation products of prebiotic fibres on gut barrier and immune functions in vitro. Journal 6,Issue (2018): 2018e5288.

(60) C. Raghu Subramanian, S. Talluri, S. U. Khan, J. A. Katz, M. Georgetson and P. Sinh. Fecal Microbiota Transplantation for Recurrent Clostridium difficile Infection in Patients With Multiple Comorbidities: Long-Term Safety and Efficacy Results From a Tertiary Care Community Hospital. Journal 13,Issue (2020): 2020138-145.

(61) A. Khan, Z. Ding, I. Muhammad, A. Bacha, I. Khan, A. Hanif, et al. Understanding the Effects of Gut Microbiota Dysbiosis on Nonalcoholic Fatty Liver Disease and the Possible Probiotics Role: Recent Updates. Journal 17,Issue (2021): 2021818-833.

(62) M. Singer, C. S. Deutschman, C. W. Seymour, M. Shankar-Hari, D. Annane, M. Bauer, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). Journal 315,Issue (2016): 2016801-10.

(63) M. D. Font, B. Thyagarajan and A. K. Khanna. Sepsis and Septic Shock - Basics of diagnosis, pathophysiology and clinical decision making. Journal 104,Issue (2020): 2020573-585.

(64) T. Abe, K. Yamakawa, H. Ogura, S. Kushimoto, D. Saitoh, S. Fujishima, et al. Epidemiology of sepsis and septic shock in intensive care units between sepsis-2 and sepsis-3 populations: sepsis prognostication in intensive care unit and emergency room (SPICE-ICU). Journal 8,Issue (2020): 202044.

(65) M. Cecconi, L. Evans, M. Levy and A. Rhodes. Sepsis and septic shock. Journal 392,Issue (2018): 201875-87.

(66) K. Reinhart, R. Daniels, N. Kissoon, F. R. Machado, R. D. Schachter and S. Finfer.

Recognizing Sepsis as a Global Health Priority — A WHO Resolution. Journal 377,Issue (2017): 2017414-417.

(67) K. Fay, M. R. P. Sapiano, R. Gokhale, R. Dantes, N. Thompson, D. E. Katz, et al. Assessment of Health Care Exposures and Outcomes in Adult Patients With Sepsis and Septic Shock. Journal 3,Issue (2020): 2020e206004.

(68) P. Pairattanakorn, N. Angkasekwinai, R. Sirijatuphat, W. Wangchinda, L. Tancharoen and V. Thamlikitkul. Diagnostic and Prognostic Utility Compared Among Different Sepsis Scoring Systems in Adult Patients With Sepsis in Thailand: A Prospective Cohort Study. Journal 8,Issue (2021): 2021ofaa573.

(69) J. E. Gotts and M. A. Matthay. Sepsis: pathophysiology and clinical management. Journal 353,Issue (2016): 2016i1585.

(70) P. Arina and M. Singer. Pathophysiology of sepsis. Journal 34,Issue (2021): 202177-84.

(71) N. L. Denning, M. Aziz, S. D. Gurien and P. Wang. DAMPs and NETs in Sepsis. Journal 10,Issue (2019): 20192536.

(72) C. Ince, P. R. Mayeux, T. Nguyen, H. Gomez, J. A. Kellum, G. A. Ospina-Tascón, et al. THE ENDOTHELIUM IN SEPSIS. Journal 45,Issue (2016): 2016259-70.

(73) S. M. Hollenberg and M. Singer. Pathophysiology of sepsis-induced cardiomyopathy. Journal 18,Issue (2021): 2021424-434.

(74) S. G. Lee, J. Song, D. W. Park, S. Moon, H. J. Cho, J. Y. Kim, et al. Prognostic value of lactate levels and lactate clearance in sepsis and septic shock with initial hyperlactatemia: A retrospective cohort study according to the Sepsis-3 definitions. Journal 100,Issue (2021): 2021e24835.

(75) M. A. Matthay, R. L. Zemans, G. A. Zimmerman, Y. M. Arabi, J. R. Beitler, A. Mercat, et al. Acute respiratory distress syndrome. Journal 5,Issue (2019): 201918.

(76) P. Kasper, F. Tacke, H. M. Steffen and G. Michels. [Hepatic dysfunction in sepsis]. Journal 115,Issue (2020): 2020609-619.

(77) J. Chen and H. Wei. Immune Intervention in Sepsis. Journal 12,Issue (2021): 2021

(78) K. M. Tourelle, S. Boutin, M. A. Weigand and F. C. F. Schmitt. Sepsis and the Human Microbiome. Just Another Kind of Organ Failure? A Review. Journal 10,Issue (2021): 20214831.

(79) J. R. Strich, E. L. Heil and H. Masur. Considerations for Empiric Antimicrobial Therapy in Sepsis and Septic Shock in an Era of Antimicrobial Resistance. Journal 222,Issue (2020): 2020S119-S131.

(80) O. A. Usman, A. A. Usman and M. A. Ward. Comparison of SIRS, qSOFA, and NEWS for the early identification of sepsis in the Emergency Department. Journal 37,Issue (2019): 20191490-1497.

(81) M. E. Celikkaya, B. Akcora, S. Hakverdi, B. Ozer, K. T. Ulutas and N. Duran. Effects of Probiotic Use on Bacterial Translocation in Created Rat Models with Biliary Obstructions. Journal 51,Issue (2019): 2019106-111.

(82) O. D. Umeh, O. O. Sanu, I. L. Utomi and F. O. Nwaokorie. Prevalence and intensity of bacteraemia following orthodontic procedures. Journal 14,Issue (2016): 201680-94.

(83) N. R. Shin, T. W. Whon and J. W. Bae. Proteobacteria: microbial signature of dysbiosis in gut microbiota. Journal 33,Issue (2015): 2015496-503.

(84) A. Sivan, L. Corrales, N. Hubert, J. B. Williams, K. Aquino-Michaels, Z. M. Earley, et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Journal 350,Issue (2015): 20151084-9.

(85) P. Sirivongrangson, W. Kulvichit, S. Payungporn, T. Pisitkun, A. Chindamporn, S. Peerapornratana, et al. Endotoxemia and circulating bacteriome in severe COVID-19 patients. Journal 8,Issue (2020): 202072.

(86) G. Gargari, G. Mantegazza, V. Taverniti, C. Del Bo, S. Bernardi, C. Andres-Lacueva, et al. Bacterial DNAemia is associated with serum zonulin levels in older subjects. Journal 11,Issue (2021): 202111054.

(87) W. Makałowski. The human genome structure and organization. Journal 48,Issue (2001): 2001587-598.

(88) S. Casagranda. Modeling, analysis and reduction of biological systems. Journal (2017): 2017

(89) R. T. Dame, F.-Z. M. Rashid and D. C. Grainger. Chromosome organization in bacteria: mechanistic insights into genome structure and function. Journal 21,Issue (2020): 2020227-242.

(90) A. Travers and G. Muskhelishvili. DNA structure and function. Journal 282,Issue (2015): 2015

(91) G. Salieb-Beugelaar. Electrokinetic Transport of DNA in Nanoslits. Journal (2022): 2022

(92) L. Panawala. Home » Science » Biology » Molecular Biology » Difference Between Prokaryotic and Eukaryotic DNA Difference Between Prokaryotic and Eukaryotic DNA. Journal (2017): 2017

(93) B. J. Shuter, J. Thomas, W. D. Taylor and A. M. Zimmerman. Phenotypic correlates of genomic DNA content in unicellular eukaryotes and other cells. Journal 122,Issue (1983): 198326-44.

(94) D. M. Klinman, A. K. Yi, S. L. Beaucage, J. Conover and A. M. Krieg. CpG motifs present in bacteria DNA rapidly induce lymphocytes to secrete interleukin 6, interleukin 12, and interferon gamma. Journal 93,Issue (1996): 19962879-83.

(95) A. S. Trevani, A. Chorny, G. Salamone, M. Vermeulen, R. Gamberale, J. Schettini, et al. Bacterial DNA activates human neutrophils by a CpG-independent pathway. Journal 33,Issue (2003): 20033164-74.

(96) R. Nakad and B. Schumacher. DNA Damage Response and Immune Defense: Links and Mechanisms. Journal 7,Issue (2016): 2016147.

(97) B. Briard, D. E. Place and T. D. Kanneganti. DNA Sensing in the Innate Immune Response. Journal 35,Issue (2020): 2020112-124.

(98) E. A. Martínez-García, M. G. Zavala-Cerna, A. V. Lujano-Benítez, P. E. Sánchez-Hernández, B. T. Martín-Márquez, F. Sandoval-García, et al. Potential Chronotherapeutic Optimization of Antimalarials in Systemic Lupus Erythematosus: Is Toll-Like Receptor 9 Expression Dependent on the Circadian Cycle in Humans? Journal 9,Issue (2018): 20181497.

(99) A. J. Talati, H. J. Kim, Y. I. Kim, A. K. Yi and B. K. English. Role of bacterial DNA in macrophage activation by group B streptococci. Journal 10,Issue (2008): 20081106-13.

(100) A. Ablasser and Z. J. Chen. cGAS in action: Expanding roles in immunity and inflammation. Journal 363,Issue (2019): 2019

(101) Z. Cheng, S. T. Abrams, J. Toh, S. S. Wang, Z. Wang, Q. Yu, et al. The Critical Roles and Mechanisms of Immune Cell Death in Sepsis. Journal 11,Issue (2020): 20201918.

(102) A. Decout, J. D. Katz, S. Venkatraman and A. Ablasser. The cGAS–STING pathway

as a therapeutic target in inflammatory diseases. Journal 21,Issue (2021): 2021548-569.

(103) G. Montamat, C. Leonard, A. Poli, L. Klimek and M. Ollert. CpG Adjuvant in Allergen-Specific Immunotherapy: Finding the Sweet Spot for the Induction of Immune Tolerance. Journal 12,Issue (2021): 2021

(104) K. Onishi, J. Mochizuki, A. Sato, A. Goto and T. Sashihara. Total RNA and genomic DNA of Lactobacillus gasseri OLL2809 induce interleukin-12 production in the mouse macrophage cell line J774.1 via toll-like receptors 7 and 9. Journal 20,Issue (2020): 2020217.

(105) M. E. Alvarez, J. I. Fuxman Bass, J. R. Geffner, P. X. Fernández Calotti, M. Costas, O. A. Coso, et al. Neutrophil signaling pathways activated by bacterial DNA stimulation. Journal 177,Issue (2006): 20064037-46.

(106) C. Bode, G. Zhao, F. Steinhagen, T. Kinjo and D. M. Klinman. CpG DNA as a vaccine adjuvant. Journal 10,Issue (2011): 2011499-511.

(107) C. F. Amorim, A. S. Souza, A. G. Diniz, N. B. Carvalho, S. B. Santos and E. M. Carvalho. Functional activity of monocytes and macrophages in HTLV-1 infected subjects. Journal 8,Issue (2014): 2014e3399.

(108) Y. H. Liu, Y. Ding, C. C. Gao, L. S. Li, Y. X. Wang and J. D. Xu. Functional macrophages and gastrointestinal disorders. Journal 24,Issue (2018): 20181181-1195.

(109) S. Y. Kim and M. G. Nair. Macrophages in wound healing: activation and plasticity. Journal 97,Issue (2019): 2019258-267.

(110) E. Uribe-Querol and C. Rosales. Phagocytosis: Our Current Understanding of a Universal Biological Process. Journal 11,Issue (2020): 2020

(111) K. Lindell. Cell-to-cell communication and virulence in Vibrio anguillarum. Journal (2022): 2022

(112) A. Lin and K. Loré. Granulocytes: New Members of the Antigen-Presenting Cell Family. Journal 8,Issue (2017): 2017

(113) C. Nicchitta. Opinion: Re-evaluating the role of heat-shock protein–peptide interactions in tumour immunity. Journal 3,Issue (2003): 2003427-32.

(114) C. Pifferi, N. Berthet and O. Renaudet. Cyclopeptide scaffolds in carbohydrate-based synthetic vaccines. Journal 5,Issue (2017): 2017

(115) S. Arora, K. Dev, B. Agarwal, P. Das and M. A. Syed. Macrophages: Their role,

activation and polarization in pulmonary diseases. Journal 223,Issue (2018): 2018383-396.

(116) J. Tur, T. Vico, J. Lloberas, A. Zorzano and A. Celada. Macrophages and Mitochondria: A Critical Interplay Between Metabolism, Signaling, and the Functional Activity. Journal 133,Issue (2017): 20171-36.

(117) M. Benoit, B. Desnues and J.-L. Mege. Macrophage Polarization in Bacterial Infections. Journal 181,Issue (2008): 20083733-3739.

(118) D. Lu, Y. Xu, Q. Liu and Q. Zhang. Mesenchymal Stem Cell-Macrophage Crosstalk and Maintenance of Inflammatory Microenvironment Homeostasis. Journal 9,Issue (2021): 2021

(119) V. Sampath. Bacterial endotoxin-Lipopolysaccharide; structure, function and its role in immunity in vertebrates and invertebrates. Journal 52,Issue (2018): 2018

(120) M. Kanost, H. Jiang and X.-Q. Yu. Kanost MR, Jiang H, Yu XQ. Innate immune responses of a lepidopteran insect, Manduca sexta. Immunological Reviews. Journal 198,Issue (2004): 200497-105.

(121) V. Sampath. Bacterial endotoxin-lipopolysaccharide; structure, function and its role in immunity in vertebrates and invertebrates. Journal 52,Issue (2018): 2018115-120.

(122) M. Suparna, G. Mallikarjun, S. Ingalhalli, V. Shyamkumar and A. Hooli. Role of antibacterial proteins in different silkworm strains against flacherie. Journal 6,Issue (2011): 2011365-369.

(123) S. Vishnupriya. Bio molecular studies in selected silkworm races of Bombyx mori L under biotic stress and their association with the genetic hardiness. Journal (2013): 2013

(124) L. Mazgaeen and P. Gurung. Recent Advances in Lipopolysaccharide Recognition Systems. Journal 21,Issue (2020): 2020379.

(125) A. Ciesielska, M. Matyjek and K. Kwiatkowska. TLR4 and CD14 trafficking and its influence on LPS-induced pro-inflammatory signaling. Journal 78,Issue (2021): 20211233-1261.

(126) X. Liu, S. Yin, Y. Chen, Y. Wu, W. Zheng, H. Dong, et al. LPS-induced

proinflammatory cytokine expression in human airway epithelial cells and macrophages via NF-κB, STAT3 or AP-1 activation. Journal 17,Issue (2018): 20185484-5491.

(127) L. Mazgaeen and P. Gurung. Recent Advances in Lipopolysaccharide Recognition Systems. Journal 21,Issue (2020): 2020

(128) Z. Nová, H. Škovierová and A. Calkovska. Alveolar-Capillary Membrane-Related Pulmonary Cells as a Target in Endotoxin-Induced Acute Lung Injury. Journal 20,Issue (2019): 2019831.

(129) Q. Li, C. Wang, C. Tang, X. Zhao, Q. He and J. Li. Identification and Characterization of Blood and Neutrophil-Associated Microbiomes in Patients with Severe Acute Pancreatitis Using Next-Generation Sequencing. Journal 8,Issue (2018): 20185.

(130) J. Wongpiyabovorn, W. Soonthornchai, A. Wilantho, M. Palasuk, S. Payungporn, P. Sodsai, et al. Effect of tacrolimus on skin microbiome in atopic dermatitis. Journal 74,Issue (2019): 20191400-1406.

(131) P. Visitchanakun, W. Kaewduangduen, A. Chareonsappakit, P. Susantitaphong, P. Pisitkun, P. Ritprajak, et al. Interference on Cytosolic DNA Activation Attenuates Sepsis Severity: Experiments on Cyclic GMP-AMP Synthase (cGAS) Deficient Mice. Journal 22,Issue (2021): 2021

(132) T. Jaroonwitchawan, P. Visitchanakun, P. C. Dang, P. Ritprajak, T. Palaga and A. Leelahavanichkul. Dysregulation of Lipid Metabolism in Macrophages Is Responsible for Severe Endotoxin Tolerance in FcgRIIB-Deficient Lupus Mice. Journal 11,Issue (2020): 2020959.

(133) K. Doi, A. Leelahavanichkul, P. S. Yuen and R. A. Star. Animal models of sepsis and sepsis-induced kidney injury. Journal 119,Issue (2009): 20092868-78.

(134) T. Rimmelé and J. A. Kellum. Clinical review: blood purification for sepsis. Journal 15,Issue (2011): 2011205.

(135) J. J. Gao, Q. Xue, C. J. Papasian and D. C. Morrison. Bacterial DNA and lipopolysaccharide induce synergistic production of TNF-alpha through a post-transcriptional mechanism. Journal 166,Issue (2001): 20016855-60.

(136) H. Ochman and A. Caro-Quintero. Genome Size and Structure, Bacterial. Journal

(2016): 2016

(137) D. Williams, W. L. Trimble, M. Shilts, F. Meyer and H. Ochman. Rapid quantification of sequence repeats to resolve the size, structure and contents of bacterial genomes. Journal 14,Issue (2013): 2013537.

(138) P. Bellot, R. Francés and J. Such. Pathological bacterial translocation in cirrhosis: pathophysiology, diagnosis and clinical implications. Journal 33,Issue (2013): 201331-9.

(139) G. P. Rodríguez-Laiz, P. Zapater, P. Melgar, C. Alcázar, M. Franco, P. Giménez, et al. Bacterial DNA translocation contributes to systemic inflammation and to minor changes in the clinical outcome of liver transplantation. Journal 9,Issue (2019): 2019835.

(140) M. Camilleri. Leaky gut: mechanisms, measurement and clinical implications in humans. Journal 68,Issue (2019): 20191516-1526.

(141) W. Panpetch, N. Somboonna, M. Palasuk, P. Hiengrach, M. Finkelman, S. Tumwasorn, et al. Oral Candida administration in a Clostridium difficile mouse model worsens disease severity but is attenuated by Bifidobacterium. Journal 14,Issue (2019): 2019e0210798.

(142) Y. C. Lu, W. C. Yeh and P. S. Ohashi. LPS/TLR4 signal transduction pathway. Journal 42,Issue (2008): 2008145-151.

(143) G. Soto-Heredero, M. M. Gómez de Las Heras, E. Gabandé-Rodríguez, J. Oller and M. Mittelbrunn. Glycolysis - a key player in the inflammatory response. Journal 287,Issue (2020): 20203350-3369.

(144) M. J. López-Armada, R. R. Riveiro-Naveira, C. Vaamonde-García and M. N. Valcárcel-Ares. Mitochondrial dysfunction and the inflammatory response. Journal 13,Issue (2013): 2013106-18.

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